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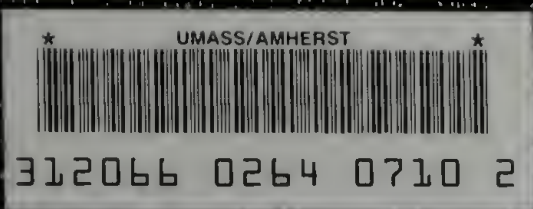
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SELF-ASSEMBLED POLYPEPTIDE-SURFACTANT COMPLEXES
IN ORGANIC SOLVENTS AND IN THE SOLID STATE:
A NEW CLASS OF COMB-SHAPED POLYPEPTIDES

A Dissertation Presented

by

EKATERINA A. PONOMARENKO

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 1997

Department of Polymer Science and Engineering

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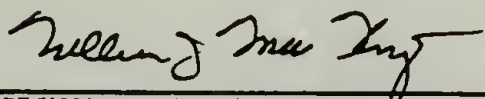
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ABSTRACT

SELF-ASSEMBLED POLYPEPTIDE-SURFACTANT COMPLEXES IN ORGANIC SOLVENTS AND IN THE SOLID STATE: A NEW CLASS OF COMB-SHAPED POLYPEPTIDES

SEPTEMBER 1997

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We describe herein the preparation and physical characterization of novel water-insoluble complexes formed by synthetic polypeptides, sodium poly(α ,L-glutamate) and poly(L-lysine) hydrobromide, and oppositely charged low molecular weight surfactants, alkyltrimethylammonium bromides and sodium alkyl sulfates with chain lengths from twelve to eighteen methylene groups. The complexes of nearly stoichiometric compositions were prepared by mixing equimolar amounts of the components in water. The goal of the research was to understand the influence of the electrostatically bound 'side chains' on properties of polypeptide chains (solubility and conformation) and the effect of polymer chains on organization of the complexed surfactants. The behavior of the complexes was compared to that of their covalent analogs, alkyl esters of poly(α ,L-glutamic acid) and acyl derivatives of poly(L-lysine).

Conformational and structural properties of the complexes in the solid state were studied via circular dichroism, infrared spectroscopy, X-ray diffraction and differential scanning calorimetry. Poly(α ,L-glutamate) chains in the complexes adopt α -helical

conformations at room temperature and disordered conformations at elevated temperatures. Poly(L-lysine) chains in the complexes adopt either β -sheet conformation (as isolated after synthesis) or α -helical conformation (in the solid films cast from chloroform - trifluoroacetic acid solutions). Organization of surfactants in the complexes depends on the surfactant chain length. Shorter chains (eight - sixteen carbon atoms) are packed with a short range order, while the longer chains (eighteen carbon atoms) crystallize on a hexagonal lattice. In complexes with mixed octyl and octadecyl sulfates, organization of the surfactants depends on the composition: the minimum octadecyl chain content required for crystallization is about 20 molar per cent. All complexes studied are organized in lamellar structures consisting of alternating layers of polypeptide chains separated by layers of surfactants.

Dilute solution properties of poly(L-lysine) - dodecyl sulfate complexes in organic solvents were studied via viscometry, ^1H NMR and ^1H NMR relaxation techniques. Poly(L-lysine) chains in the complexes in chloroform - trifluoroacetic solution adopt either α -helical (1 - 2 volume per cent trifluoroacetic acid) or disordered (4 - 10 volume per cent trifluoroacetic acid) conformations.

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CHAPTER I

INTRODUCTION

Concept of Molecular Self-Assembly

One of the goals of chemical synthesis is to prepare materials with tailored macroscopic properties. A promising strategy offering control over the prepared structures is to utilize the capability of molecules to self-organize. Self-assembly is defined as spontaneous intermolecular association via non-covalent bonds (e.g., electrostatic interactions, hydrogen bonds or hydrophobic interactions), resulting in thermodynamically stable, well-defined supramolecular structures with dimensions ranging from 10 nm to 10 μm .^{1,2} Self-organizing systems are widely represented in Nature, e.g., double-helical structures of nucleic acids and bilayers of lipids within cell membranes, with their organization responsible for their function.^{3,4} Assembly through non-covalent interactions offers a number of advantages over chemical synthesis involving covalent bonds: it does not require complicated preparative procedures, the reactions are typically characterized by fast kinetics,⁵ and the resulting structures may be capable of reversible adaptive rearrangement in response to changes in environment (e.g. solvent or temperature). Control over organization of synthetic supermolecular structures by tuning the assembly processes opens a fascinating possibility to manipulate material properties on a molecular scale. This may be particularly important for fabrication of multifunctional materials for technological applications where a high degree of control over properties is essential, e.g., electronic devices, microsensors, separation membranes, catalysts and biomaterials.

Complexes of Synthetic Polyelectrolytes and Oppositely Charged Surfactants

Types and Preparation of Complexes

Among the best-known synthetic self-assembling polymeric systems are complexes of polyelectrolytes and oppositely charged small amphiphilic molecules (surfactants) consisting of a polar headgroup and a nonpolar 'tail'.⁶ The complexation process is an ion-exchange reaction driven by electrostatic attraction between the polymer chain units and the surfactant ions.

Several major types of polyelectrolyte - surfactant complexes are described in the literature. Complexes of the first type are formed at the air - water interface if an amphiphile is spread on the aqueous solution of an oppositely charged polyelectrolyte.⁷⁻¹² Complexation occurs on the water surface and results in monolayer films considerably more stable than those of the amphiphile alone, as indicated by the increased collapse pressure.^{7,11,13} Complexes of another class are prepared by successive adsorption of a surfactant (typically with two headgroups separated by a nonpolar part) and a polyelectrolyte on a solid substrate from their solutions, resulting in multilayer films.¹⁴⁻¹⁶ Complexes of the third type consist of polyelectrolytes with flexible chains and oppositely charged small amphiphiles with mesogenic groups, prepared by mixing solutions of the two components in polar organic solvents.¹⁷⁻¹⁹ Such complexes exhibit liquid crystalline mesophases formed by the bound amphiphile, while the polymer chains enhance the thermal stability of the ordered structures.

Complexes of polyelectrolytes and oppositely charged surfactants with aliphatic chains are formed spontaneously if dilute aqueous solutions of the two components are mixed.^{6,20,21} The complexation reaction occurs at concentrations considerably lower than the critical micelle concentration (CMC) of the surfactant, and is highly cooperative. The electrostatic driving force for complexation is reinforced by hydrophobic self-association of the surfactant chains in water.

Depending on the polymer to surfactant ratio in aqueous solution, the complexes formed are either stoichiometric or nonstoichiometric^{6,20} (Figure 1.1). Nonstoichiometric complexes containing excess of either charged polymer chain units or surfactant molecules are generally soluble in water. The formation and structure of water-soluble polyelectrolyte - surfactant complexes containing an excess of polyelectrolyte chain units, have been studied in detail.^{6,20} Such complexes form 'mixed micelles' consisting of clusters of hydrophobic surfactant chains surrounded by the polar polyelectrolyte backbone (Figure 1.1 a). An interesting property of such clusters is their ability to solubilize nonpolar organic molecules in water solutions, used for stabilization of colloidal suspensions.⁶

If equimolar amounts of charged polymer chain units and surfactant molecules are mixed in water, stoichiometric complexes are formed (Figure 1.1 b). Such complexes are insoluble in water. Until recently, the interest in water-insoluble polyelectrolyte - surfactant complexes has been quite limited. However, the simplicity of synthesis of such complexes and the wide variety of available polyelectrolytes and surfactants provide attractive opportunities for preparation of materials with adjustable macroscopic properties.

Stoichiometric polyelectrolyte - surfactant complexes can be viewed as a new type of comb-shaped polymers, in which every polymer chain unit has an electrostatically bound 'side chain'. Such compounds possess a unique combination of polymeric nature with properties of low molecular weight amphiphiles. Polymeric components can provide mechanical strength, good thermal stability, etc., while surfactants bring about their tendency to assemble in layered structures and the ability to crystallize. The main research efforts in the area of water-insoluble polyelectrolyte - surfactant complexes have been focused on the understanding of the influence of electrostatically attached 'side chains' on properties of polymer chains (solubility, conformation), and the effect of polymer chains on the organization of the complexed surfactants.

Stoichiometric Polyelectrolyte - Surfactant Complexes in Organic Solvents

To understand the solution properties of polyelectrolyte - surfactant complexes, it is important to realize that they are amphiphilic compounds. They consist of a non-polar, hydrophobic part (surfactant alkyl chains) and a polar part (ionic groups). The presence of structural elements of different polarity and, therefore, of different solubility, allows one to manipulate the solution properties of complexes, such as solubility and conformation of polymer chains.

Stoichiometric complexes are insoluble in water, because the ionic groups of polyelectrolyte and surfactant are shielded from solvent by non-polar parts of the complex. However, stoichiometric complexes formed by polyelectrolytes with hydrophobic side chains²² or even linear synthetic polyelectrolytes²³⁻²⁶ (e.g., poly(styrene sulfonate), poly(methacrylic acid), etc.) and oppositely charged single or double-chain surfactants can be soluble in organic solvents of both lower (ϵ 2-10) and higher (ϵ 10-40) polarity. In solvents of low polarity (e.g., benzene, chloroform, dichloroethane), the complexes are soluble without dissociation, as shown by the linear dependence of the reduced viscosity on concentration in dilute solutions.²⁴ In solvents of higher polarity (e.g., dimethylformamide, ethanol, isopropyl alcohol), the complexes partially dissociate into polyelectrolyte and surfactant ions, as indicated by a non-linear increase in solution viscosity with dilution.²⁶ This effect is similar to that of polyelectrolyte solutions in water and is attributed to increased charge repulsion upon dilution.

Conformational properties of polymer chains in the polyelectrolyte-surfactant complexes in non-aqueous solutions have been investigated both theoretically²⁷ and experimentally.²⁴ Conformation of the polymer chains in the complexes of conventional polyelectrolytes and surfactants in organic solvents is governed by two main factors: dipole-dipole interactions of the ion pairs and steric interactions of the surfactant chains.

Theory predicts a considerable stiffening of a flexible polymer backbone upon complexation with surfactants (at complex compositions close to stoichiometric), owing to crowding of bound surfactant chains. In a good solvent for surfactant chains, the complex conformation is described as a semiflexible rod, with persistence length considerably exceeding its diameter. Based on calculation of the stiffness of polymer chains in such complexes, the theory predicts formation of liquid crystalline phases in solutions.²⁷

However, experimental studies of complexes of poly(N-ethyl-4-vinyl pyridinium) cations and dodecyl sulfate anions in dilute solutions in low-polarity solvents suggest that the conformation of polyelectrolyte chains in the complex is that of a flexible coil, based on the value of the Huggins' constant of 0.25 and the persistence length of about 5 nm, estimated from viscometry and flow birefringence data, respectively.²⁴

Stoichiometric Polyelectrolyte - Surfactant Complexes in the Solid State

The solid state organization of complexes of flexible chain polyelectrolytes and oppositely charged surfactants is dominated by the tendency of amphiphilic molecules to assemble in layered structures. Most complexes studied, formed by linear^{25,28-30} or cross-linked³¹ polyelectrolytes and oppositely charged single or double chain surfactants, spontaneously adopt lamellar structures, consisting of alternating layers of polymer chains separated by layers of surfactant (Figure 1.2). The long period of the lamellae depends on organization of surfactant molecules within the layers, governed by alkyl chain length and chemical structure of the amphiphile. Surfactants with shorter chains (less than sixteen methylene groups), are typically disordered in the complexes,^{25,31,32} while surfactants with longer chains (of at least sixteen methylene groups) can crystallize in the complexes.³¹ In the complexes of cross-linked polymethacrylate anions and hexadecyltrimethyl ammonium cations, the surfactant chains crystallize on a hexagonal lattice.³¹ Surfactant crystallites in the complexes are considerably smaller than those of

the uncomplexed surfactants, as indicated by their lower melting temperatures. For complexes of poly(styrene sulfonate) with alkyltrimethyl ammonium surfactants, no crystalline order was observed for chains with up to eighteen methylene groups.^{25,32} These data suggest that polyelectrolyte chains impose restrictions on surfactant chain packing, thus decreasing the tendency of the surfactant to form ordered structures.

Mechanical properties of polyelectrolyte - surfactant complexes were shown to depend on the chemical structure of the amphiphile.³⁰ Complexes with double chain surfactants in the amorphous state can exhibit mechanical properties similar to high performance rubbery polymers, with elastic modulus in the range of 20 - 200 MPa.³³

Stoichiometric polyelectrolyte complexes can be easily processed by casting from solution in organic solvents, but their melt processability is limited. The presence of ionic groups results in a high glass transition temperature (T_g), exceeding the temperature of decomposition. An interesting approach to decreasing T_g by complexing surfactants with copolymers of ionic and nonionic monomers has been reported.²⁹ However, a significant reduction in T_g of complexes of alkyltrimethylammonium surfactants with polyacrylic acid was only observed at high contents of N-alkylacrylamide (80 weight per cent) in the copolymer.

Complexation of polyelectrolytes with surfactants capable of reversible structural rearrangement has been used to prepare membranes with controlled permeability. Membranes with permeability switched by small electric fields has been prepared based on complexes of poly(styrene sulfonate) and viologen-containing dialkyl sulfates, with temperatures of liquid - solid transitions of the alkyl chains controlled by redox reactions of the headgroups.^{34,35}

Comb-Shaped Polymers

Polymers with covalently attached side chains (comb-shaped polymers) are covalent analogs of stoichiometric polyelectrolyte - surfactant complexes. Polymers with

comb-shaped structure have been extensively studied in the last two decades.³⁶⁻⁴² A wide variety of such polymers is known, consisting of either relatively flexible or rigid main chains with long aliphatic and/or mesogenic side groups in each chain unit. Polymers of this type combine the properties of low molecular weight compounds and the superior mechanical properties of macromolecules. One of the most important features of such polymers is their ability to form mesophases.

For flexible chain polymers, e. g., homologs of poly-n-alkyl acrylates, poly-n-alkylvinyl ethers, etc., two types of ordering are observed in the solid state.^{36,43} While polymer chains are arranged in layers separated by layers of side chains, packing of the side chains with lengths below a critical value is characterized by only short range order. Longer alkyl side chains (e.g., consisting of ten or more methylene groups for poly-n-alkyl methacrylates) form alkane-type crystals. If mesogenic groups are attached to a flexible polymer backbone, thermotropic liquid crystalline mesophases are formed by mesogenic groups distributed in a 'solvent' of disordered main chains. Smectic mesophases consisting of alternating layers of macromolecules and layers of side chains are the most common. The presence of polymer chains results in a sharp increase in the thermal stability of mesophases, compared to the mesophases formed by the corresponding low molecular weight molecules. One also observes a slowing down of all relaxational processes and a significant increase in the imperfection of the structures.³⁶

In the case of rigid chain polymers, e. g., stiff chain polyesters with aliphatic side chains⁴⁴ or alkyl esters of poly(α ,L-glutamic acid),^{40,41,45} etc., rod-like main chains promote formation of liquid crystalline mesophases, while flexible side chains promote solubility in organic solvents or play the role of 'solvent' in the solid state.

Synthetic polypeptides with alkyl side chains, e.g., alkyl esters of poly(α ,L-glutamic acid) (PALGs) and acyl derivatives of poly(L-lysine) (PALLs), are of special interest, owing to the ability of polypeptides to adopt highly ordered conformations. The most common structures are the α -helix and β -sheet.⁴⁶ The α -helix is stabilized by

hydrogen bonds connecting amino acid residues along the polypeptide chain, and provides a rod-like character to the molecule, which is often responsible for formation of liquid crystalline phases.⁴⁰ β -sheets are formed by nearly extended polypeptide chains connected by hydrogen bonds and are assembled in layers. Control over formation and disruption of hydrogen bonds (e.g., by solvent or temperature) may allow one to manipulate polypeptide chain conformation and the overall material properties.

PALGs are the best studied, at present.^{40,41,45} PALGs adopt an α -helical conformation owing to the hydrogen bonds within the polypeptide chains in most organic solvents. Addition of solvents capable of breaking hydrogen bonds, e. g., trifluoroacetic acid, results in disruption of the α -helices. Helical polypeptide chains form liquid crystalline mesophases in a wide variety of organic solvents. In the solid state, the polypeptide backbone of PALGs is either in the α -helical or β -sheet conformation.⁴⁰ Most PALGs adopt the α -helical conformations. β -sheet conformation can be achieved by casting low molecular weight benzyl ester of poly(α , L-glutamic acid) from organic solvents or by stretching the films of the methyl ester. The organization of PALGs in the solid state depends on the alkyl side chain length: for homopolymers with alkyl chains of less than ten methylene groups, the helices are packed hexagonally; longer alkyl chains form paraffin-type crystallites forcing the helices to pack in layers with crystallites located between the layers. Melting of side chain crystallites results in formation of cholesteric liquid crystals.⁴¹

PALLs have been reported to adopt α -helical conformations in such organic solvents as aliphatic alcohols and in their mixtures with hydrocarbons, while disordered conformations are observed upon addition of trifluoroacetic acid.^{38,39} With shorter alkyl side chains, the helices are organized hexagonally, while with longer side chains, consisting of nine or more methylene groups, which form alkane type crystallites, the helices are arranged in layers separated by layers of side chains.³⁷

Owing to their unique combinations of physical properties, such as high solubility and the ability to form liquid crystalline mesophases, rod-like polypeptides with flexible side chains show promise as materials for optoelectronics, separation membranes and molecular composites.

Motivation and Objectives

As described above, most work in the area of water-insoluble polyelectrolyte - surfactant complexes has addressed complexes of conventional synthetic polyelectrolytes and low molecular weight amphiphiles. However, biopolymers may offer special advantages in the development of new polymer-surfactant complexes with useful properties. Only a few reports concerning complexes of polypeptides with oppositely charged surfactants in non-aqueous media can be found in the literature.^{12,47} Copolymers consisting of a block of poly(α ,L-glutamic acid) and two blocks of poly(styrene sulfonate) complexed with alkyl trimethylammonium surfactants were found to undergo a pH-sensitive helix-coil transition at the air-water interface.¹² Complexes of poly(L-lysine) with oppositely charged lipids have been reported to adopt lamellar structures in the solid state, consisting of layers of polypeptide chains in the β -sheet conformation sandwiched between lipid bilayers.⁴⁷

In this work, we describe an investigation of stoichiometric complexes formed by synthetic polypeptides, sodium poly(α ,L-glutamate) and poly(L-lysine) and the oppositely charged low molecular weight surfactants, alkyltrimethyl ammonium bromides and sodium alkyl sulfates, respectively. We compare the behavior of these complexes to that of their covalent analogs - synthetic polypeptides with covalently attached alkyl side chains, alkyl esters of poly(α ,L-glutamic acid) and acyl derivatives of poly (L-lysine). The goal of this study is to investigate the effect of electrostatically attached 'side chains' on the conformation of the polypeptide chains and the influence of the polypeptide chains on the organization of the complexed surfactant. We attempt to gain a better

understanding of the roles of hydrogen bonds, electrostatic interactions, and solvent polarity on the conformation and aggregation of the polypeptide chains and on the supramolecular order in the polyelectrolyte - surfactant complexes.

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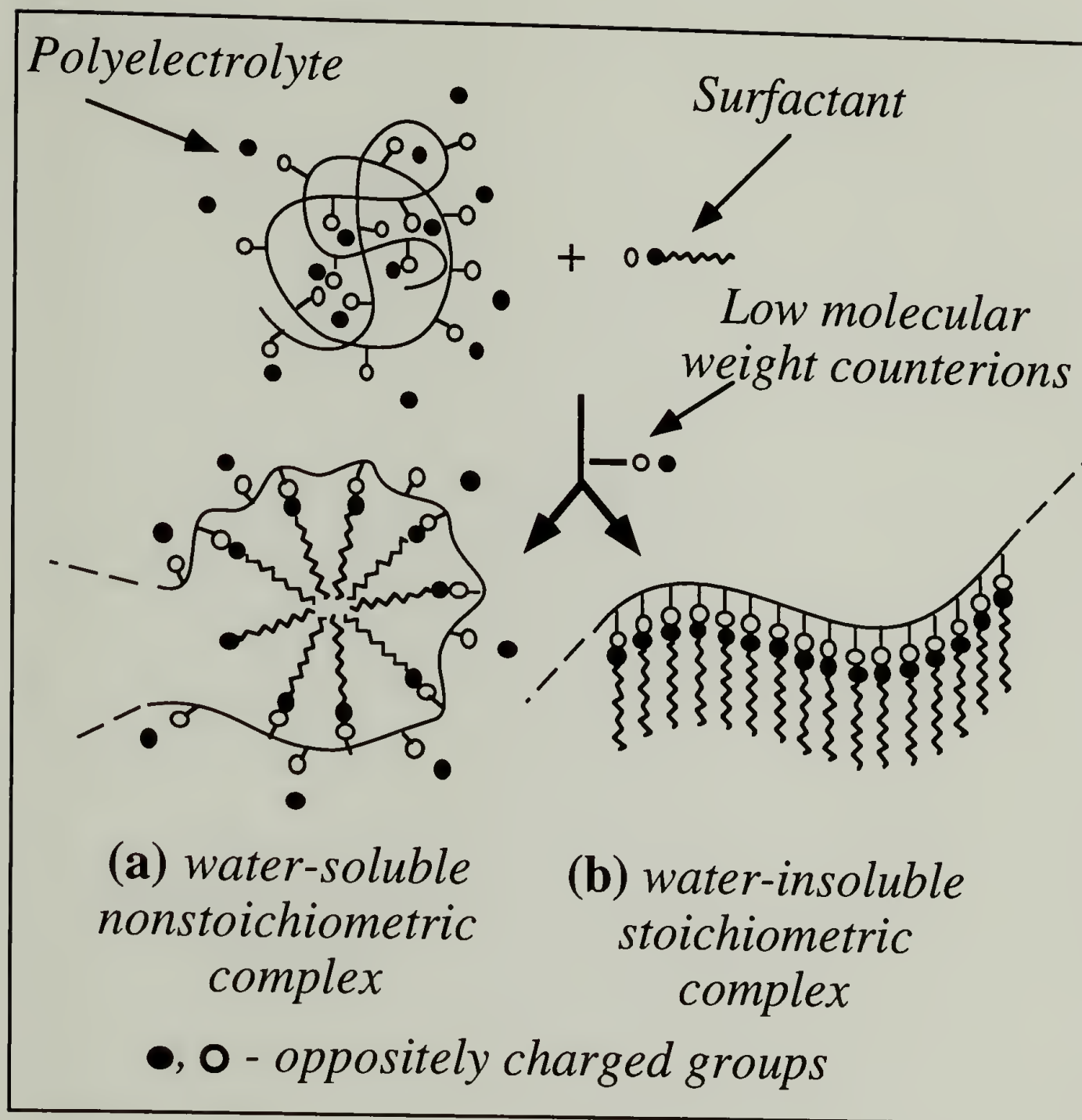


Figure 1.1 Scheme of formation of polyelectrolyte - surfactant complexes in water solutions.

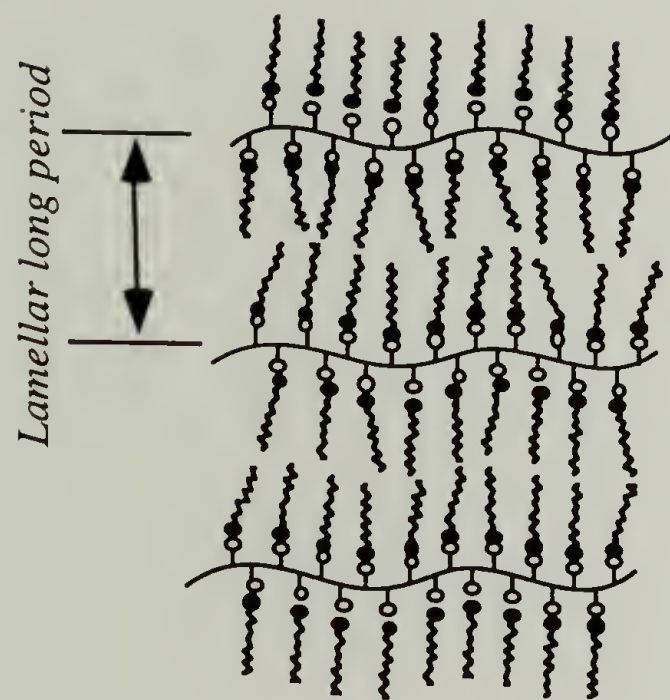


Figure 1.2 Scheme of a typical lamellar structure of a stoichiometric polyelectrolyte - surfactant complex in the solid state.

CHAPTER II

STOICHIOMETRIC COMPLEXES FORMED BY POLY(α ,L-GLUTAMATE) ANIONS AND ALKYL TRIMETHYLAMMONIUM CATIONS

Introduction

In this chapter we report an investigation of the solid state properties of stoichiometric complexes formed by a synthetic polypeptide - sodium poly(α ,L-glutamate) and oppositely charged low molecular weight surfactants.^{1,2} We compare the behavior of these complexes with that of their covalent analogs, the alkyl esters of poly(α ,L-glutamic acid). The objective of this study is to investigate the effect of the electrostatically attached 'side chains' on the solubility and conformation of the polypeptide chains, and on the supramolecular order in the system.

Poly(γ -alkyl α ,L-glutamates) have been widely investigated as liquid crystals and ordered monolayers.³⁻⁶ The stiff, helical polyglutamate backbone promotes formation of ordered structures in solution and in the solid state, while the flexible side chains promote solubility in a wide range of organic solvents. In the solid state, hexagonal or layered packing of α -helices is observed.³ If the side chains are long enough, they may crystallize at low temperatures and melt at higher temperatures, to provide a liquid-like environment for the rods and thus enable the formation of thermotropic liquid crystals. Owing to their unique combinations of physical properties, such as high solubility and the ability to form liquid crystal phases, rod-like polypeptides with flexible side-chains show promise as materials for optoelectronics, separation membranes and molecular composites.

We report herein an investigation of stoichiometric complexes formed from sodium poly(α ,L-glutamate) and the oppositely charged surfactants dodecyl, cetyl and octadecyl trimethylammonium bromides (Figure 2.1). We refer to such complexes as PGD, PGC and PGO, respectively.

Experimental Section

Materials

Sodium poly(α ,L-glutamate) (PGNa) with weight-average degree of polymerization (provided by the supplier) of about 600, and cationic surfactants dodecyl, cetyl and octadecyl trimethylammonium bromides (DDTAB, CTAB and ODTAB, respectively) were used as received from Sigma Chemical Co.

Complexes were prepared by mixing equal amounts of 0.05 M (for PGD complex) or 0.01 M (for PGC complex) aqueous solutions of PGNa and surfactant at room temperature at pH 8. The PGO complex was prepared by mixing equimolar quantities of 0.01 M PGNa and 2×10^{-3} M ODTAB solutions in water. The molarity of the polymer solutions was based on the repeating unit equivalent weight. The resulting white precipitates were isolated by filtration or centrifugation, washed several times with water to remove low molecular weight counterions and dried in vacuum for at least 48 hours at 45 °C. The complexes thus obtained were resuspended in water, filtered and dried in vacuum under the same conditions as above. The compositions of the complexes were estimated by elemental analysis and by high resolution ^1H NMR spectroscopy. Elemental analysis showed $(\text{C/N})_{\text{calcd}}=8.57$, $(\text{C/N})_{\text{found}}=8.72$, $\text{Na}_{\text{found}}<0.05\%$ (PGD complex); $(\text{C/N})_{\text{calcd}}=10.24$, $(\text{C/N})_{\text{found}}=10.58$, $\text{Na}_{\text{found}}<0.05\%$ (PGC complex); $(\text{C/N})_{\text{calcd}}=11.56$, $(\text{C/N})_{\text{found}}=11.67$, $\text{Na}_{\text{found}}<0.1\%$ (PGO complex). From the ratios of the integrated signal intensities of the α -CH protons ($\delta=4.0$ ppm) of the polymer and the protons of the surfactant alkyl chains (δ 0.82 (CH_3 , 3H), δ 1.19-1.26 (CH_2 , 16 H (PGD), 24 H (PGC) or 28H (PGO)), and δ 3.37 (CH_2 , 2H) it was found that $97 \pm 4\%$ of the polymer chain units were paired with surfactant ions.

The complexes were either analyzed immediately or stored in vacuum at room temperature. Upon storage, irreversible changes in properties were observed, indicating formation of small amounts of unbound crystalline surfactants. Formation of surfactant

molecules free of poly(α ,L-glutamate) chains was promoted by film casting from chloroform solutions and/or by heating of the samples above room temperature.

Powder samples for X-ray analysis were sealed in thin glass capillaries. Films of the complex for X-ray and differential scanning calorimetry (DSC) analyses were prepared by evaporation of chloroform solutions on Teflon plates at room temperature. Films were stretched by hand to prepare oriented samples. The draw ratio did not exceed two. For circular dichroism (CD) and Fourier transform infrared (FTIR) measurements, films were cast from chloroform solutions on quartz and KBr windows, respectively.

Measurements

^1H NMR spectra were obtained on a Bruker AMX 500 MHz instrument. CD spectra were recorded with an Aviv 62DC spectrometer. FTIR spectra were obtained using a Nicolet IR 44 spectrometer. The samples for infrared experiments were purged with nitrogen for one hour prior to measurements. X-ray diffraction patterns of powder samples were recorded using a Siemens D 500 diffractometer in transmission mode with a scintillation counter scanning in the desired 2Θ range (where Θ is the Bragg angle). Oriented films of the complexes were analyzed with an evacuated X-ray Statton camera with subsequent digitization of the X-ray films using an Optronics C4500 microdensitometer. In both types of X-ray experiments, Ni-filtered $\text{CuK}\alpha$ radiation was used with wavelength $\lambda=1.5418 \text{ \AA}$. DSC experiments were performed on a Perkin-Elmer DSC 7 system at a scanning rate of $10 \text{ }^\circ\text{C}/\text{min}$. Heating and cooling scans were performed three times for each sample and in all cases melting temperatures were essentially identical in the subsequent heating scans.

Results and Discussion

Solubility in Organic Solvents

All three complexes (PGD, PGC, PGO) are soluble in chloroform and in a variety of more polar solvents, including benzyl alcohol, methanol, dimethylsulfoxide (DMSO) and dimethylformamide (DMF). In polar solvents polyelectrolyte-surfactant complexes are likely to be partially dissociated,⁷ while in solvents of low polarity such complexes are expected to remain tightly associated.⁸

The solubility behavior of the complexes is in agreement with results published recently for complexes consisting of conventional synthetic polyelectrolytes and oppositely charged surfactants.⁷⁻⁹ The solubility behavior of the corresponding esters of poly(α ,L-glutamic acid) is quite different: poly(γ -alkyl α ,L-glutamate)s (PALGs) are soluble in most common organic solvents, including n-alkanes and aromatic hydrocarbons,⁴ which are nonsolvents for the complexes. The difference in the solubility behavior of the complexes and their covalent analogs can be attributed to the higher polarity of the complexes, owing to the presence of the ionic groups.

Conformation of the Polypeptide Chains

In the solid complexes, the polypeptide chains are in the α -helical conformation, as indicated by the circular dichroism and FTIR spectroscopy. CD spectra of the chloroform-cast films of the complexes exhibit a positive band at about 190 nm and two negative bands at 210 and 220 nm (Figure 2.2). The signs and positions of the major bands in the CD spectra of the complexes essentially coincide with those reported for polypeptides in α -helical conformation.¹⁰ This conclusion is supported by the FTIR spectra of the complexes, in which the amide I and amide II bands are observed at 1653 and 1549 cm^{-1} , respectively, consistent with α -helical conformation.⁴ This behavior is similar to that of the alkyl esters of poly(α ,L-glutamic acid).⁴

We investigated the thermal stability of the α -helical conformation in the PGD and PGC complexes by FTIR spectroscopy. As the temperature is increased, the amide I bands of the complexes shift to higher frequency, while the amide II band shifts to lower frequency (Figure 2.3). These effects are accompanied by a broadening and decrease in intensity of the amide I and amide II peaks (Figure 2.4) and by a dramatic decrease in intensity of the amide A band (Figure 2.5). All of these spectral changes are completely reversible on cooling and all of these effects are identical for the PGD and PGC complexes.

The observed effects can be explained by weakening or even disruption of intramolecular hydrogen bonds as the temperature is increased. Disruption of hydrogen bonds is expected to shift the amide I band to higher energy and the amide II band to lower energy.¹¹ Broadening of all peaks upon heating may result from a broadening of the distribution of hydrogen bonds of different geometries and strengths.

Similar effects for the amide I and amide II bands have been reported for the benzyl and methyl esters of polyglutamic acid.¹¹ The values of the shifts in vibrational frequencies with temperature have not been reported, but changes in the IR spectra with temperature were correlated with increases in the X-ray diffraction spacings characteristic of the residue translation of the α -helix.¹¹ The overall effect is ascribed to weakening of the intramolecular hydrogen bonds leading to the expansion of α -helices along their long axes.

We carried out FTIR experiments with poly(γ -benzyl α ,L-glutamate) (PBLG) for comparison. The shift in amide I and amide II band positions with temperature is much less pronounced than in the case of the PGD complex, in the temperature range studied (Table 2.1).

The difference in the behavior of the esters of polyglutamic acid and the complexes of polyglutamate with surfactants is likely to be related to the differences in stability of the α -helical conformation. The repulsive dipole-dipole interactions in the

complexes is likely to destabilize the helix and render the intramolecular hydrogen bonds more susceptible to thermal disruption. This effect finds analogy in the difference in the conformations of poly(α ,L-glutamic acid) and its salts in the solid state. While poly(α ,L-glutamic acid) itself can adopt both α -helical¹² and β -sheet¹³ conformations in the solid state at room temperature, the sodium salt is essentially disordered under the same conditions.¹⁴ On the other hand, in the case of multivalent cations, such as calcium, strontium or barium, poly(α ,L-glutamic acid) salts can be crystallized in the β -form.¹³

Conformation of Surfactant Chains

FTIR spectra also provide information about the conformation of the surfactant alkyl chains in the complexes. The C-H asymmetric and symmetric stretching vibrations of the surfactant chains are observed at 2921 and 2853 cm^{-1} (PGD complex) and at 2919 and 2851 cm^{-1} (PGC complex), respectively at room temperature. This indicates that in the case of the PGC complex, the surfactant chains are in a 'solid-like', extended conformation, while in the case of the PGD complex the surfactant chains are likely to be slightly less extended.^{15,16} As the temperature is increased, the C-H stretching vibrations are shifted to higher frequencies (Figure 2.6) indicating the transformation of the surfactant alkyl chains to a 'liquid-like' state.^{15,16} Similar shifts in C-H stretching vibrations are observed in the case of pure surfactants upon melting of their crystals; e.g., DDTAB exhibits asymmetric and symmetric C-H stretching vibrations at 2919 and 2851 cm^{-1} , respectively in the temperature range 25 - 90°C, while a shift of these bands to 2925 and 2855 cm^{-1} is observed at 100°C. Disordering of the alkyl chains upon heating appears to precede the loss of helicity of the polyglutamate backbone in the case of the PGD complex; e.g., the transition monitored by the C-H stretching region is complete by 80° C (Figure 2.6) at which point only minor changes are apparent in the amide stretching vibrations (Figure 2.3).

Supramolecular Structure

The wide angle X-ray diffractograms (WAXD) of the PGD and PGC complexes (Figure 2.7 curve a) consist of a broad halo, corresponding to a Bragg spacing of about 4.6 Å, which is close to the value known for the lateral packing of alkyl chains of various amphiphiles.¹⁷ The broad halo indicates that the packing of the 'side chains' in these complexes is characterized by only short range order.

In contrast, the WAXD pattern of the PGO complex (Figure 2.7 curve b) exhibits a relatively sharp peak, corresponding to a Bragg spacing of 4.2 Å, superimposed on a broad halo centered at a spacing of 4.6 Å. The sharp peak on the WAXD pattern of the PGO complex indicates that a portion of the surfactant chains is organized on a well-defined crystal lattice; a second fraction of the chains remains noncrystalline, as shown by the shoulder with the Bragg spacing of 4.6 Å. A spacing of 4.2 Å has been observed previously in comb-like poly(alkylmethacrylate)s with twelve or more carbon atoms in the side chains,¹⁸ as well as in complexes consisting of cross-linked sodium polyacrylate and cetyl trimethylammonium bromide,¹⁹ and is believed to indicate hexagonal packing of the alkyl chains. A small peak on the WAXD pattern of the PGO complex corresponding to the Bragg spacing of 3.6 Å ($2\Theta=24.5^\circ$) is characteristic of free surfactant and, therefore, is attributed to the presence of trace amounts of uncomplexed crystalline surfactant. We do not believe that this peak arises from orthorhombic crystals of the 'side chains' in the PGO complex, owing to its extremely low intensity.

The 'side chain' crystals in the PGO complex differ from crystals formed by alkyl trimethylammonium bromides, as well as from those of the side chains of poly(α ,L-glutamate)s: the alkyl trimethylammonium bromides crystallize in a monoclinic lattice,²⁰ and PALGs with side chain lengths of 10 or more carbon atoms form two-dimensional alkane-type crystallites.³ These two-dimensional lattices are believed to resemble the subunit cell attained by the projection of the triclinic unit cell in crystals of low molecular weight n-alkanes.

We estimated the degree of crystallinity of the PGO complex based on the WAXD data. Assuming that the broad reflection with the Bragg spacing of about 4.6 Å results from noncrystalline 'side chains', and that the surfactant chains in the PGD complex are fully amorphous, fitting the amorphous halo of the PGD complex to the semicrystalline curve of the PGO complex indicates that about 30 per cent of the 'side chains' are crystalline.

For the PGD and PGC complexes, no thermal transitions were observed by DSC in the temperature range 15 - 200 °C. In contrast, the PGO complex undergoes an endothermic first order transition upon heating at 48 °C (Figure 2.8 curve a). The transition is reproducible on heating and cooling. This transition correlates with considerable broadening of the WAXD peak of the complex and probably corresponds to the melting of the 'side chain' crystallites. This conclusion is supported by the fact that no thermal transitions were observed for the PGD and PGC complexes in the temperature range 0-170° C, consistent with the observation of only broad halos in the WAXD patterns of the complexes. The melting transition of the PGO crystallites is broader than that of the corresponding surfactant (Figure 2.8 curve b) and occurs at a lower temperature. Depression of the melting temperature of surfactants upon complexation with oppositely charged polyelectrolytes has also been observed in complexes formed by cross-linked polyacrylate anions and cetyl trimethylammonium cations¹⁹ and by poly(N-ethyl-4-vinylpyridinium) cations and cetyl sulfate anions.²¹ The melting enthalpy ΔH_m of the PGO crystallites, estimated from the first heating scan, is about 2.1 kcal/mol of surfactant ions. On the basis of the known value of about 1 kcal/mol of CH₂ units reported for the triclinic-to-liquid transition of n-alkanes and for melting of the side chain crystallites of PALGs with ten or more carbon atoms,³ we estimated that only about 10 per cent of the 'side chains' are crystalline in the PGO complex. The low value of the melting enthalpy is consistent with the prominent halo on the WAXD pattern of the PGO complex. However, for hexagonal phases, like the one observed in the case of the PGO

complex, the melting enthalpy per mole of CH₂ may be lower than for triclinic crystals. For example, the melting enthalpies of hexagonal crystallites formed by the side chains of poly(α -alkyl β , L-aspartate)s, were reported to be 0.65 kcal/mol of CH₂.²² This value was obtained by plotting the values of the transition enthalpies observed by DSC vs the number of carbon atoms in the side chains and calculating the slope of the line. The lower melting enthalpies of the hexagonal phases than for triclinic phases (used in our calculations) may account for the difference in the degree of crystallinity estimated from the WAXD and DSC data.

The PGO complex does not undergo any thermal transitions in the temperature range 10-170° C, other than melting of the 'side chain' crystallites. The thermal behavior of the polypeptide - surfactant complexes is quite different from that of the alkyl esters ($n \geq 10$) of polyglutamic acid, which undergo two first order transitions.³ The first transition corresponds to the melting of the side chain crystallites and the transition from hexagonal to layered packing of the α -helices. At the temperature of the second transition, flow is observed and melts with cholesteric order are formed. Unlike the PALGs with crystalline side chains, the PGO complex does not flow upon heating before decomposition. No ordered melts are observed.

The small angle X-ray diffractograms (SAXD) of the PGD, PGC and PGO complexes exhibit narrow peaks of high intensity with Bragg spacings of 31 ± 0.6 , 37 ± 0.8 and 39 ± 0.9 Å, respectively, and reflections of low intensity, corresponding to Bragg spacings of about 15.6, 19.0 and 19.4 Å, respectively (Figure 2.9). The ratio of the spacings of the inner and outer reflections is equal to two, suggesting that these signals arise from lamellar structures of the complexes with long periods of 31.6, 37.6 and 39.3 Å, respectively. The second order signal arising from the long period of the lamellae of the PGO complex is much more pronounced than in the patterns of the PGD and PGC complexes (compare curves a, b and c in Figure 2.9). The increase in the intensity of the second order of the lamellar spacing coincides with the onset of

crystallization of the surfactant chains in the complex, consistent with the formation of the structure in which lamellar interface is more sharply defined.²³

It is reasonable to propose that all three complexes adopt lamellar structures consisting of alternating layers of α -helical polyglutamate chains and bimolecular layers of surfactant molecules. Films of the complexes are transparent and appear optically isotropic between crossed polarizers, suggesting that the stacks of lamellae are randomly oriented within the sample and are much smaller than the wavelength of visible light.

The diameter of the rod-like poly(α ,L-glutamic acid) chain, including both the polypeptide backbone and the side chain carboxyethyl groups, is about 13 Å.⁶ The lengths of fully extended DDTAB, CTAB and ODTAB chains are about 16, 21 and 23 Å, respectively.²⁴ Thus, the experimental values of the long periods of the lamellae of the complexes suggest that the surfactant tails bound to the polypeptide chains lying in adjacent layers should be either interdigitated and perpendicular to the lamellar surface or tilted with respect to the layers without being interdigitated.

Figure 2.10 curve a presents the dependence of the long period of the lamellae on the number of carbon atoms in the surfactant chains in the complexes studied. The dependence can be represented as a straight line, indicating that the three complexes studied possess similar lamellar structures. The slope of the line is about 1.3 Å per CH₂ group, suggesting that the surfactant chains in the complexes are nearly fully extended, interdigitated and perpendicular to the lamellar surface.¹⁸ The dependence of the long period of the lamellae of alkyl trimethylammonium bromides on the number of carbon atoms in the chain is presented for comparison (Figure 2.10 curve b). In this case, the increment of the lamellar thickness is about 1.1 Å per CH₂ group, indicating that the surfactant chains are tilted with respect to the lamellar surfaces,¹⁸ in agreement with the literature.²⁴

Additional information about the orientation of the surfactant chains with respect to the lamellar surfaces is provided by comparison of the WAXD and SAXD patterns of

stretched films of the complexes. It should be noted that, while the PGD and PGC complexes are plastic and deformable, the PGO films are quite brittle, probably owing to the presence of the 'side chain' crystallites. Figure 2.11 presents the dependence of the X-ray intensity of the reflections corresponding to the Bragg spacings of 4.2 Å (a) and 19.4 Å (b) on the azimuthal angle Φ of the stretched PGO film. The oriented X-ray patterns are essentially identical for all complexes studied. It is clear that the reflections corresponding to the lateral packing of the surfactant chains (Figure 2.11 curve a) and to the lamellar spacing (Figure 2.11 curve b), exhibit orthogonal orientations, with the lamellae aligned parallel to the draw direction. The presence of only two peaks on the oriented WAXD pattern and the same azimuthal width of the peaks on the oriented WAXD and SAXD patterns suggest again perpendicular orientation of the "side chains" with respect to the lamellae.²³

Thus, the stoichiometric polypeptide - surfactant complexes are characterized by lamellar structures consisting of layers of α -helical polymer chains separated by layers of surfactant. It is plausible that the side chains associate on opposite sides of the α -helices in order to form a layer, similar to the alkyl groups of polyglutamic acid esters, as proposed by Watanabe and coworkers.³ The surfactant molecules are likely to be perpendicular to the lamellar surface and are significantly interdigitated (Figure 2.12). We have no information concerning the packing of the α -helical polymer chains within the layers.

The structures of the complexes are similar to those of complexes formed by conventional linear synthetic polyelectrolytes and oppositely charged low molecular weight surfactants,^{19,25,26} but differ from those of the corresponding esters of polyglutamic acid. In the latter case, the α -helices are arranged in layers with the side chains located between the layers and interdigitated,³ but those with alkyl side chains of ten or more carbons form alkane-like crystallites. The structures of the PGD and PGC complexes are also strikingly different from those of the corresponding surfactants in

their bromide forms. Both DDTAB and CTAB adopt crystalline structures in the solid state²⁰ and DDTAB is reported to form a monoclinic lattice²⁰ with interdigitated chains.

The SAXD spacings corresponding to the lamellar organization of the PGD and PGC complexes are identical at 20 and 120 °C. Therefore, we conclude that the lamellar structure of the complexes remains intact in this temperature range, although disordering of alkyl tails and loss of helicity of the polyglutamate backbone are observed upon heating. Films prepared from the complexes remain mechanically stable until thermal degradation begins, with onset of weight loss at about 200°C. The lamellar structure of the complexes is stable at temperatures higher than the melting temperatures of the corresponding surfactants (according to our experiments, 103°C for DDTAB and 107°C for CTAB); thus the complexes can be considered as lamellar phases of surfactants stabilized by the polymer.

Conclusions

Stoichiometric complexes formed by poly(α ,L-glutamate) anions and alkyl trimethylammonium cations with chain lengths of twelve to eighteen carbon atoms adopt α -helical conformations similar to those of the alkyl esters of poly(α ,L-glutamic acid). Intramolecular hydrogen bonds in the complexes are more sensitive to temperature changes than those characteristic of the esters.

In the complexes studied, two types of surfactant organization were observed: shorter chains consisting of twelve or sixteen carbon atoms are extended but positionally disordered, while the longer chains of the PGO complex crystallize in a hexagonal lattice. The 'side chain' crystals, in the latter case, are different from those of the PALGs and the corresponding surfactants.

In the solid state, the complexes are organized in lamellae consisting of alternating layers of the polypeptide chains separated by bimolecular layers of surfactant, with the surfactant chains aligned perpendicular to the lamellar surfaces and interdigitated. The

lamellar structure is not subservient to the state of the surfactant chains, as it is in the alkyl esters of poly(α ,L-glutamic acid), where hexagonal packing of the α -helices is observed if the side chains are crystalline and lamellar packing dominates only if the side chains are disordered.³

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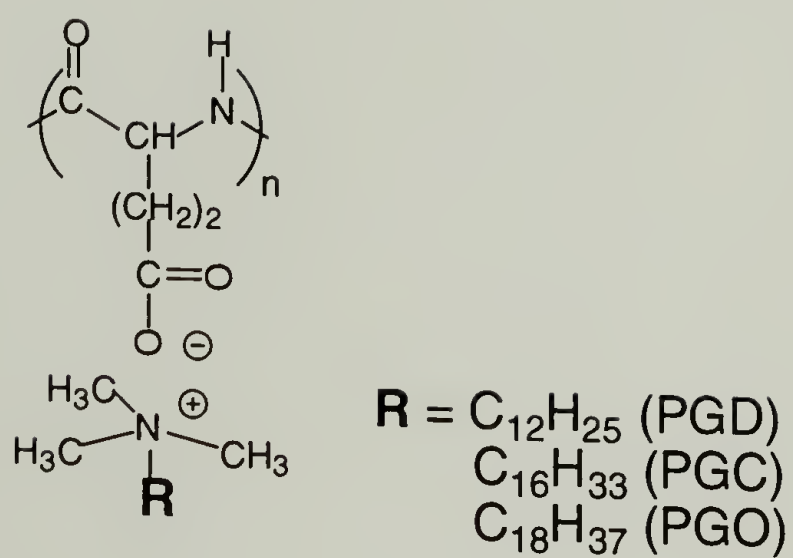


Figure 2.1 Chemical structures of the PGD, PGC and PGO complexes.

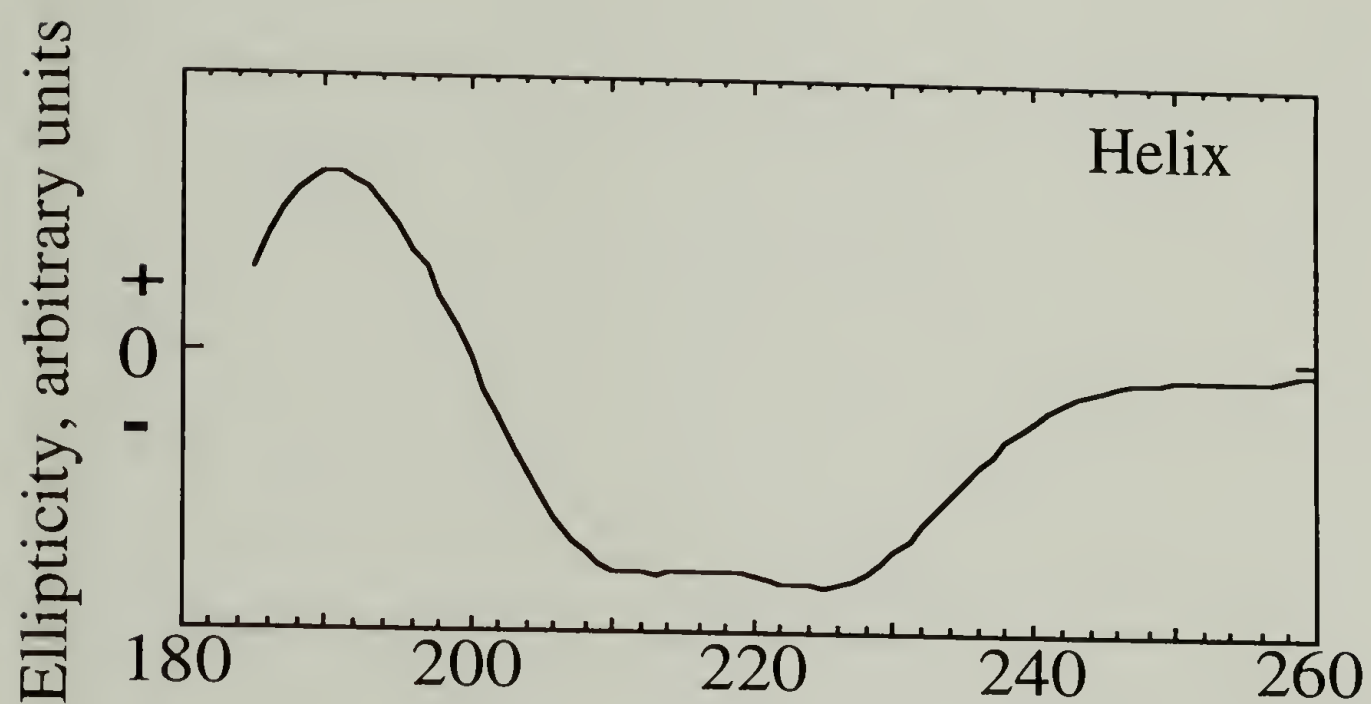


Figure 2.2 Circular dichroism spectrum of PGD film at 20° C. The spectra of the PGC and PGO complexes are identical.

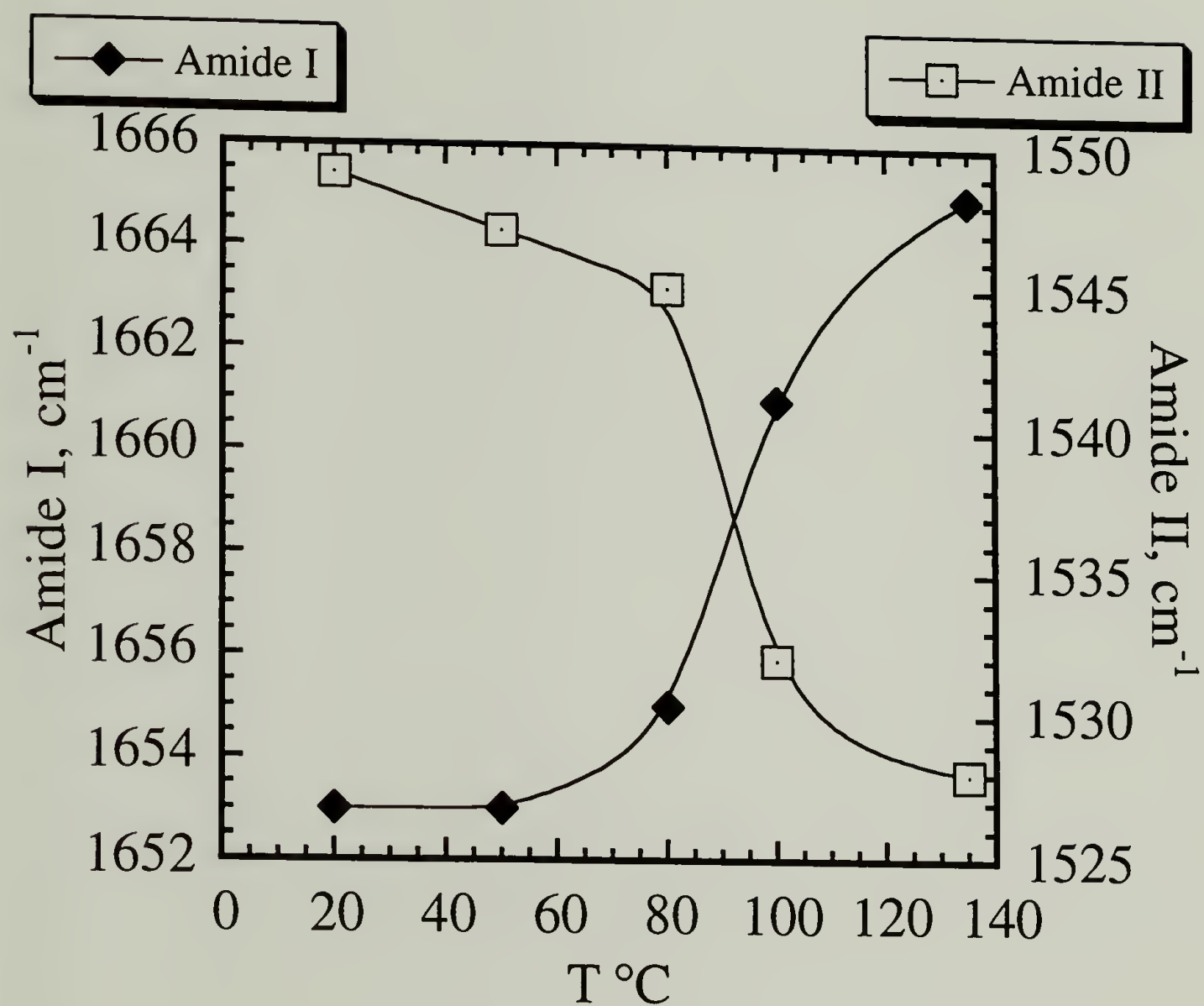


Figure 2.3 Temperature dependence of the frequencies of the amide I and amide II vibrations in the infrared spectra of PGD films. The behavior of the PGC films is similar.

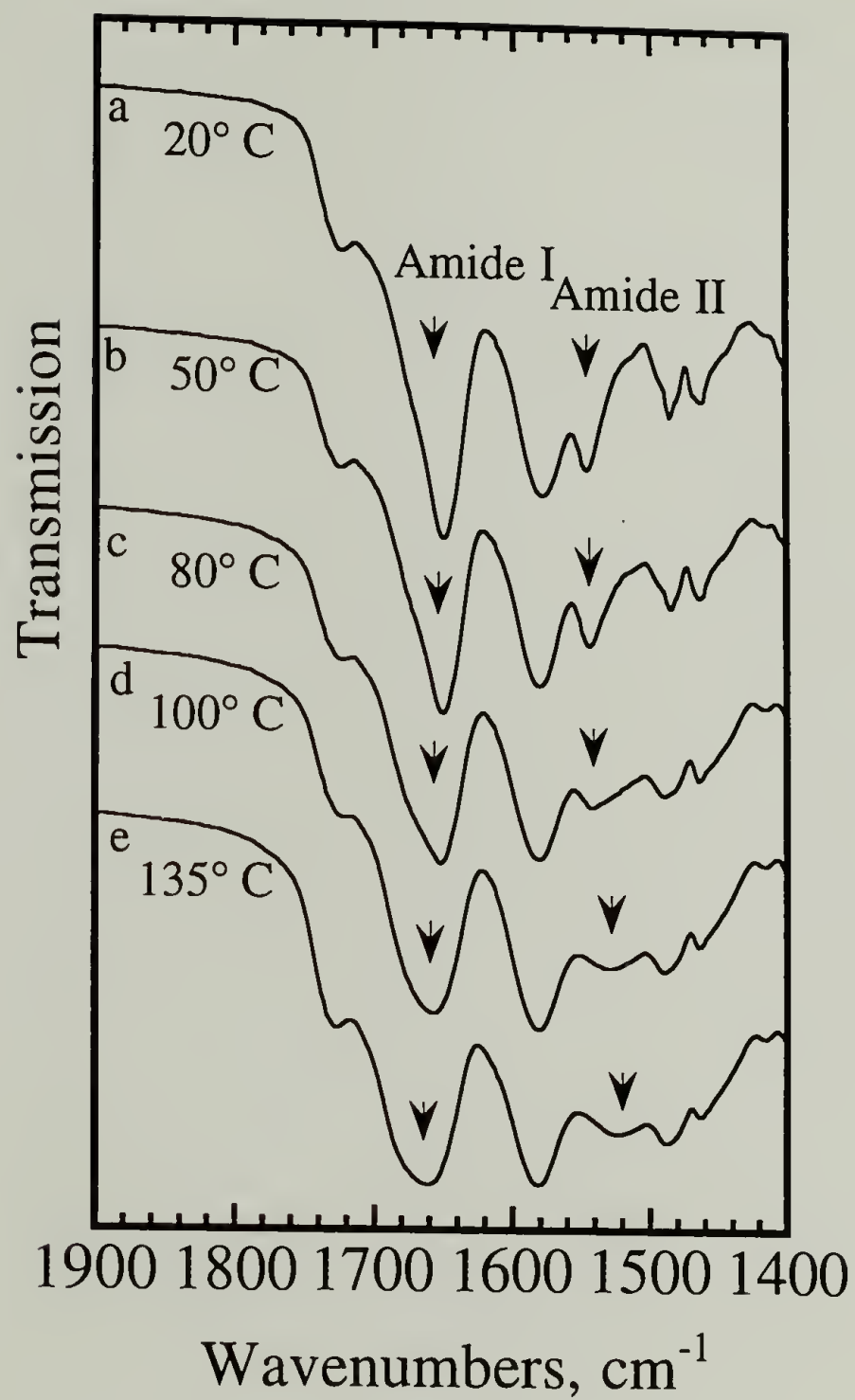


Figure 2.4 FTIR spectra of PGD films at 20°C (a), 50°C (b), 80°C (c), 100°C (d), 135°C (e) in the 1400 - 1900 cm⁻¹ region. The behavior of the PGC films is similar.

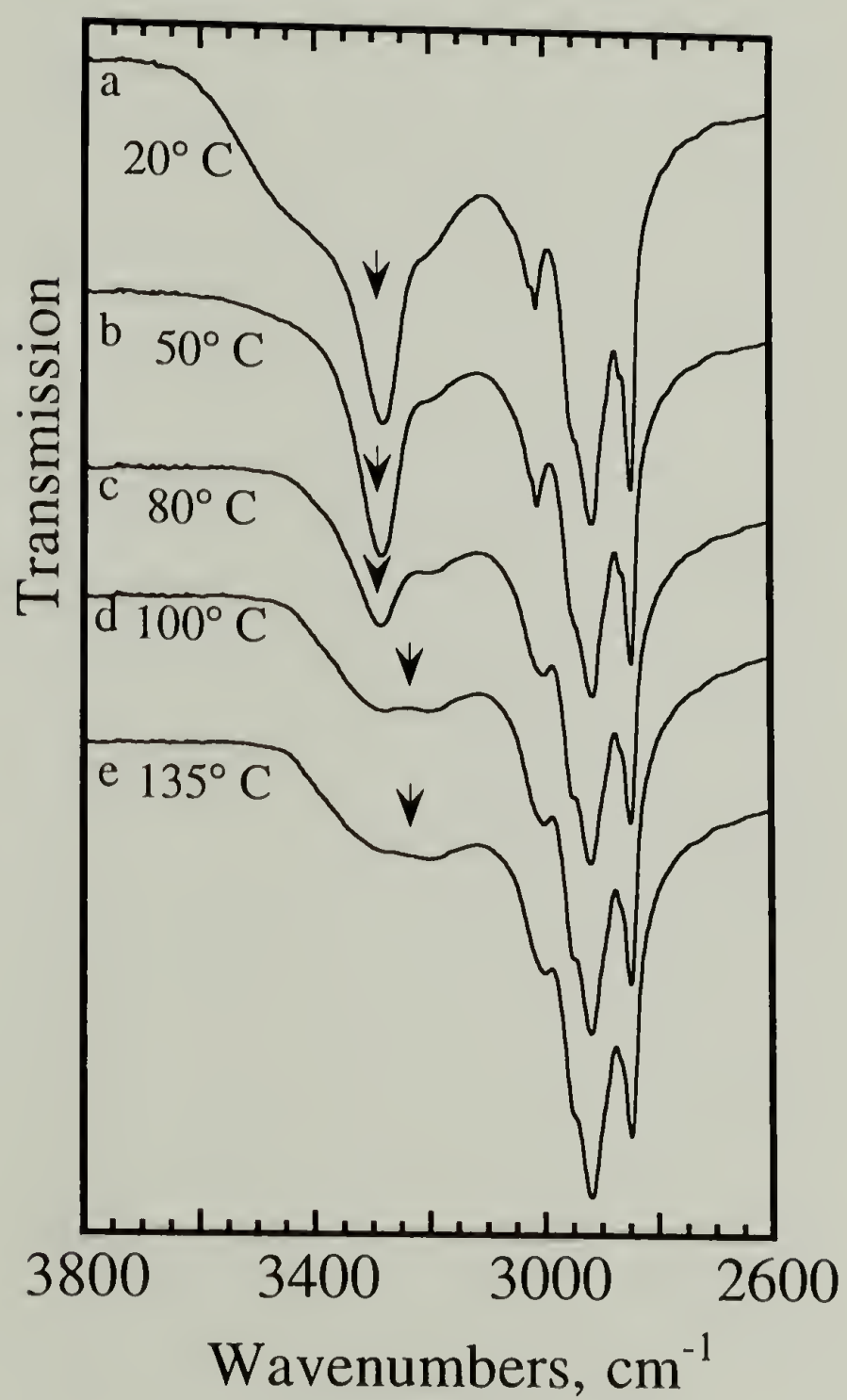


Figure 2.5 FTIR spectra of PGD films at 20°C (a), 50°C (b), 80°C (c), 100°C (d), 135°C (e) in the 2600 - 3800 cm⁻¹ region; arrow indicates amide A band.

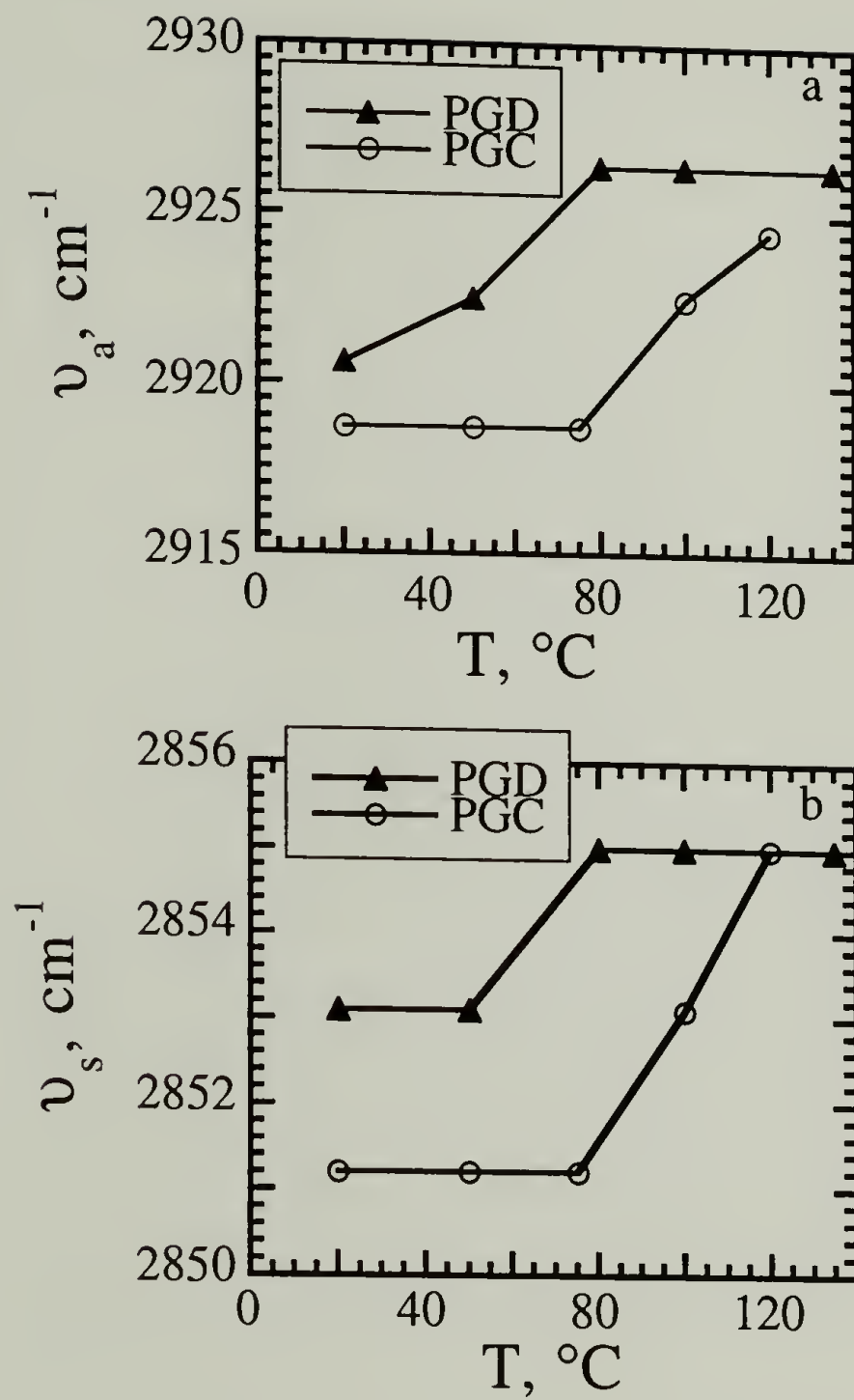


Figure 2.6 Temperature dependence of the frequencies of asymmetric (a) and symmetric (b) C-H stretching vibrations in the infrared spectra of PGD and PGC films.

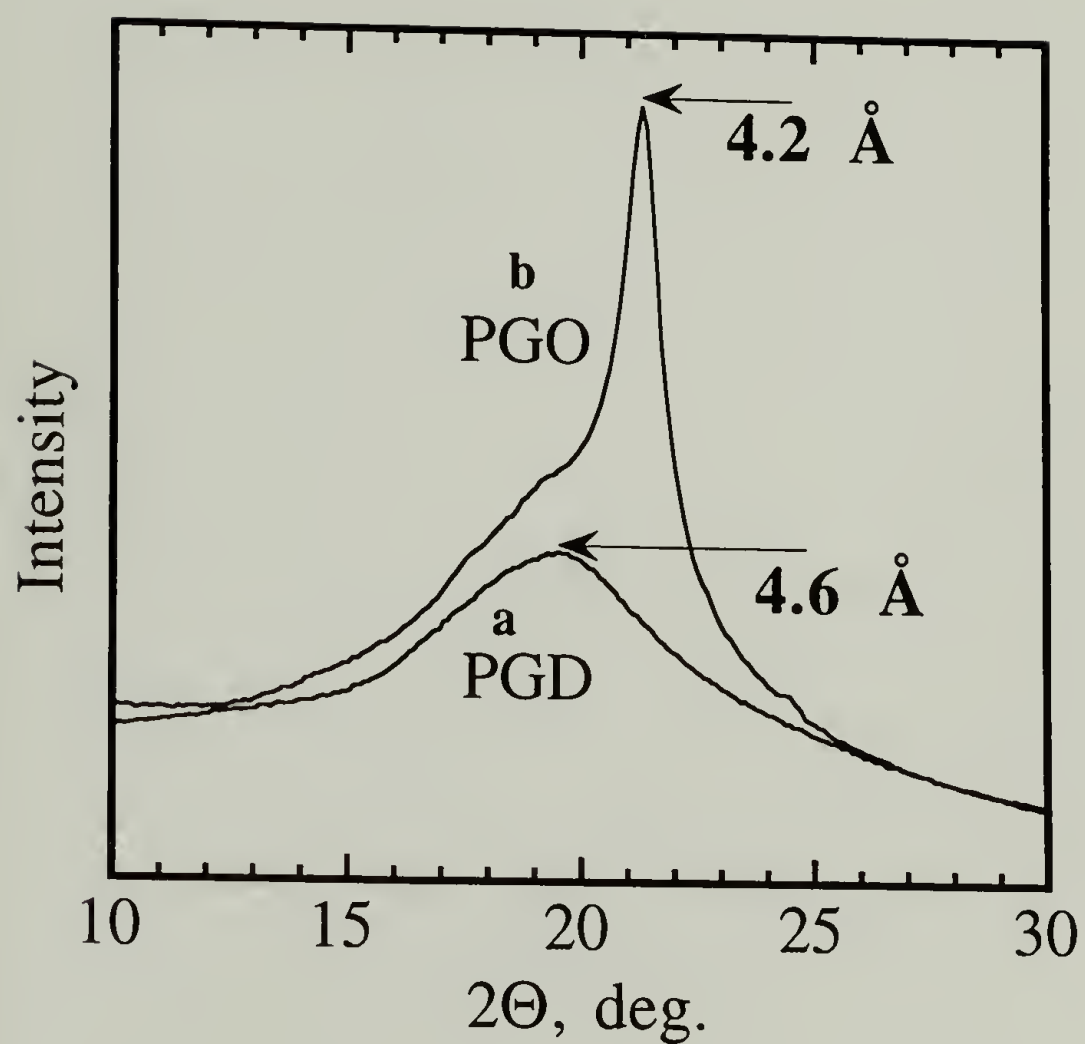


Figure 2.7 Wide angle X-ray diffractometer traces of PGD (a) and PGO (b) powders.

The diffractograms of the PGD and PGC powders are identical.

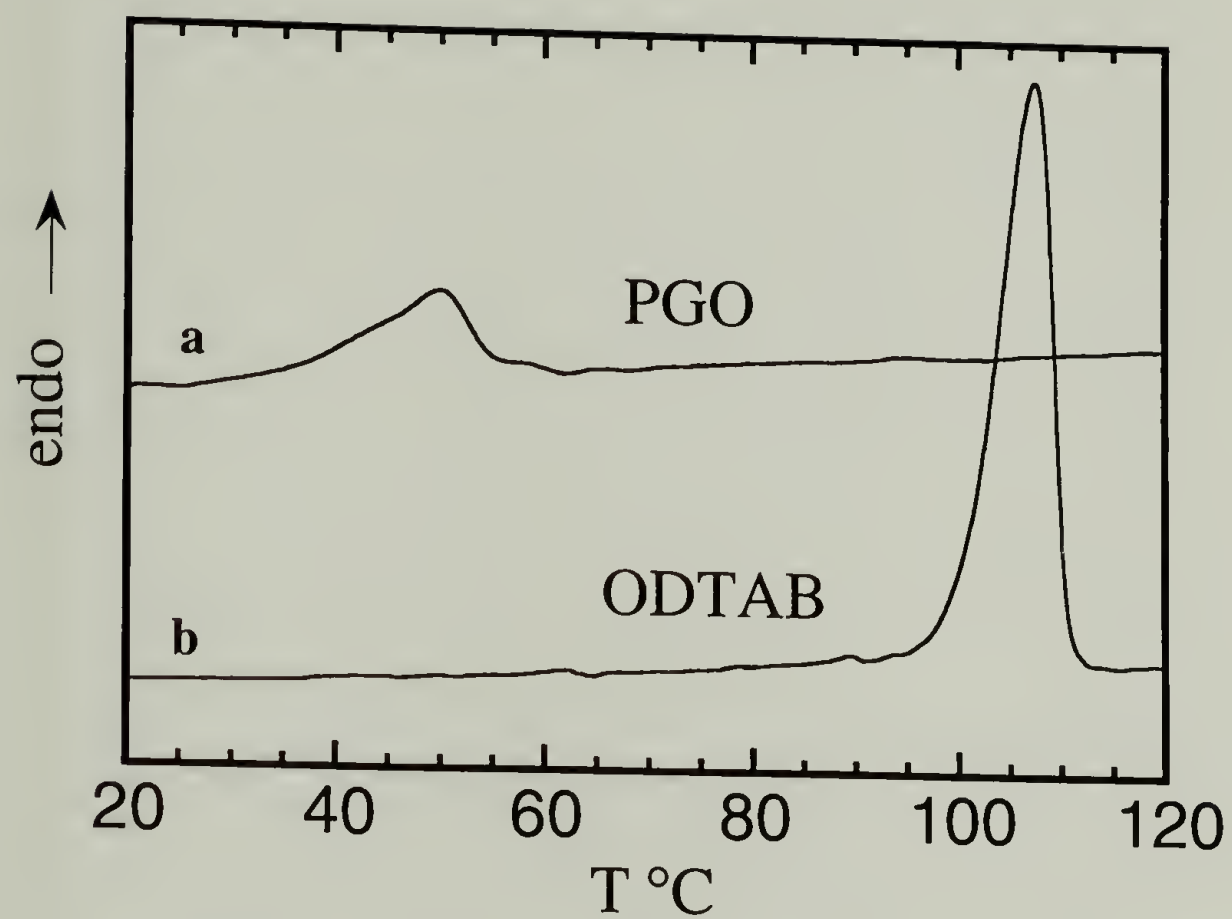


Figure 2.8 DSC thermograms of PGO film (a) and ODTAB powder (b) on heating.

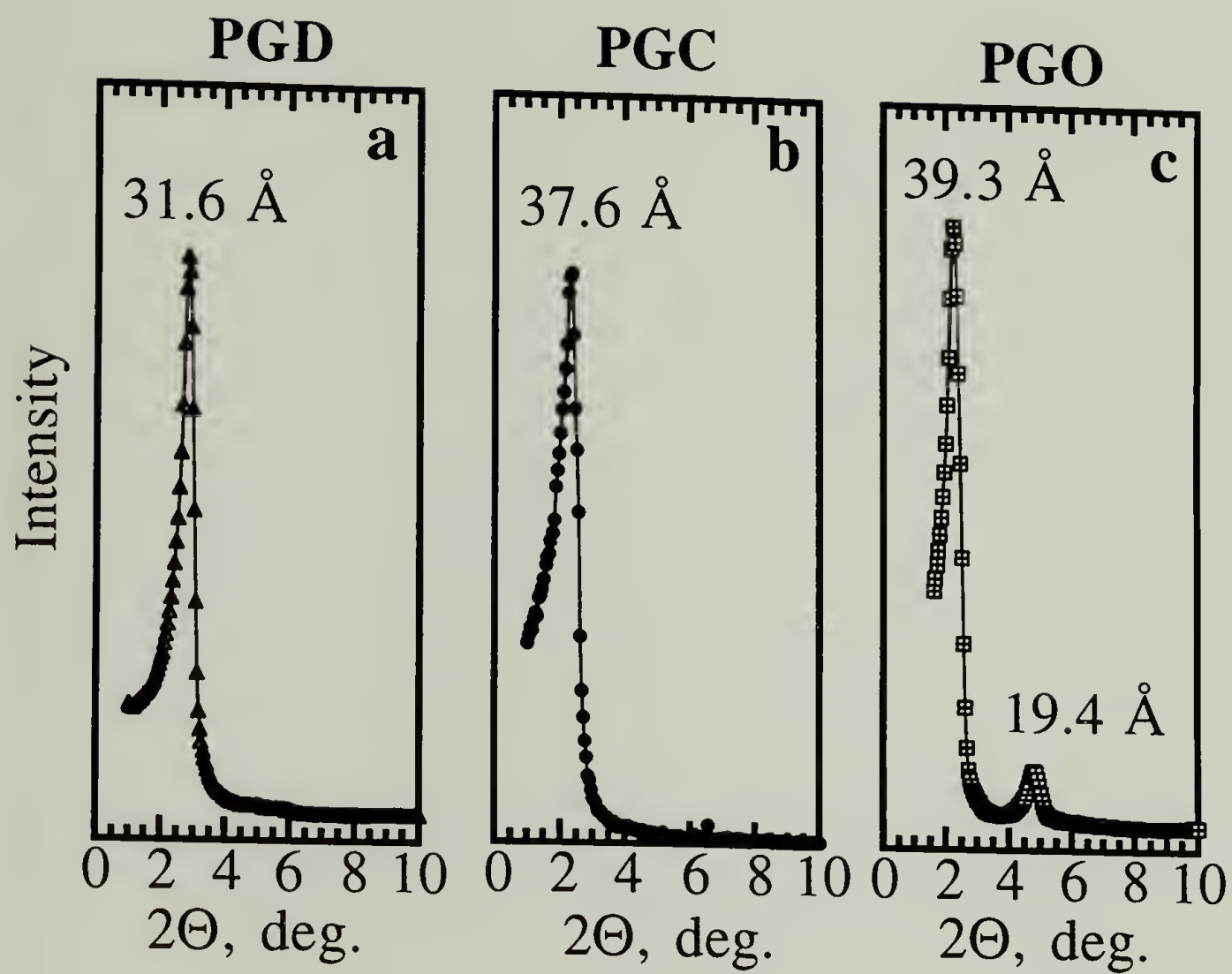


Figure 2.9 Small angle X-ray diffractometer traces of PGD (a), PGC (b) and PGO (c) powders.

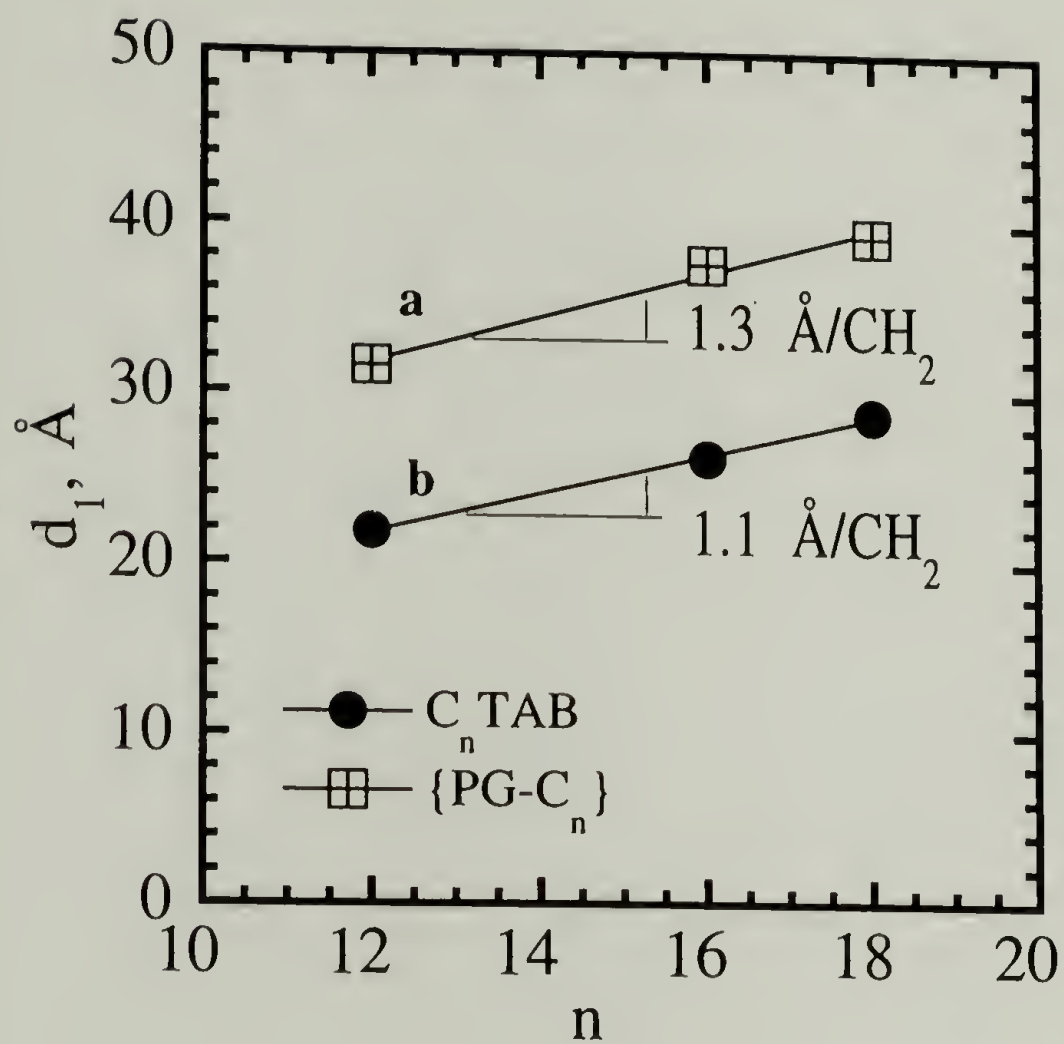


Figure 2.10 Dependence of the long period of the lamellae of the PGX complexes (a) and of the corresponding surfactants (b) on the number of carbon atoms (n) in the surfactant chains.

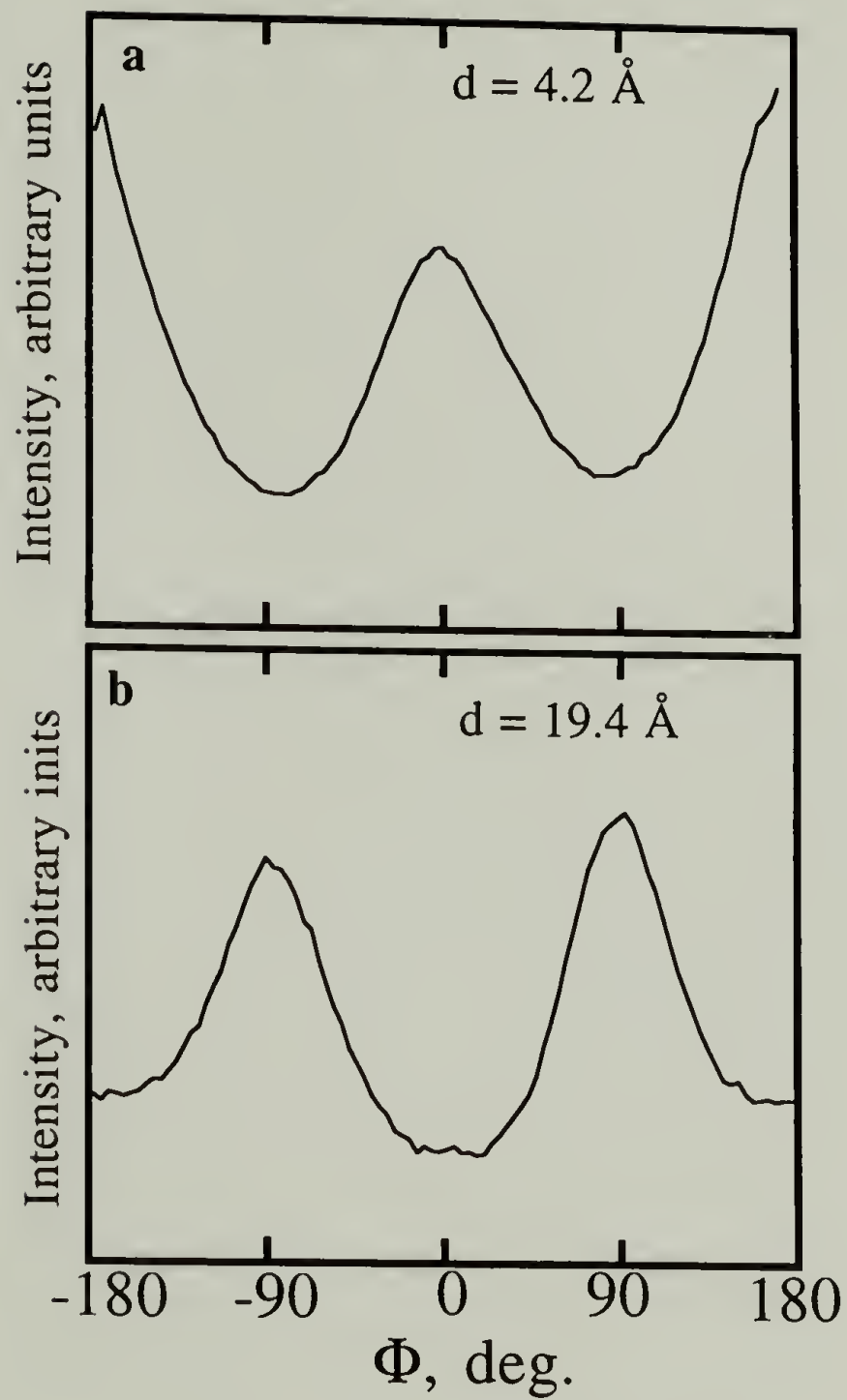


Figure 2.11 Dependence of the X-ray intensity of the reflections corresponding to the Bragg spacings of 4.2 Å (a) and 19.4 Å (b) on the azimuthal angle Φ of the stretched PGO film. The patterns of the PGD and PGC complexes are similar.

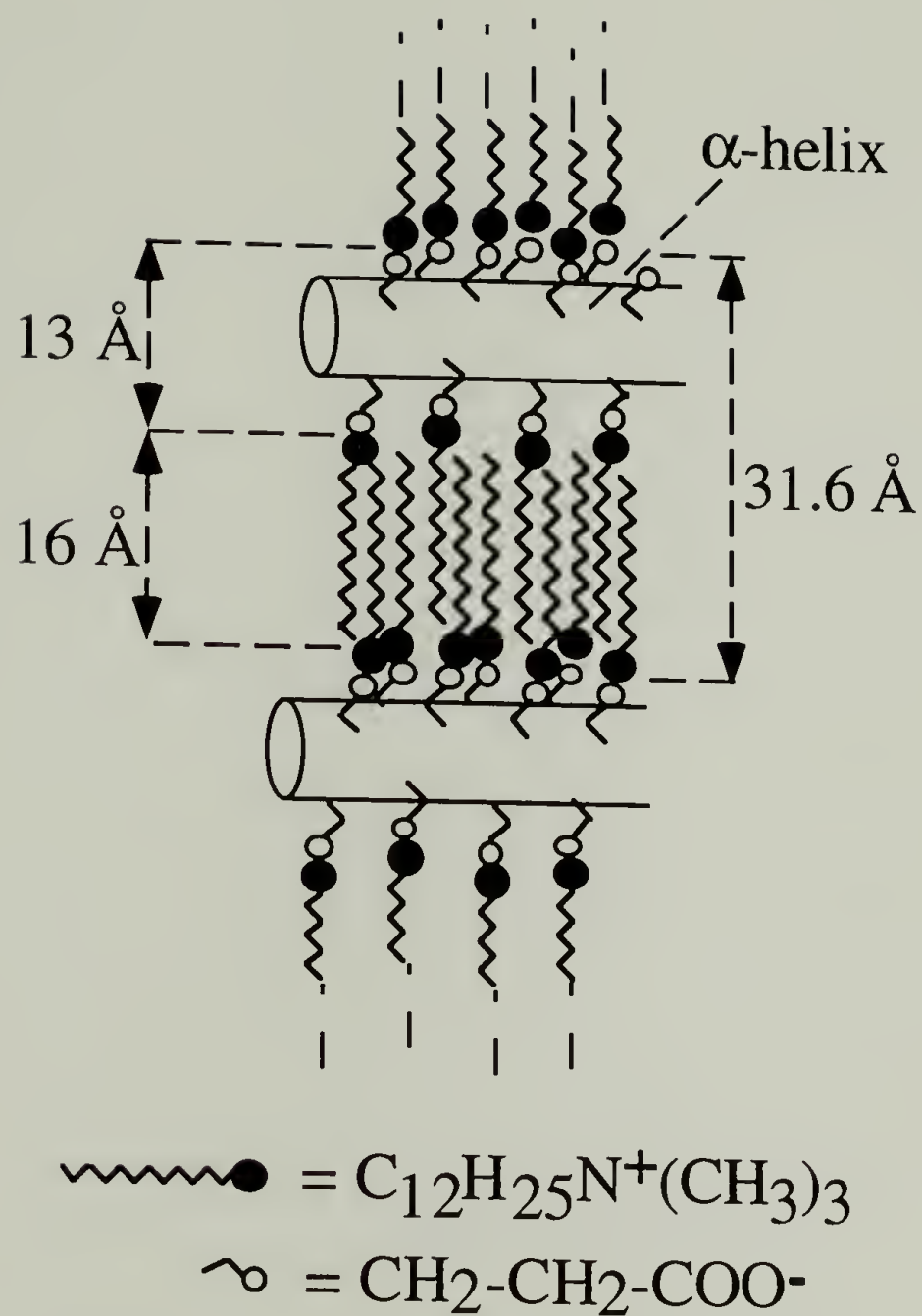


Figure 2.12 Proposed scheme of the lamellar structure of the PGD complex, viewed parallel to the layers and perpendicular to the long axes of the α -helices. The structures of the PGC and PGO complexes are believed to be similar.

Table 2.1

The Frequencies of Amide Bond Vibrations (cm^{-1}) of PBLG and of the PGD Complex at Different Temperatures

	20°C		115°C	
	PBLG	PGD	PBLG	PGD
amide I	1654	1653	1657	1663
amide II	1549	1549	1547	1527

CHAPTER III

STOICHIOMETRIC COMPLEXES FORMED BY POLY(L-LYSINE) CATIONS AND ALKYL SULFATE ANIONS IN ORGANIC SOLVENTS AND IN THE SOLID STATE

Introduction

In this chapter, we report an investigation of the properties of the stoichiometric complex formed by poly(L-lysine) hydrobromide and an oppositely charged surfactant - sodium dodecyl sulfate - in organic solvents and in the solid state.¹ We refer to this complex as PLD. The chemical structure of the complex is presented in Figure 3.1.

We compare the behavior of the complex with that of its covalent analogs - poly(L-lysine)s bearing covalently attached acyl side chains (PALLs). The goal of this study is to understand the roles of hydrogen bonds, electrostatic interactions, and solvent polarity on the conformation and aggregation of the polypeptide chains and on the supramolecular order in the system.

Experimental Section

Materials

Poly(L-lysine) hydrobromide (PLys HBr) with viscosity average degree of polymerization (provided by the supplier) of about 2400 (Sigma) and the anionic surfactant sodium dodecyl sulfate (SDS) (Research Plus) were used as received. Chloroform (Aldrich), deuterated chloroform (Aldrich) and trifluoroacetic acid (Acros) of the highest purity available were purchased and used as received.

The complex consisting of poly(L-lysine) cations and dodecyl sulfate anions (PLD) was prepared by mixing equimolar quantities of 0.05 M aqueous solutions of PLys HBr and SDS. After stirring the mixture for one hour, the resulting white precipitate was isolated by centrifugation, washed several times with water to remove low molecular weight counterions and dried in vacuum at 45 °C for at least 36 hours.

Elemental analysis showed good agreement between the experimental and calculated contents of C, H and N corresponding to the stoichiometric composition: C_{calcd} 54.8, C_{found} 54.1, H_{calcd} 9.6, H_{found} 9.6, N_{calcd} 7.1, N_{found} 7.1, $Na_{\text{found}} < 0.1 \%$.

Solutions of the PLD complex in organic solvents were prepared by dissolution with stirring for 12 - 24 hours prior to measurements. Solutions for viscosity measurements were filtered through 0.5 μm Millipore Lillex-LCR filters (modified hydrophilic PTFE membranes in polyethylene housing, product number SLCR 025 NS). ^1H NMR relaxation experiments were carried out with solutions sealed in air. To elucidate the effect of oxygen on the spin - lattice relaxation times, two degassed solutions of the PLD complex in deuterated chloroform with 1 and 10 volume per cent TFA were prepared. Degassed samples were prepared on a vacuum line using three freeze - pump - thaw cycles and then sealed under vacuum. Spin - lattice relaxation times were identical within the experimental error of the measurements in the degassed samples and in the samples which were not degassed. Powder samples for X-ray analysis were sealed in thin glass capillaries. Films of the complex for X-ray analysis were prepared by evaporation of chloroform - TFA solutions on Teflon plates at room temperature. For circular dichroism (CD) and Fourier transform infrared (FTIR) measurements, films were cast from chloroform solutions on quartz and KBr windows, respectively. The concentration of the PLD solutions for film casting was 1 weight per cent. For FTIR measurements of the PLD powder, KBr pellets were prepared.

Measurements

^1H NMR spectra were recorded on a Bruker AMX 500 MHz instrument. The measurements were performed at 20 °C, unless otherwise specified. Proton spin - lattice relaxation times (T_1) were determined using an inversion - recovery technique with a $180^\circ - \tau - 90^\circ$ pulse sequence and a delay time of 10 seconds. The accuracy of T_1 determination for different samples was estimated to be ± 15 per cent for the polypeptide peaks and ± 5

per cent for the surfactant peaks. T_1 measurements at different temperatures were carried out with the same samples. Viscosity measurements were performed in a standard Ubbelohde viscometer at 25° C. Circular dichroism spectra were recorded using an Aviv 62DC spectrometer. FTIR spectra were obtained on a Perkin Elmer 1600 series spectrometer. X-ray diffraction patterns were recorded using either an evacuated Statton camera or a Siemens D500 diffractometer in transmission mode with a scintillation counter scanning through the appropriate 2Θ range (where Θ is the Bragg angle). In both cases Ni-filtered $\text{CuK}\alpha$ ($\lambda = 1.5418 \text{ \AA}$) radiation was used.

Results and Discussion

Complex Stability

In contrast to the stoichiometric complexes consisting of poly(α ,L-glutamate) anions and alkyl trimethylammonium cations (PGX),^{2,3} which undergo irreversible changes in properties upon storage with formation of small amounts (5 -10 per cent) of unbound crystalline surfactants (detected via X-ray and DSC analysis), the PLD complex is quite stable. No free surfactant has been detected by polarized optical microscopy or X-ray analysis upon storage of the complex in the air for months, and no changes in properties with time have been observed.

The dramatic differences in the stabilities of the PGX and the PLD complexes may arise from either or both of two factors. First, the low acid strength of carboxylic polyacids such as poly(α , L-glutamic acid) may render the PGX complexes particularly susceptible to hydrolysis. Second, it is known that alkyl trimethylammonium surfactants absorb water readily to form lyotropic liquid crystals, while alkyl sulfates with chain lengths greater than eight methylene groups form solid hydrates.⁴ The stability of the hydrates may prevent hydrolysis of the complexes examined in this work. The stability of these complexes will become an important consideration if new materials based on such complexes are to be developed.

Solubility in Organic Solvents

The solubility behavior of the PLD complex in organic solvents is quite different from that of the PGX complexes, discussed above (Table 3.1), and from that of the stoichiometric complexes formed by conventional synthetic polyelectrolytes and oppositely charged surfactants.^{5,6} PLD is insoluble in most common organic solvents; however, it is soluble in mixtures of organic solvents of low polarity ($\epsilon=2-6$) with small amounts (at least 1-2 volume per cent) of trifluoroacetic acid (TFA). In relatively polar solvents (e.g., dimethylformamide or methanol), in which the stoichiometric complexes of conventional polyelectrolytes and oppositely charged surfactants exhibit a polyelectrolyte effect (and therefore are believed to dissociate⁵), PLD is insoluble, even if TFA is added. The solubility behavior of the PLD complex is also different from that of its covalent analogs - the poly(N- ϵ -acyl-L-lysine)s (PALLs). PALLs are soluble in a wider range of solvents, e.g., in halogenated hydrocarbons, hydrocarbons, aliphatic alcohols and of mixtures of these solvents with TFA.^{7,8}

Conformational Transitions in Chloroform - Trifluoroacetic Acid Solutions

We performed a systematic study of dilute solutions of the PLD complex in chloroform - TFA mixtures.

Addition of up to 10 volume per cent of TFA to chloroform solutions of the PLD complex does not cause dissociation of the complex, as indicated by the absence of a polyelectrolyte effect on solution viscosity (Figure 3.2); the reduced viscosity shows an approximately linear dependence on the concentration of the complex. The Huggins constants, estimated from the slopes of the lines for the PLD complex in chloroform mixtures with 1 and 10 volume per cent TFA, are 0.12 and 0.66, respectively. An increase in the TFA content causes an increase in the Huggins constant, indicating a change from relatively rigid polymer chains to more flexible chains in a "poor" solvent.

The low viscosity of the solutions of the PLD complex, the linear dependence of the reduced viscosity on concentration in the concentration range of 1 - 10 mg/ml, and the low values of the Huggins constants (<1) suggest the an absence of interchain aggregation in dilute solutions of the PLD complex.

Evidence that the polypeptide chains in the PLD complex can adopt either ordered or disordered conformations is provided by ^1H NMR spectroscopy. Figure 3.3 presents ^1H NMR spectra of the PLD complex in deuterated chloroform - TFA mixtures. At low TFA contents (1 volume per cent) in chloroform solutions of the PLD complex, the $\alpha\text{-CH}$ resonances of the polypeptide chains and $\alpha\text{-CH}_2$ resonances of the surfactant are observed as one peak at 4.00 ppm, and the NH resonances are observed at 8.15 ppm, consistent with hydrogen bonded (α -helix or β -sheet) conformations of the polypeptide chains⁹ (Figure 3.3 a). It is reasonable to assume that the poly(L-lysine) chains are in the α -helical conformation, considering the absence of aggregation in dilute solutions and the known propensity of β -sheets to aggregate in solution. The possibility of formation of other types of helices cannot be excluded, based on these data; however, because the α -helix is most common for synthetic homopolypeptides, we refer to the main chain conformation in the PLD complex as an α -helix. PALLs also adopt α -helical conformations in organic solvents, if no TFA is added.^{7,8,10}

Addition of TFA to chloroform solutions of the PLD complex results in a considerable narrowing of all resonances (Figure 3.3 b), an upfield shift of the NH resonances, and a downfield shift of the $\alpha\text{-CH}$ resonances of the polypeptide backbone (Figure 3.4). Upon further addition of TFA at concentrations above 7.5 volume per cent, the poly(L-lysine) proton frequencies remain constant. These effects are consistent with disruption of hydrogen bonds and formation of a disordered conformation of the polypeptide.⁹ The shifts in the proton resonances of the poly(L-lysine) backbone upon addition of TFA coincide with an abrupt decrease in viscosity of the PLD solution between 4 and 6 volume per cent TFA (Figure 3.5). Conformational transitions induced

by TFA have been observed previously by optical rotary dispersion for poly(L-lysine) derivatives with covalently attached acyl side chains^{8,10,11} and for other synthetic polypeptides in organic solvents.¹² For poly(L-lysine) derivatives in chloroform - TFA mixtures, the helix-coil transition is half complete at 20 - 30 volume per cent TFA; higher TFA contents are necessary to disrupt the helical forms of the PLLs with shorter side chains. The difference in the behavior of PLLs and the PLD complex may be related to the lower stability of the α -helix of the latter due to electrostatic repulsion between 'side chain' ionic sites. Additionally, the acyl side chains in PLLs are believed to be associated via hydrogen bonds,¹¹ which may further stabilize the α -helical conformation.

There are at least two driving forces for the conformational transition of the PLD complex in chloroform - TFA mixtures: the disruption of hydrogen bonds by TFA, as occurs in solutions of PLLs,^{8,10} and the increase in polarity of the solvent upon addition of TFA. Increasing solvent polarity is expected to increase ion pair separation and, in some cases, is known to promote dissociation of polyelectrolyte - surfactant complexes.⁵ Thus increasing ion pair separation with increasing solvent polarity would be expected to destabilize the α -helical conformation of the poly(L-lysine) chains in the complex, and may account for the sensitivity of PLD to low concentrations of added TFA.

Additional control over the helix - coil transition of the PLD chains in chloroform - TFA mixtures is provided by temperature. At low and high TFA contents, when the poly(L-lysine) chains are in the helical or disordered conformations, respectively, the positions of the polypeptide proton resonances remain constant in the temperature range 5 - 35 °C (Figure 3.6). In contrast, in CDCl₃ - TFA mixtures with 4 - 5 volume per cent TFA, when the poly(L-lysine) chains are in an intermediate state at room temperature (Figures 3.4 and 3.5), a transition to disordered chains is observed at lower temperatures and to helical chains at higher temperatures, as indicated by shifts of the α -CH resonance (Figure 3.6).

These observations allow us to conclude that the α -helical conformation of the PLD chains is stabilized at higher temperatures in chloroform - TFA solutions, similar to the case of other synthetic polypeptides, e.g., PBLG¹³ and poly(δ -N-carbobenzoxy - L-lysine)¹⁴. The coil - helix transition induced by temperature is believed to be due to the entropy gain of the solvent associated with release of TFA from the polypeptide chains upon transition of the solvated coil of the polypeptide chain to the helical form.

Effect of Trifluoroacetic Acid on Segmental Mobility in Solutions

To determine the effect of TFA on the mobility of the PLD chain segments, we measured the proton spin - lattice relaxation times of the poly(L-lysine) backbone and of the dodecyl chains in deuterated chloroform - TFA mixtures.

At all TFA contents in the range 1 - 10 volume per cent, the relaxation times of the dodecyl sulfate methylene protons at about 1.26 ppm are larger than those of the polypeptide (Figure 3.7), consistent with the suggestion that the mobility of the surfactant chains is higher than that of the polymer chains. This conclusion is supported by the fact that in both the α -helical and the disordered conformations of poly(L-lysine) chains, the proton resonances of the polypeptide backbone are broader than those of the 'side chains' (Figure 3.2).

An increase in the TFA content from 1 to 10 volume per cent does not affect the spin - lattice relaxation times of the surfactant protons (Figure 3.7), suggesting that there is neither dissociation of the complex nor aggregation of the 'side chains' accompanying the helix - coil transition induced by TFA. Therefore, in the disordered conformation, as well as in the α -helical conformation of the polypeptide, the 'side chains' are likely to be exposed to the solvent, shielding the polypeptide backbone and the ionic groups (Figure 3.8).

Increasing the TFA concentration from 1 to 10 volume percent gradually decreases the spin-lattice relaxation times of the polypeptide backbone protons (Figure

3.9), concurrent with the helix - coil transition of the poly(L-lysine) chains. Transition from the helical to the disordered conformation is expected to result in an increase in the mobility of the polypeptide chain segments, as observed for poly(γ -benzyl α ,L-glutamate) in CDCl₃ - TFA mixtures.¹⁵ In general, T₁s decrease with decreasing mobility, reach a minimum value, and then increase.¹⁶ In order to elucidate the mobility changes accompanying the helix - coil transition of the PLD complex, we first measured the temperature dependence of the proton spin - lattice relaxation times. Increasing temperature is expected to increase the mobility of both the polypeptide chains and the 'side chains', provided no conformational changes occur.¹⁷

At relatively high TFA contents when the poly(L-lysine) chains are in the disordered conformation in the temperature range 0 - 35° C, a decrease in T₁ of the polypeptide protons is observed with increasing temperature (Figure 3.9), suggesting that T₁ decreases as mobility is increased. This allows us to conclude that the decrease in T₁ of the poly(L-lysine) protons observed at the helix - coil transition (Figure 3.7) can be ascribed to an increase in segmental mobility, as observed for other synthetic polypeptides.¹⁸

At low TFA contents, when the polypeptide chains are predominantly α -helical in the temperature range 0 - 35° C, the relaxation times of the poly(L-lysine) protons are essentially constant, within the experimental error of the measurements. However, the poly(L-lysine) resonances in the α -helical conformation are extremely broad, and the error in the T₁ determination may be large compared to the small temperature effects.

Increasing temperature results in an increase in T₁s for the surfactant protons, regardless of TFA concentration (Figure 3.10), suggesting that the spin - lattice relaxation times of the PLD 'side chains' increase with mobility.

Thus, in chloroform - TFA solutions, the poly(L-lysine) chains of the PLD complex undergo a coil - helix transition upon heating. The transition is entropically

driven, but is accompanied by a decrease in the mobility of the polypeptide chain segments. The mobility of the 'side chains' remains unchanged.

The absence of the polyelectrolyte effect on viscosity of the PLD solutions in chloroform - TFA mixtures, the insensitivity of the mobility of the PLD 'side chains' to addition of TFA, and the fact that the helical conformation is favored at elevated temperature, allow us to conclude that a significant driving force in the solvent-dependent helix - coil transition is the disruption of hydrogen bonds within the polypeptide chains by TFA, similar to other synthetic polypeptides with covalently attached side chains.^{8,10-12}

Solid State Structure

In the powder form of the PLD complex as isolated after synthesis, the poly(L-lysine) chains adopt a β -sheet conformation, as shown by the positions of the amide I and amide II vibrations in the FTIR spectrum¹⁹ (observed at 1629 and 1534 cm^{-1} , respectively, Figure 3.11 curve a). The β -sheet conformation of the polypeptide chains in the PLD complex is likely to account for its insolubility in organic solvents. Poly(L-lysine) also adopts a β -sheet conformation in complexes with negatively charged lipids²⁰ and in the protonated form in the solid state.^{19,21,22} However, covalent analogs of the PLD complex (PALLs) are in α -helical conformations in solid samples.²³

Casting of films from chloroform - TFA mixtures results in the appearance of the new amide I and amide II bands at 1652 and 1542 cm^{-1} , respectively (Figure 3.11 curves b and c), suggesting disruption of the β -sheet architecture.¹⁹ If the films are cast from solutions with 10 volume per cent TFA, in which the poly(L-lysine) chains are disordered, the polypeptide chains are predominantly α -helical, as shown by a positive CD band at about 190 nm and negative bands at about 210 and 220 nm, respectively (spectra not shown).²⁴

Conversion of the main chain conformation from the β -sheet to the α -helix is accompanied by a conformational transition of the surfactant alkyl chains. In the PLD powder, the CH asymmetric and symmetric stretching vibrations are observed at 2924 and 2854 cm^{-1} , respectively, indicating an intermediate state of the alkyl chains between 'liquid-like' and 'solid-like',²⁵ (Figure 3.12 curve a). In PLD films cast from chloroform solutions containing 1 volume per cent TFA, the CH vibrations are shifted to 2920 and 2851 cm^{-1} (Figure 3.12 curve b), indicating that the surfactant alkyl chains become progressively more 'solid-like', and in the films cast from with 10 volume per cent TFA, the positions of the CH vibrations coincide with those reported for the alkyl chains in the fully extended state; i. e., 2918 and 2850 cm^{-1} , respectively.²⁵ (Figure 12 curve c).

The WAXD pattern of the PLD powder (Figure 3.13 curve a) consists of a broad halo centered at about 4.6 Å and a shoulder at 3.9 Å. These spacings are similar to those of the 010 and 100 reflections (4.3 - 4.4 and 3.9 - 4.0 Å, respectively) of the two dimensional crystal lattices formed by alkane chains of ten or more methylene groups in PALGs.²⁶ These two-dimensional lattices are believed to resemble the subunit cell attained by the projection of the triclinic unit cell in crystals of low molecular weight n-alkanes. However, because the WAXD peaks of the PLD complex are considerably broader than those observed for PALG crystallites, we conclude that the packing of dodecyl sulfate chains in the complex is characterized by only short-range order. The WAXD pattern of the pure crystalline SDS is presented for comparison (Figure 3.13 curve b). The most prominent signals (Bragg spacings of 4.33 and 4.06 Å) correspond to the 220 and the 400 reflections of the monoclinic unit cell of SDS.²⁷ The absence of crystalline SDS reflections in the PLD X-ray pattern suggests that the complex is uncontaminated by unbound surfactant.

The SAXD pattern of the PLD powder consists of a relatively sharp peak of high intensity with a Bragg spacing of about 43 Å, and multiple peaks of low intensity with ratios of Bragg spacings of 1:1/2:1/3:1/5:1/6:1/7 (Figure 3.14 curve a), indicating a

lamellar structure with the long period spacing of 43 Å. The SAXD pattern of the SDS powder is presented for comparison (Figure 3.14 curve b). The peak corresponding to the lamellar spacing of SDS (39 Å), is much sharper than that of PLD. Additionally, the higher orders of the SDS lamellae are of significantly higher intensity, relative to the first order peak, in SDS than in PLD, consistent with higher order organization of pure SDS compared to the PLD complex.

The lamellar structures of PLD films cast from chloroform solutions with TFA contents of 1 - 10 volume per cent, are similar to that of the PLD powder, as shown by diffraction experiments with the X-ray beam perpendicular to the plane of the film. However, when the X-ray beam is directed parallel to the plane of the film, differences in lamellar orientation become apparent. For samples cast from chloroform containing 1 volume per cent TFA (where the polypeptide chains are predominantly in the β -sheet conformation), the reflections corresponding to the lamellar spacings became equatorial, indicating anisotropic orientation of stacks of the lamellae within the film (Figure 3.15 a). At high TFA content, (when the polypeptide chains are in the α -helical conformation in the solid state), the films are characterized by isotropic orientation of stacks of lamellae (Figure 3.15 b). The long period spacing of the lamellae does not depend on the conformation of the polypeptide chains.

The lamellar aggregates of the PLD complex are likely to consist of layers of β -sheets or α -helices separated by layers of surfactant chains. The length of the fully extended dodecyl sulfate chain is about 16 Å, and the distance between the α -carbon and the terminal nitrogen atom in poly(L-lysine) is about 6 Å,²¹ if the methylene chains are fully extended. Thus, the value of the long period of the lamellae (43 Å) is approximately twice the length of the fully extended 'side chain' in the PLD complex. Considering that the thickness of the polymer backbone layer is small and that the alkyl chains in the complex may not be fully extended, we conclude that there is very little, if any, interdigitation of the surfactant chains lying in adjacent layers and that the surfactant

chains are roughly perpendicular to the planes of the lamellae, though the possibility of a small tilt cannot be entirely excluded. Our structure factor calculations for this lamellar structure, carried out assuming that the length of the dodecyl sulfate chain is about 16 Å and the length of the poly(L-lysine) alkyl chains is about 6 Å, suggest that the fourth order of the lamellar long period should have a very low intensity. This is supported by experiment (Figure 3.14 a), indicating that the proposed lamellar structure is plausible. A schematic illustration of the structure is presented in Figure 3.16.

The organization of the dodecyl sulfate chains within the lamellae of the PLD complex resembles that of the pure surfactant. Pure SDS crystallizes tail to tail in double layers,^{27,28} with the hydrocarbon chains fully extended and slightly tilted (tilt angle reported is 79 degrees²⁸) with respect to the layer plane.

For α -helical polypeptides with covalently attached alkyl side chains in the amorphous state, hexagonal packing of the helices is often observed, while lamellar packing is reported when the side chains are crystalline.^{8,26} As we have shown previously, complexes of polypeptides with oppositely charged surfactants are organized in lamellae in the case of both amorphous and crystalline 'side chains'.^{2,3} Therefore, the lamellar packing typical of low molecular weight amphiphiles is likely to govern the organization of the complexes. In complexes consisting of poly(α ,L-glutamate) anions and alkyl trimethylammonium cations, described by us previously, the lamellae consist of layers of α -helical polypeptide chains and layers of surfactant molecules, with surfactant chains perpendicular to the lamellar surfaces and significantly interdigitated. It is interesting to note that pure crystalline alkyl trimethylammonium salts are also organized in layers with interdigitated chains tilted (tilt angle reported is 67 degrees) with respect to the planes of the layers.

Conclusions

The stoichiometric complex consisting of poly(L-lysine) cations and dodecyl sulfate anions is soluble in mixtures of organic solvents of low polarity ($\epsilon = 2 - 6$) with TFA. Addition of up to 10 volume per cent TFA to chloroform solutions does not cause either dissociation or interchain aggregation of the complex. At low TFA contents (less than 5 volume per cent), the poly(L-lysine) chains are in the α -helical conformation in dilute solutions; a transition to disordered chains occurs at higher TFA contents. The helix - coil transition in chloroform - TFA mixtures is accompanied by an increase in the mobility of the polypeptide chain segments, while the mobility of the surfactant chains remains unchanged. A significant driving force in the helix - coil transition of the PLD main chains in chloroform - TFA mixtures is the disruption of intrachain hydrogen bonds by TFA, as in the case of common synthetic polypeptides in organic solvents. However, the stability of the PLD α -helices in the chloroform - TFA mixtures is considerably lower than that of the analogous poly(L-lysine) derivatives with covalently attached acyl groups.

In the powder form of the complex as isolated after synthesis, the polypeptide chains are in the β -sheet conformation. In the films cast from chloroform - TFA mixtures, in which the polypeptide chains are α -helical, the predominant film architecture is the β -sheet. In films cast from chloroform solutions in which the poly(L-lysine) chains are disordered, the PLD main chains are α -helical. Upon conversion of the main chain conformation from the β -sheet to the α -helix, the surfactant chains become progressively more 'solid - like'. The solid PLD complex is organized in a lamellar structure, consisting of layers of polypeptide chains separated by layers of surfactant molecules arranged tail to tail.

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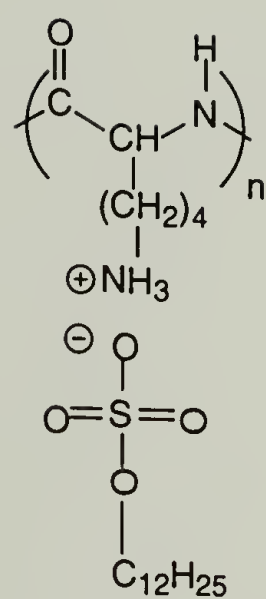


Figure 3.1 Chemical structure of the PLD complex.

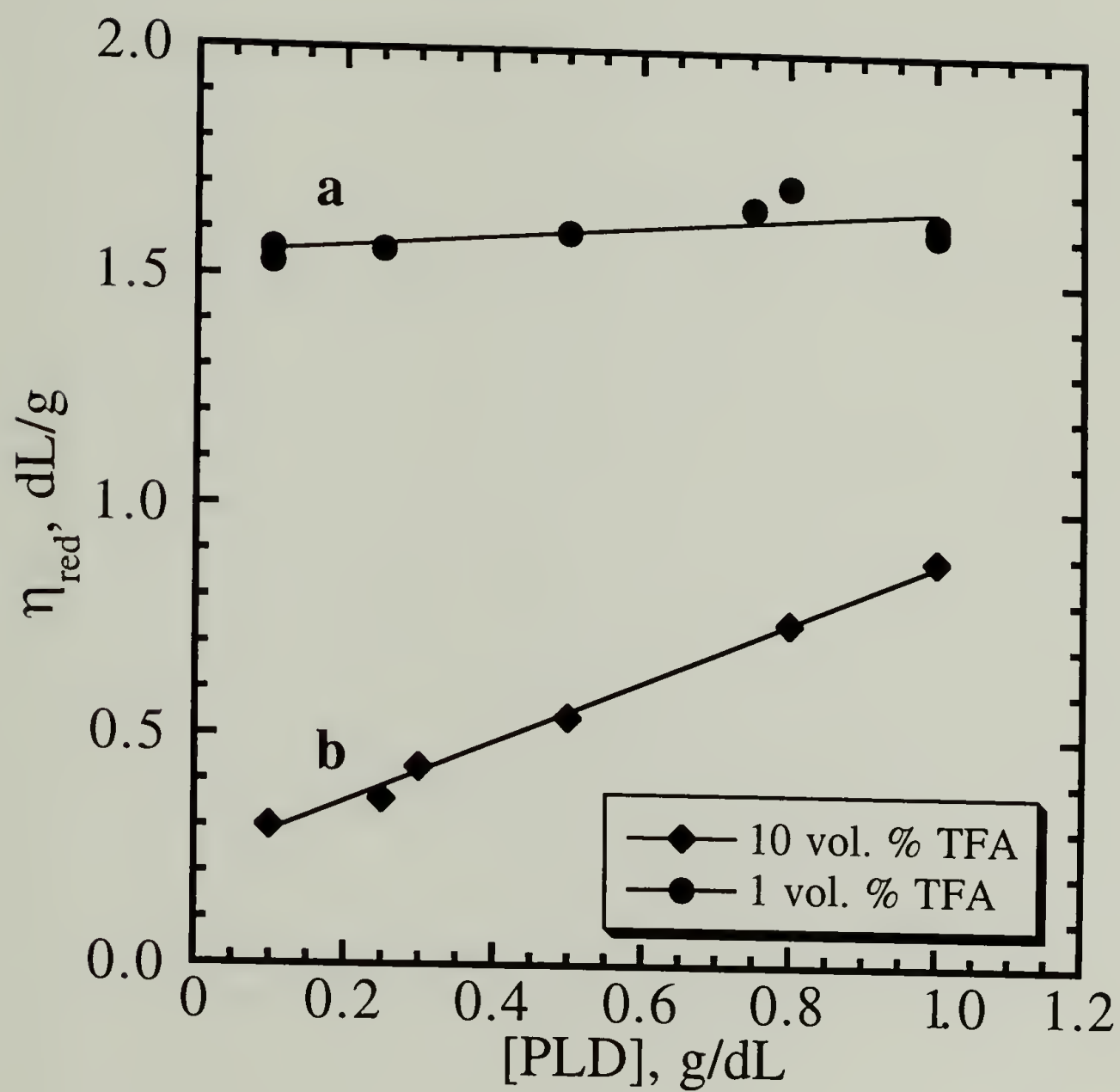


Figure 3.2 Concentration dependence of reduced viscosity of the PLD complex in chloroform solutions containing 1 volume per cent (a), 10 volume per cent (b) trifluoroacetic acid.

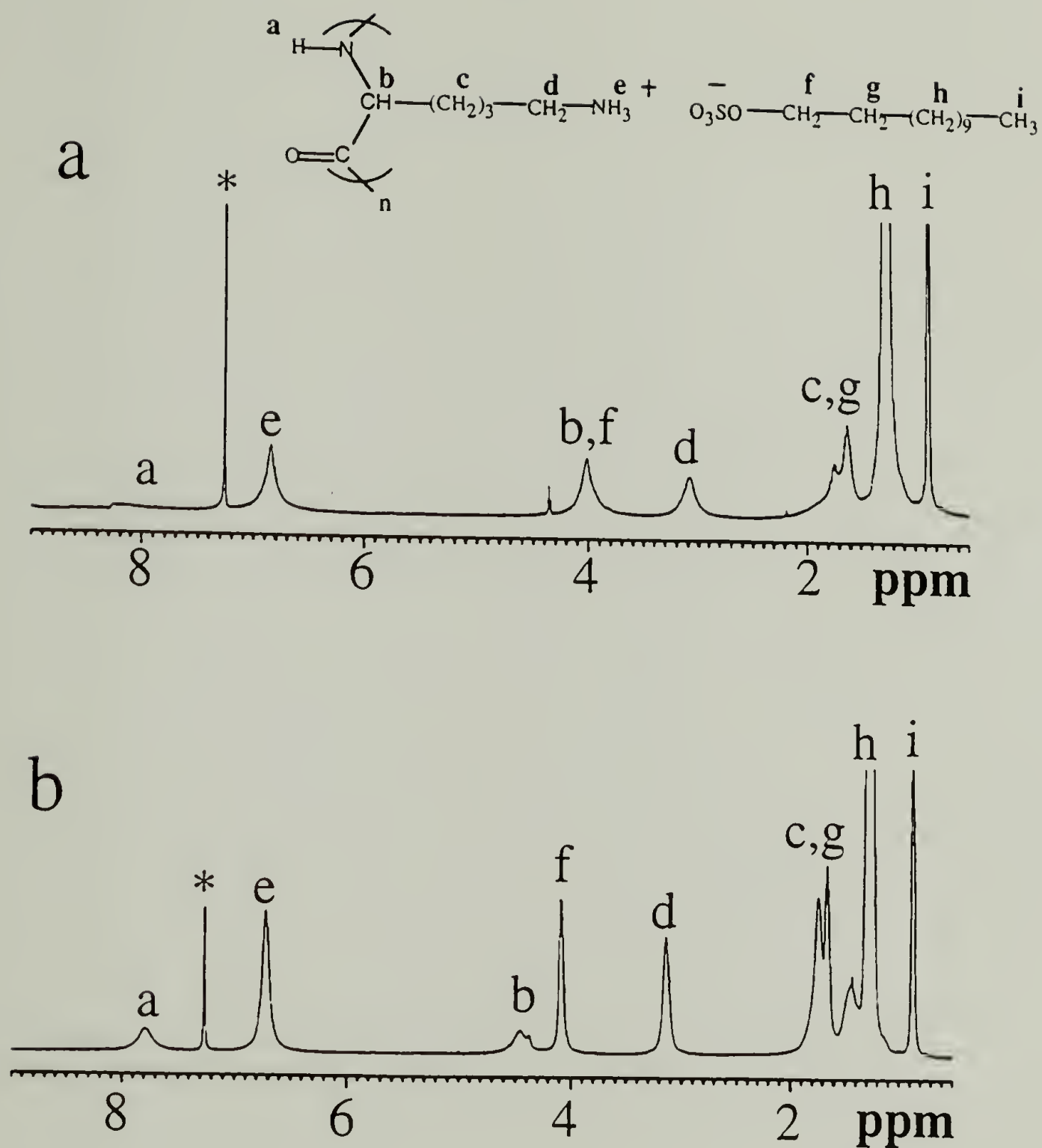


Figure 3.3 500 MHz ^1H NMR spectrum of PLD solution in deuterated chloroform containing 1 (a) and 10 (b) volume per cent trifluoroacetic acid; concentration of PLD solutions is 10 mg/ml; asterisks indicate signals due to solvents.

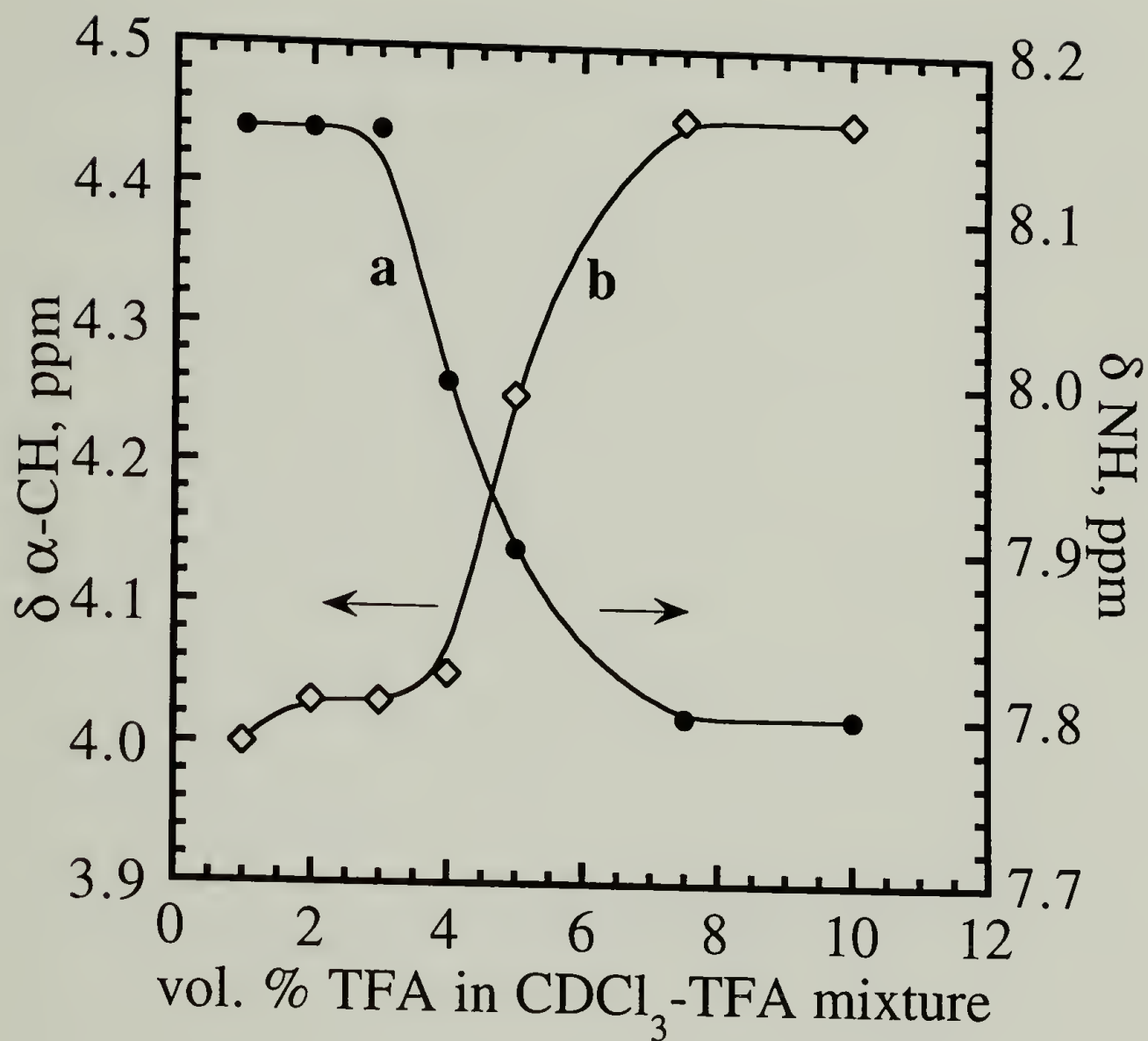


Figure 3.4 Dependence of the positions of NH (a) and α -CH (b) resonances of the PLD complex on the trifluoroacetic acid content in deuterated chloroform solutions; concentration of PLD solutions is 10 mg/ml.

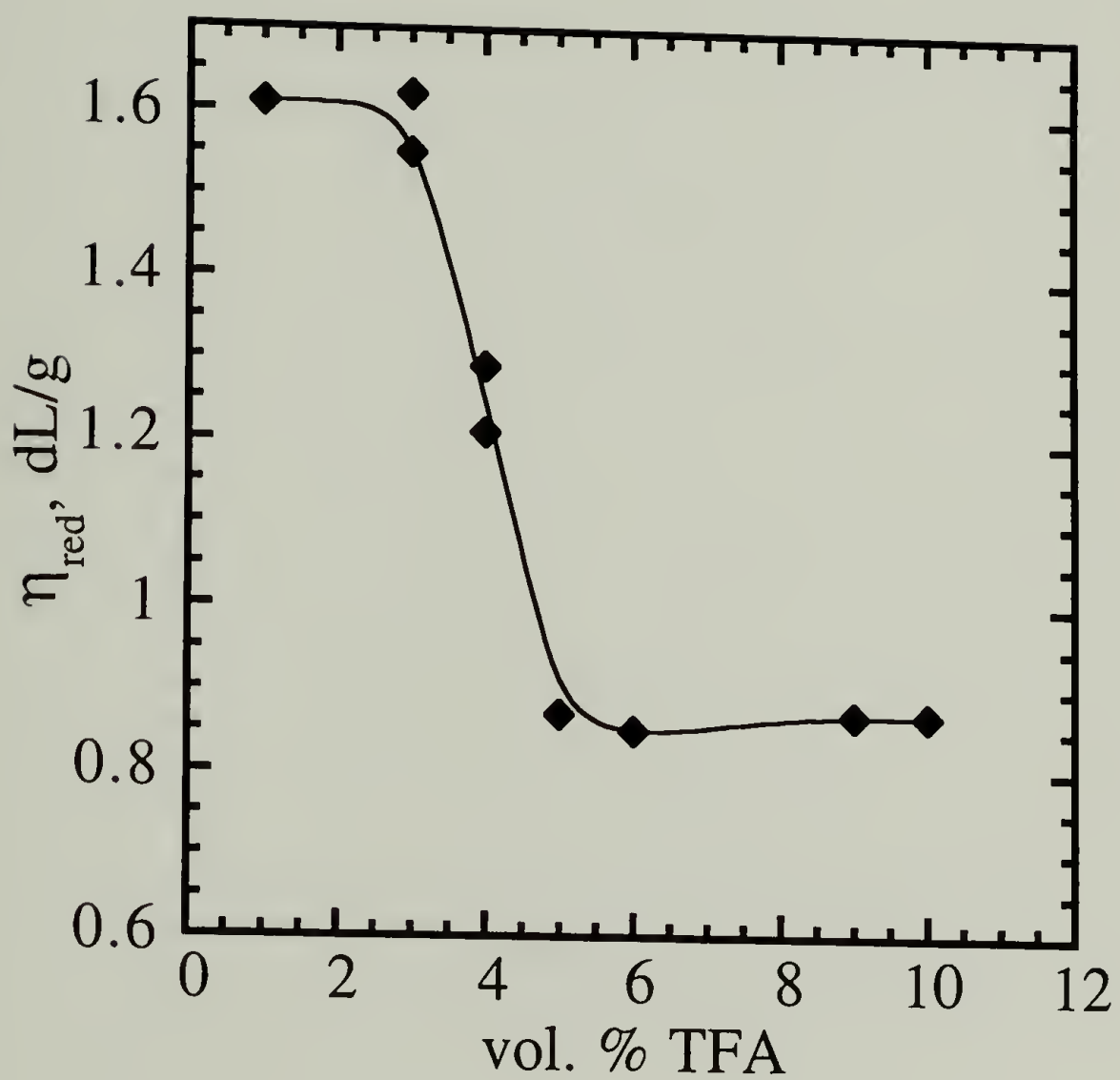


Figure 3.5 Dependence of reduced viscosity on the trifluoroacetic acid content in chloroform solutions of the PLD complex; concentration of PLD solutions is 10 mg/ml.

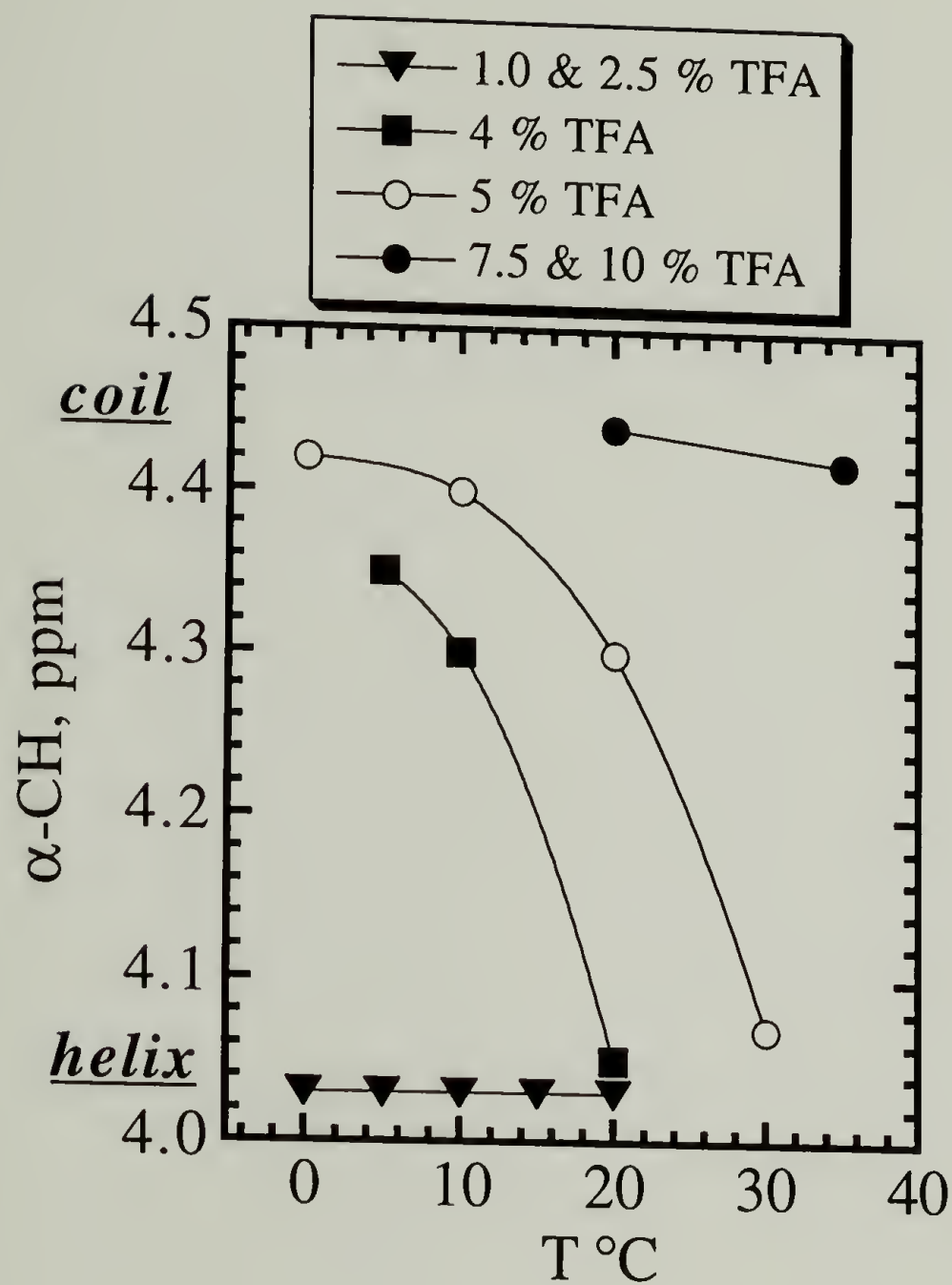


Figure 3.6 Dependence of the positions of the α -CH resonances of the PLD complex on temperature in deuterated chloroform solutions; concentration of PLD solutions is 10 mg/ml.

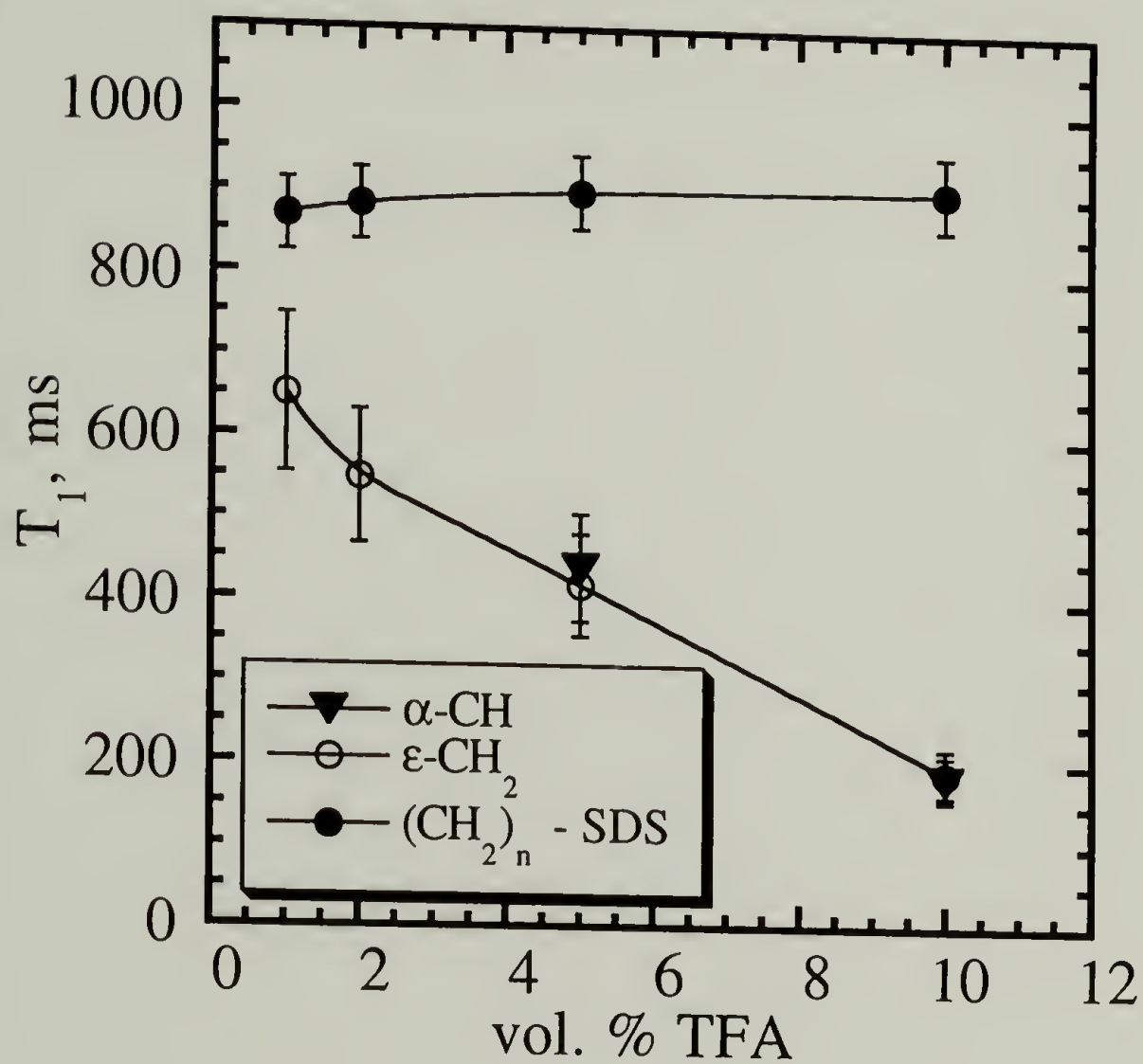


Figure 3.7 Dependence of the proton spin - lattice relaxation times (T_1) of the PLD complex on the trifluoroacetic acid content in deuterated chloroform solutions; concentration of PLD solutions is 10 mg/ml.

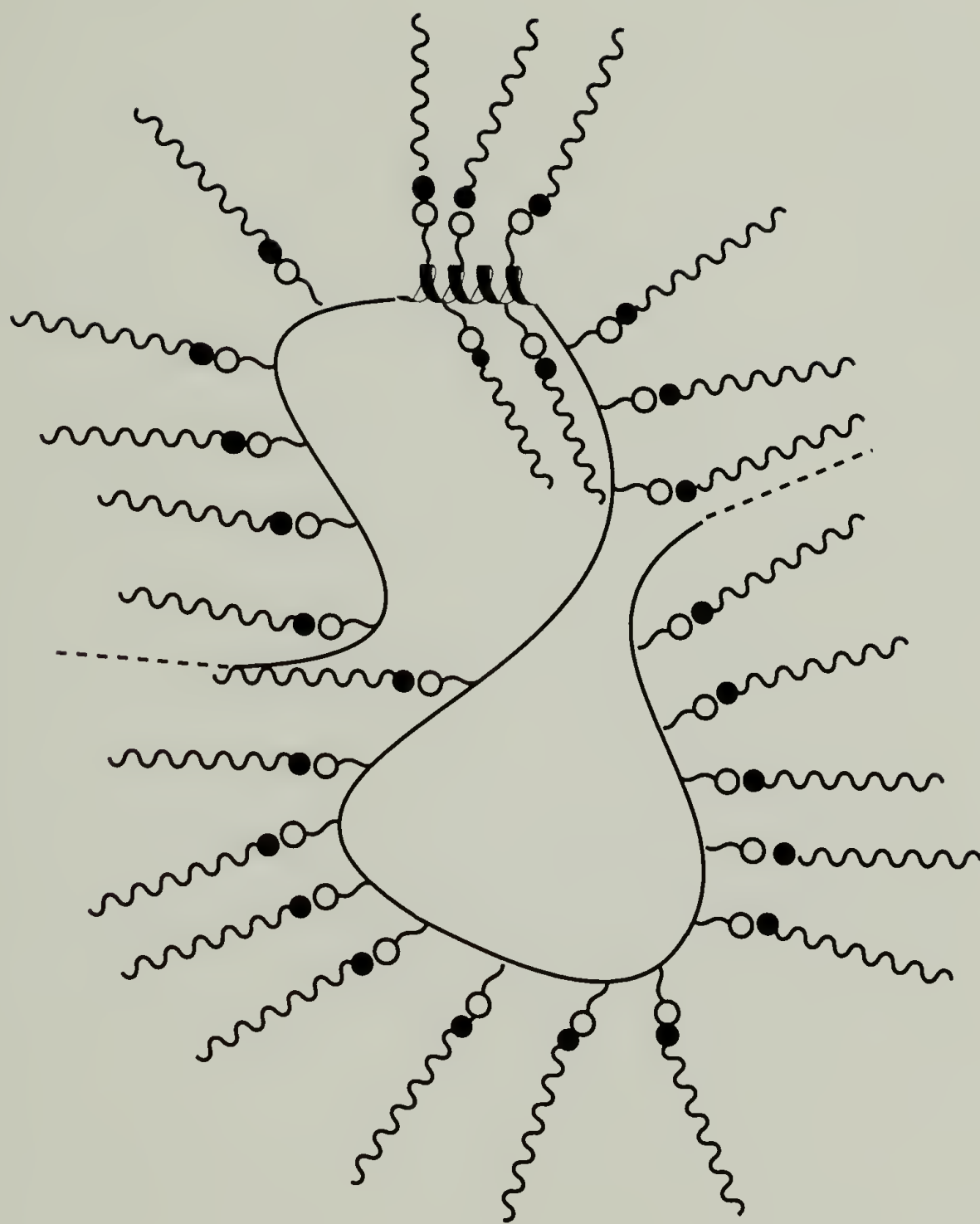


Figure 3.8 Schematic representation of the structure of the PLD complex in dilute chloroform solutions containing about 10 volume per cent trifluoroacetic acid.

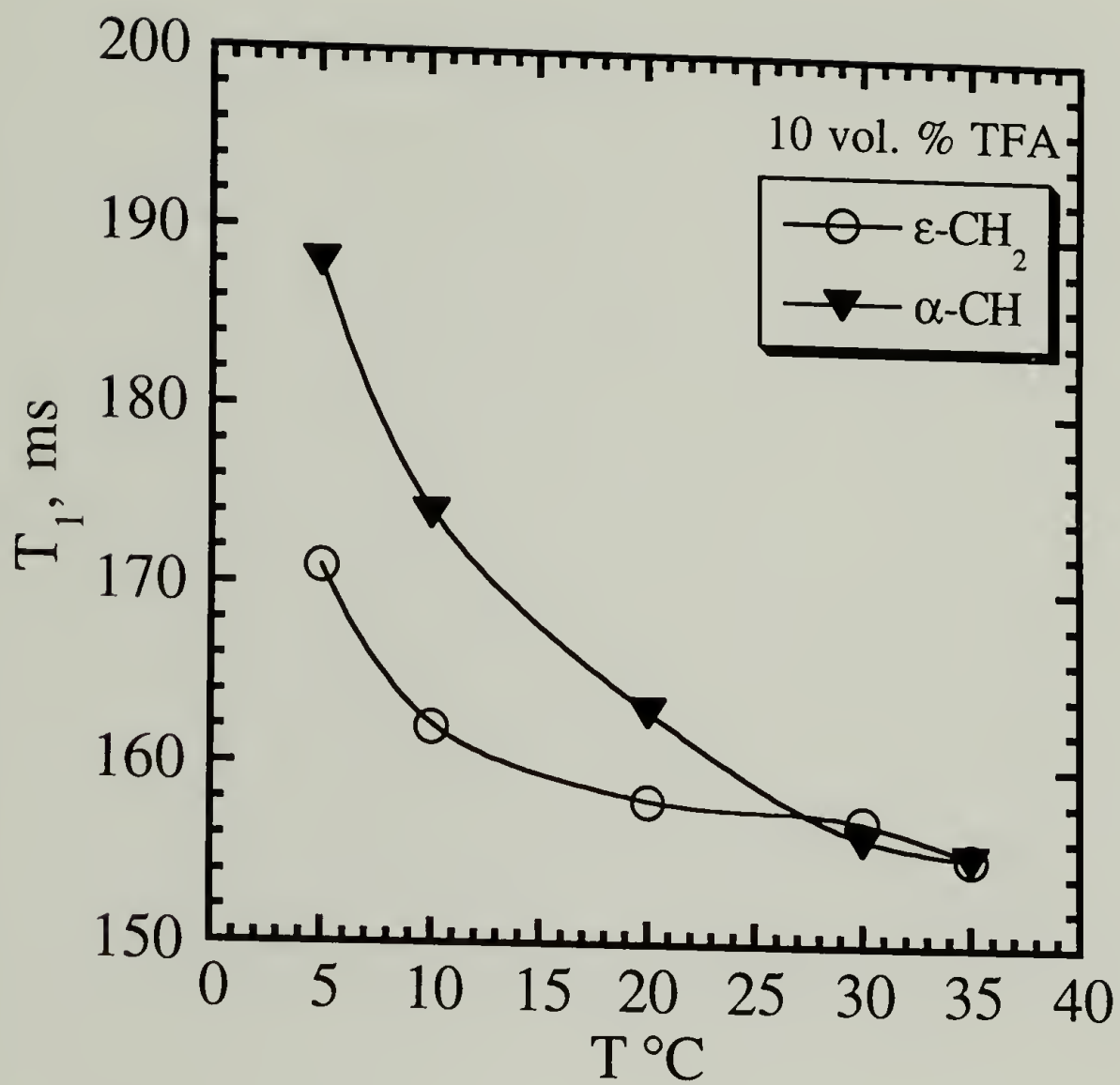


Figure 3.9 Temperature dependence of the proton spin - lattice relaxation times (T_1) of poly(L-lysine) chains in the PLD complex in deuterated chloroform - trifluoroacetic acid solutions with 10 volume per cent TFA; concentration of PLD solutions is 10 mg/ml.

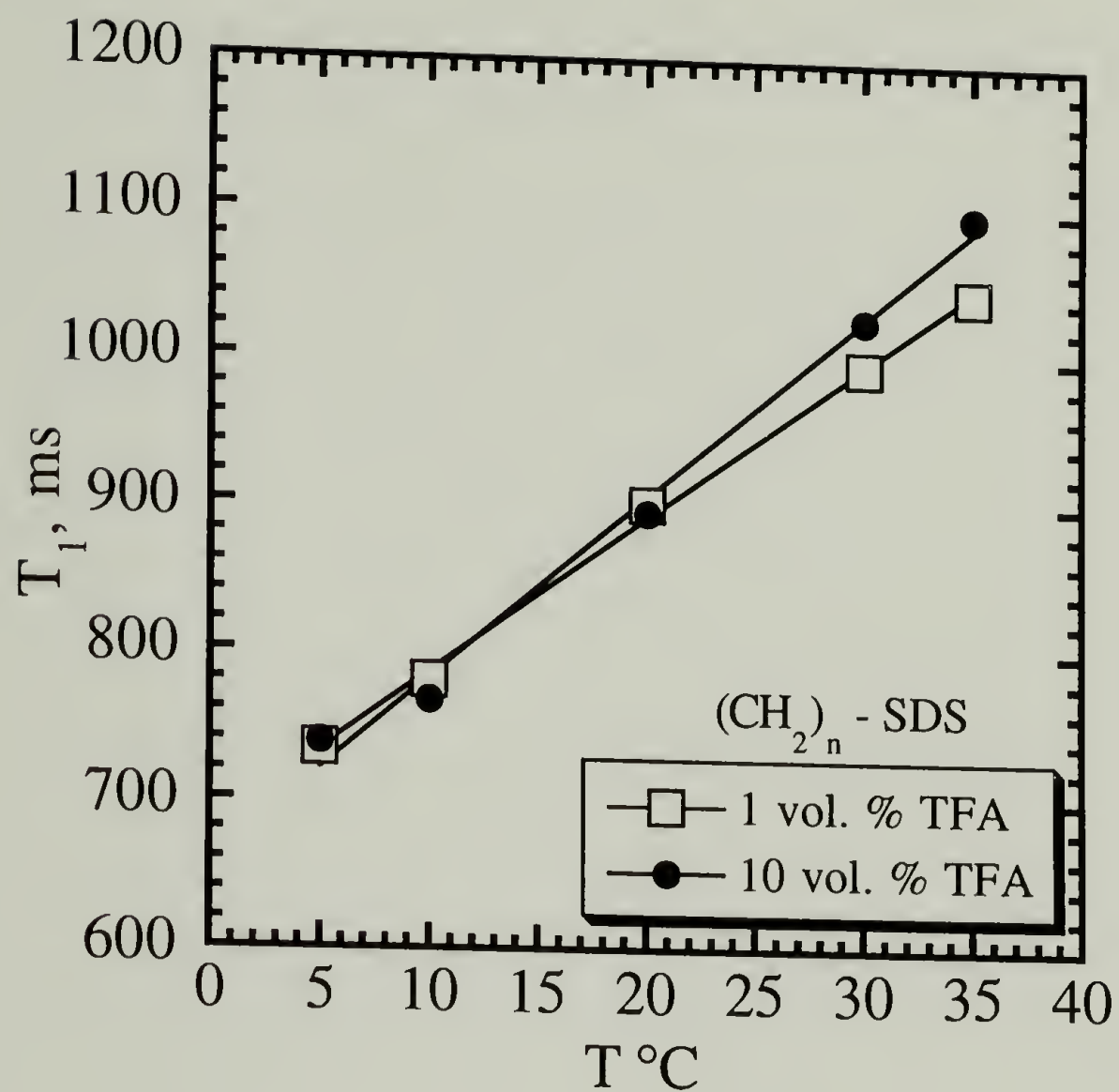


Figure 3.10 Temperature dependence of the proton spin - lattice relaxation times (T_1) of dodecyl sulfate chains in the PLD complex in deuterated chloroform - trifluoroacetic acid solutions with 1 and 10 volume per cent TFA; concentration of PLD solutions is 10 mg/ml.

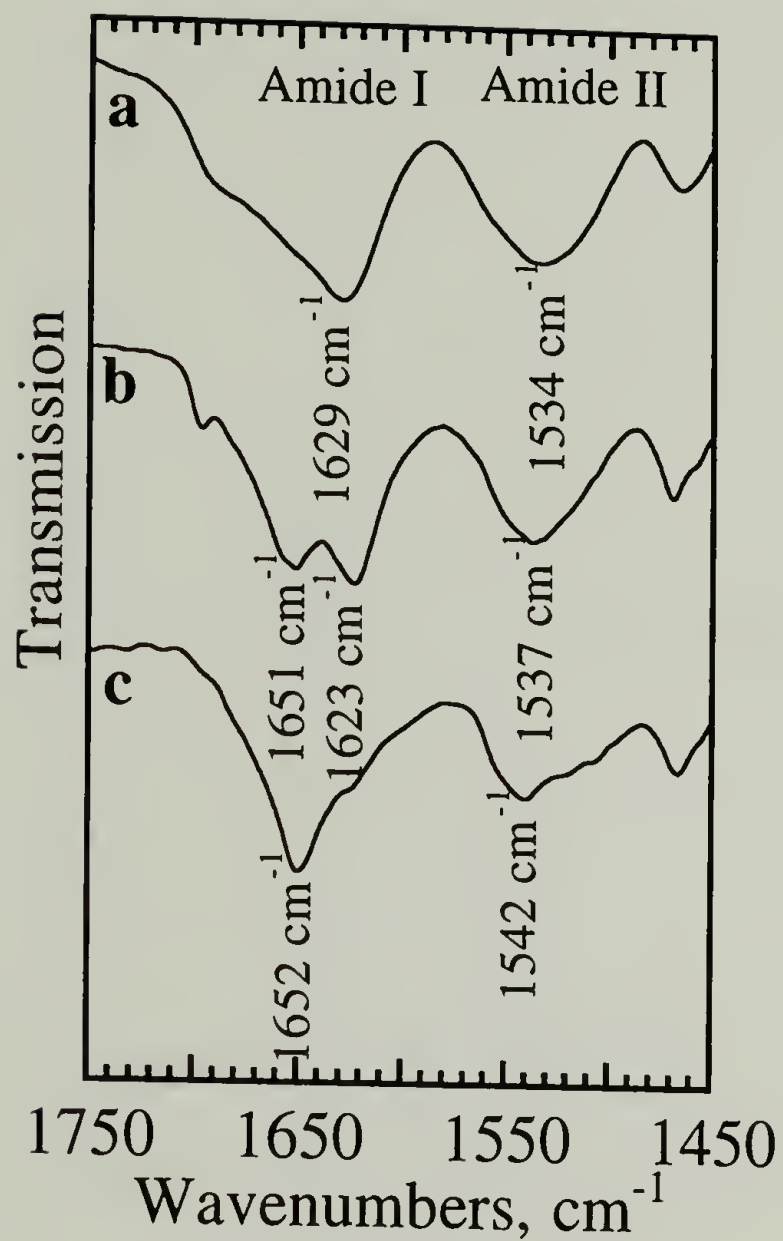


Figure 3.11 FTIR spectra of the PLD complex in the amide region: KBr pellet (a); films cast from chloroform solutions containing 1 volume per cent (b) and 10 volume per cent (c) trifluoroacetic acid.

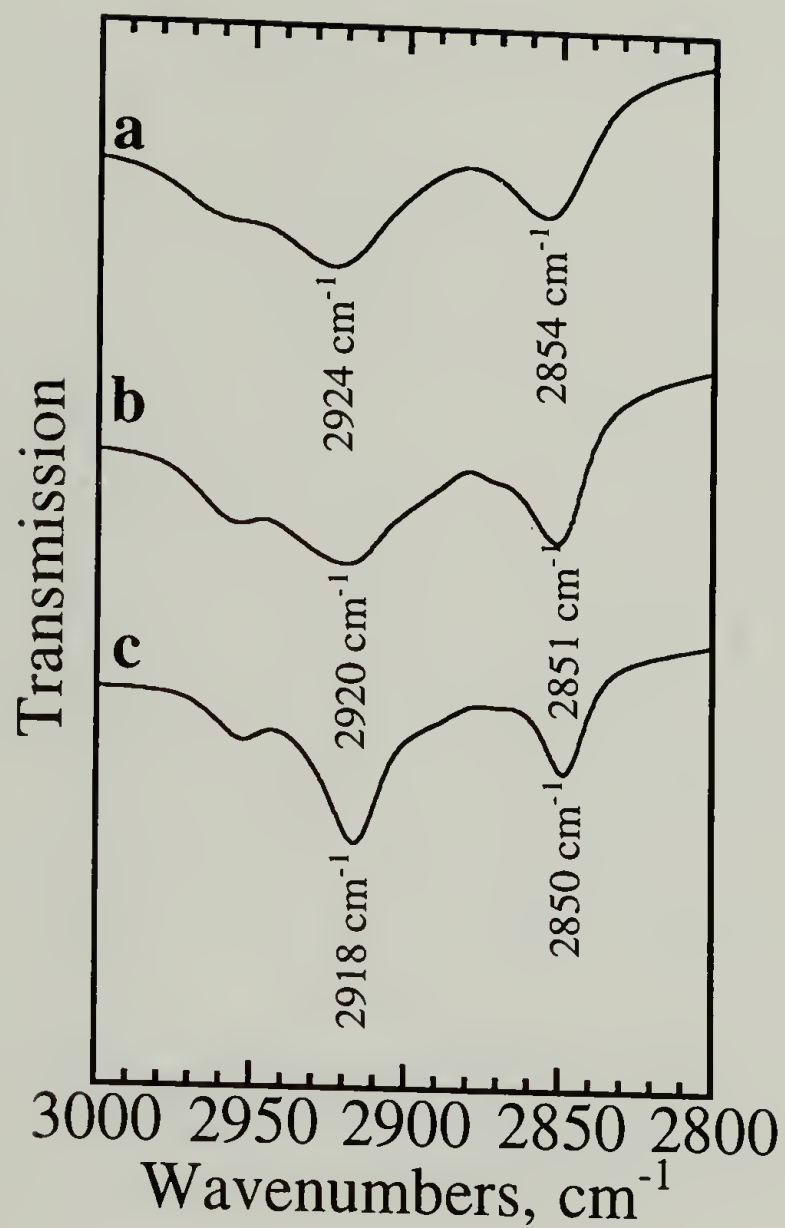


Figure 3.12 FTIR spectra of the PLD complex in the CH region: KBr pellet (a); films cast from chloroform solutions containing 1 volume per cent (b) and 10 volume per cent (c) trifluoroacetic acid.

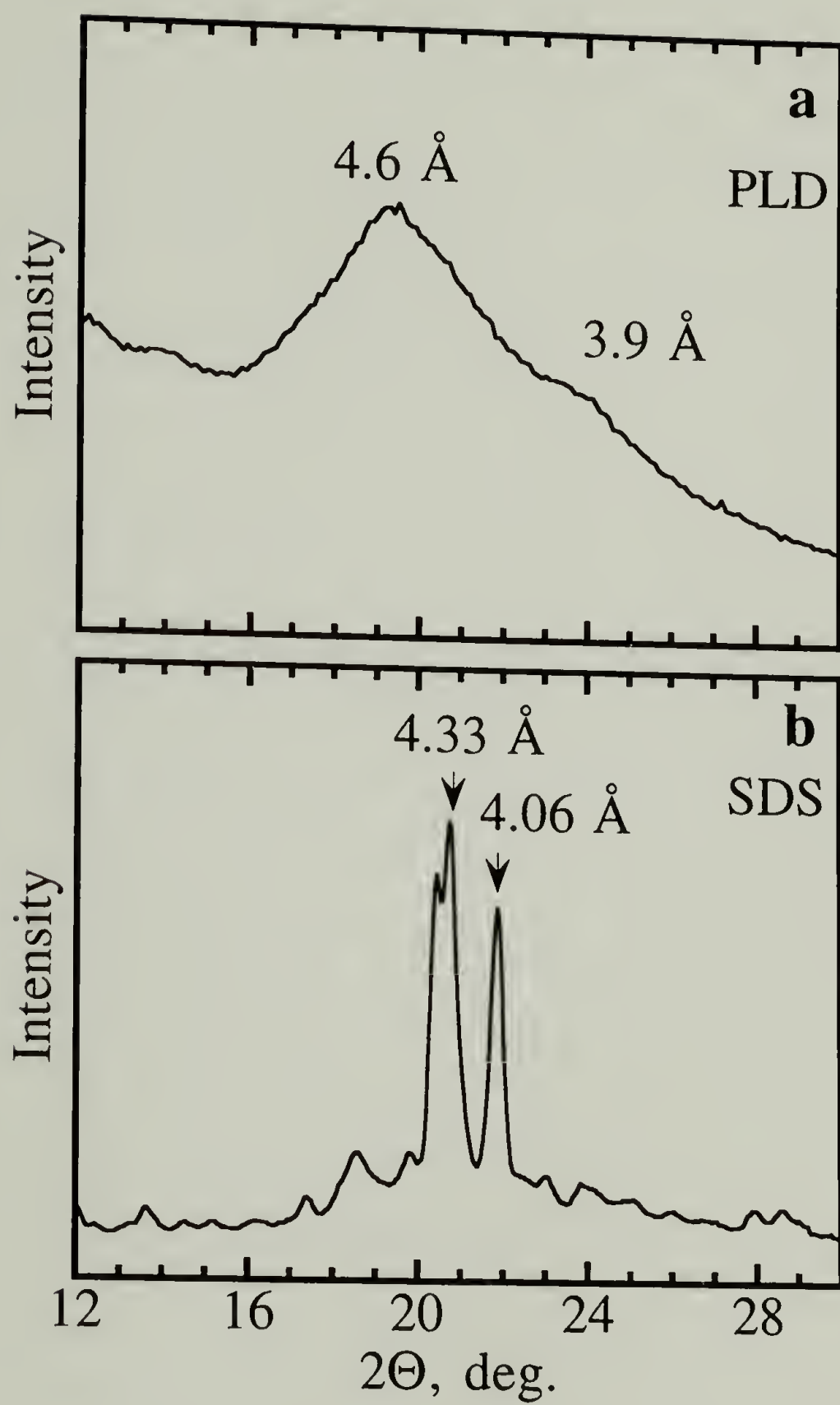


Figure 3.13 Wide angle X-ray diffractometer traces of the PLD (a) and SDS (b) powders; for the SDS sample, only the signals of highest intensity are marked.

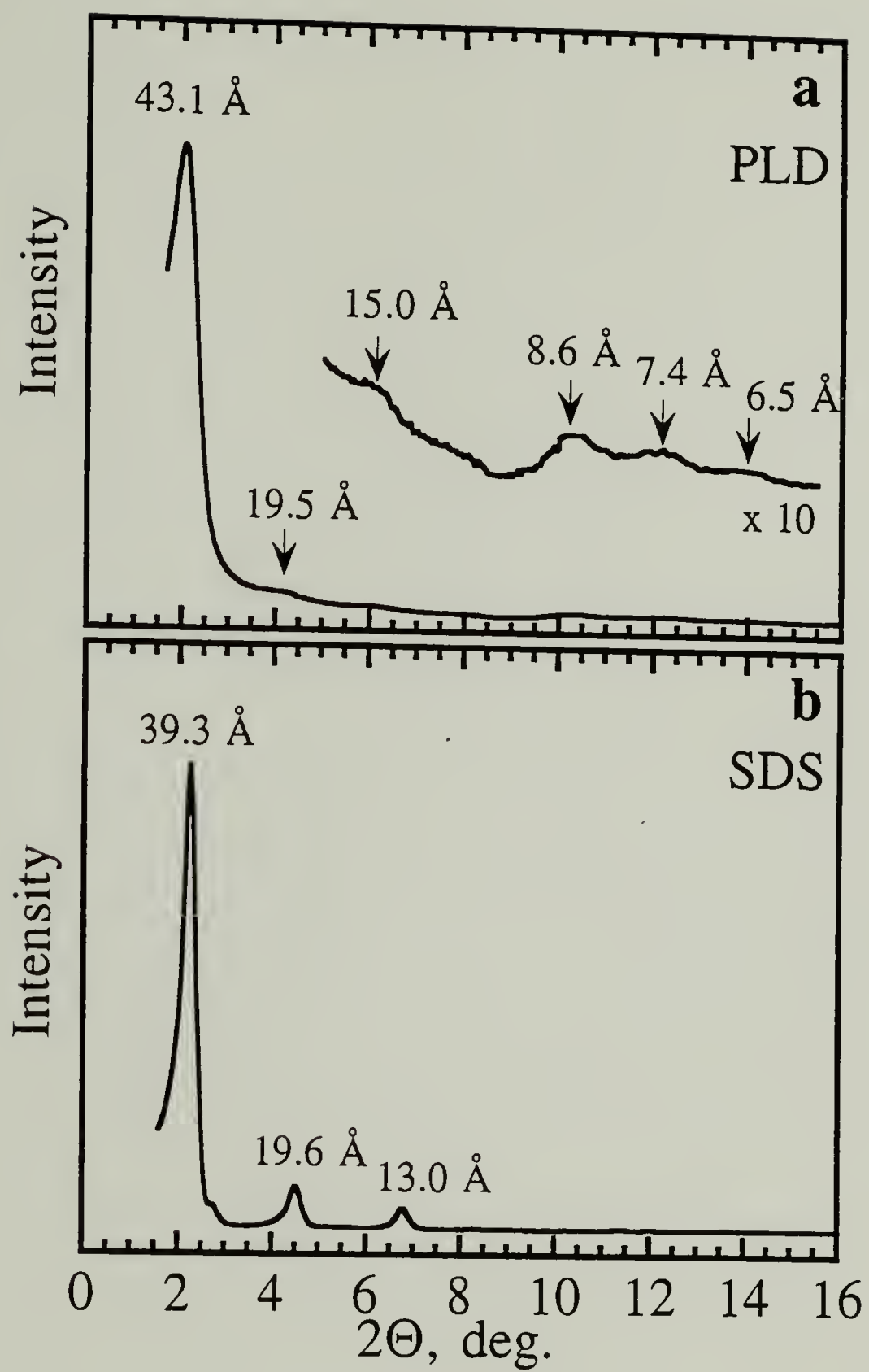


Figure 3.14 Small angle X-ray diffractometer traces of the PLD (a) and SDS (b) powders.

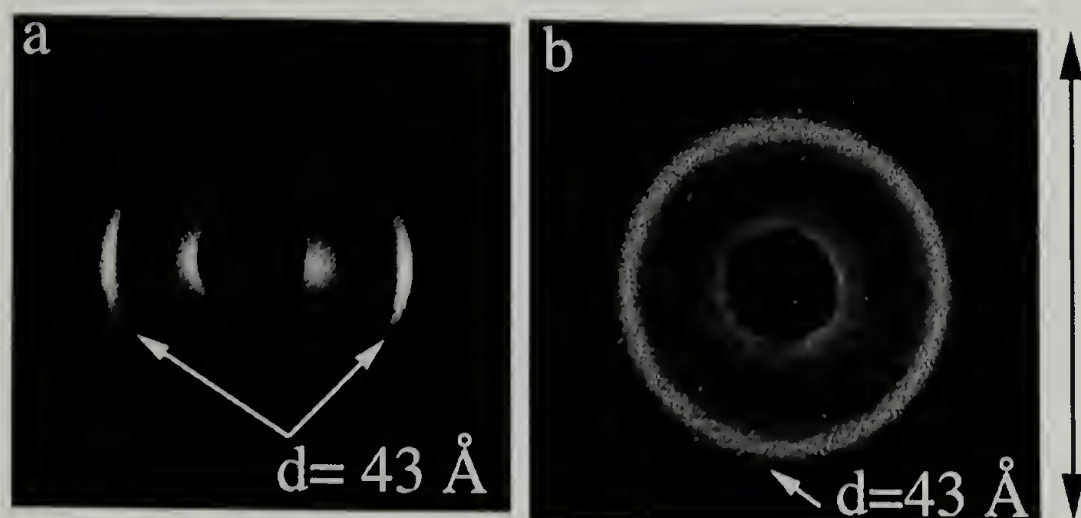


Figure 3.15 Small angle X-ray diffraction patterns of the PLD films cast from chloroform solutions containing 1 volume per cent (a) and 10 volume per cent (b) trifluoroacetic acid; X-ray beam was parallel to the plane of the films; arrow indicates the orientation of the film.

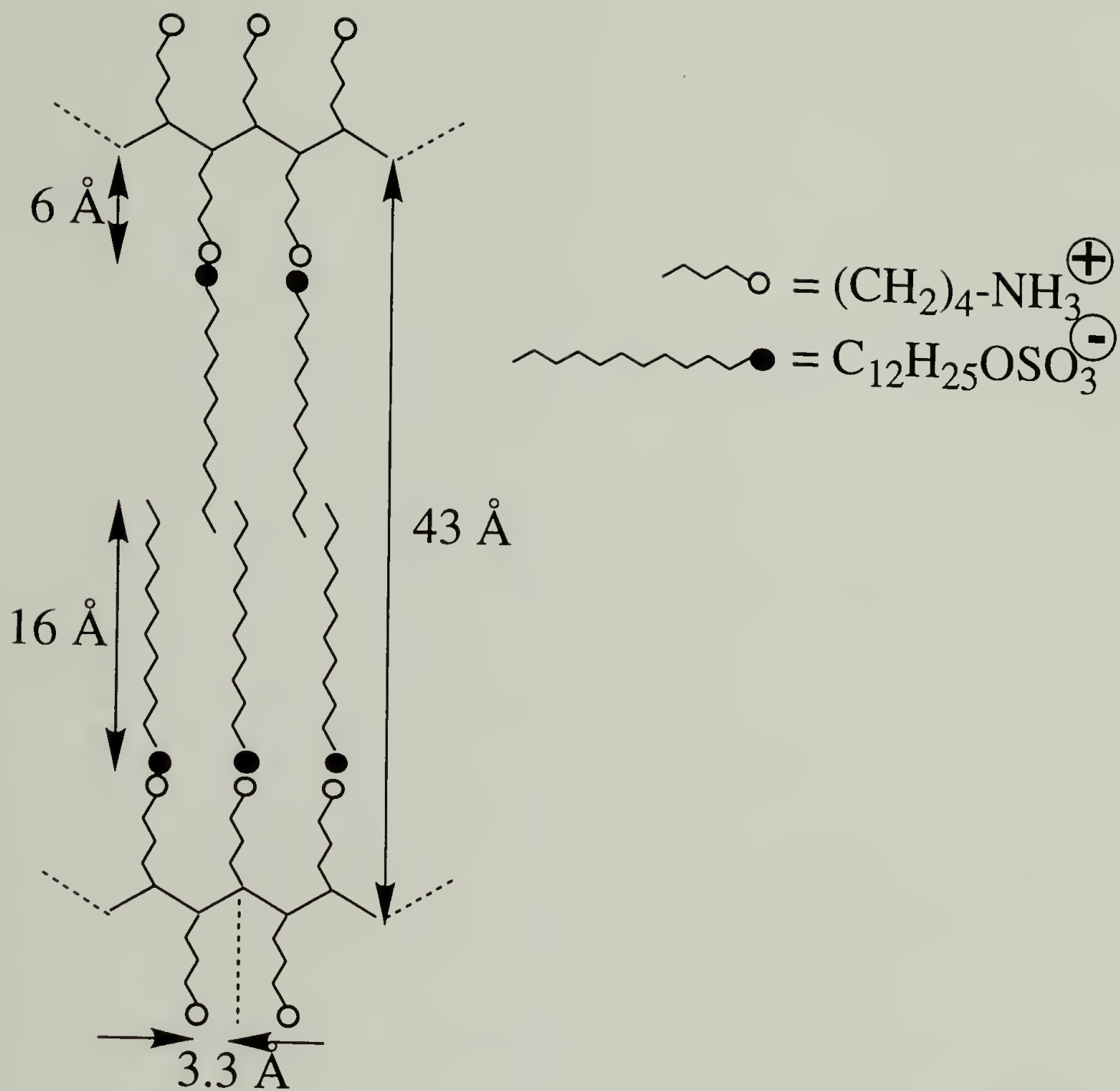


Figure 3.16 Schematic representation of the lamellar structure of the PLD complex in the β -sheet conformation.

Table 3.1

Solubility of the PLD and the PGX Complexes in Organic Solvents^a

solvent	ϵ	PLD solubility in the mixture with 2- 3 vol % TFA	PGX solubility
iso-octane	$\sim 1.95^b$	+	-
cyclohexane	2.02	+	-
carbon tetrachloride	2.23	+	
p-xylene	2.27	+	
benzene	2.28	+	
chloroform ^c	4.70	+	+
chlorobenzene	5.62	+	-
THF	7.32	-	-
1,1,2,2- tetrachloroethane	8.20	-	
benzyl alcohol	13.0	-	+
1-octanol	$\sim 15.0^d$	-	
acetone	20.7	-	
methanol	32.6	-	+
DMF	36.7	-	+
trifluoroacetic acid	39.0	-	-

^a Concentration of the PLD solutions was 10 mg/ml; for higher PLD concentrations, higher TFA contents were necessary to achieve dissolution of the complex

^b The ϵ value is reported for n-octane

^c PLD is soluble in chloroform - TFA mixtures with TFA content up to 80 vol. per cent

^d The ϵ value is reported for 1-hexanol

CHAPTER IV

WATER-INSOLUBLE COMPLEXES OF POLY(L-LYSINE) WITH MIXED ALKYL SULFATES: COMPOSITION-CONTROLLED SOLID-STATE STRUCTURES

Introduction

One of the attractive features of the stoichiometric polyelectrolyte - surfactant complexes is the simplicity of their synthesis, and thus by modifying the complex composition it may be possible to fine-tune the resulting structures. In this study, we attempt to control the solid state structures of the polypeptide - surfactant complexes by complexing the polypeptide chains with mixtures of oppositely charged surfactants of different chain lengths. We compare the resulting structures to those of the complexes with one type of surfactant and to structures of the covalent analogs of the complexes - copolypeptides with covalently attached side chains, i.e. copolyesters of poly(α ,L-glutamic acid). Copolyglutamates are usually synthesized from poly(γ -benzyl α ,L-glutamate) by ester interchange reactions with the corresponding alkyl alcohols¹⁻⁴ or by copolymerization of the N-carboxyanhydrides.⁵ Both procedures yield copolymers with random distributions of monomer chain units. Polypeptide - surfactant complexes of stoichiometric composition are prepared by mixing equimolar amounts of the two components in water.⁶⁻⁸ Hydrophobic self-association of the surfactants in water and the ability of electrostatically attached 'side chains' to move along the polypeptide chains may provide a unique way to synthesize 'block copolymers' of this type. The goal of this research is to investigate the distribution of surfactants of different chain lengths on the polypeptide chains and the effect of the content and distribution of electrostatically attached 'side chains' on the supramolecular structure of the polypeptide - surfactant complexes.

In this chapter we report a study of the complexes formed by poly(L-lysine) cations and mixed ethyl and octadecyl sulfates, as well as with mixed octyl and octadecyl

sulfates (Figure 4.1). For comparison with the complexes with mixed surfactants, we also prepared the complexes containing one type of each surfactant. The choice of the surfactant chain lengths and the compositions of the complexes were based on the following considerations: i. the structures of the analogous poly(γ -methyl L-glutamate-co-stearyl L-glutamate)s are well-studied,^{3,9,10} ii. the minimum octadecyl chain content required for crystallization in poly(γ -methyl L-glutamate-co-octadecyl-L-glutamate) is about 35 mole per cent, and⁹ iii. the octadecyl chains are known to crystallize in the complexes with polypeptides, while the ethyl and octyl chains are expected to be amorphous.⁶ Differences in the crystallization behavior of the surfactants of different chain lengths will allow us to distinguish their structures in the complexes.

Experimental Section

Materials

Poly(L-lysine) hydrobromide (PLys HBr) with viscosity average degree of polymerization (provided by the supplier) of about 1800 was purchased from Sigma Chemical Co. Anionic surfactants octadecyl sodium sulfate (ODSNa), octyl sodium sulfate (OSNa) (Research Plus), ethyl sodium sulfate (ESNa) (Pfaltz and Bauer, Inc.) were used as received. Isopropanol was purchased from Fisher Chemical Co.

Measurements

X-ray diffraction patterns of powder samples were recorded using an evacuated Statton X-ray camera. Ni-filtered $\text{CuK}\alpha$ radiation was used with wavelength $\lambda=1.5418$ Å. FTIR spectra were obtained using an IBM 30 series FTIR spectrometer. Transmission spectra were recorded by coadding 64 scans at 4 cm^{-1} resolution at room temperature. Samples were analyzed in the form of KBr pellets. DSC experiments were performed on a Perkin-Elmer DSC 7 system at scanning rates of 10 and 20 °C/min. The values of the melting temperatures and enthalpies were independent of the scanning rate.

The transitions were reproducible on heating and cooling; the transition temperatures and enthalpies were lower on the second heating, but remained constant on the second and third heatings. Transition temperatures and enthalpies were estimated based on the first heating scans.

Results and Discussion

Preparation of Complexes

PL-C₁₈ was prepared by mixing equimolar quantities of a 0.05 M solution of PLys HBr in water and a 0.003 M solution of ODSNa in water containing 35 volume per cent isopropanol.¹¹ After stirring the mixture for 1 hour, the resulting white precipitate was isolated by centrifugation, washed 2 times with water and dried in vacuum at 45 °C for 36 hours. Elemental analysis showed good agreement between the experimental and calculated contents of all elements corresponding to the stoichiometric composition (Table 4.1), indicating quantitative interaction between the polylysine chain units and the octadecyl sulfate ions.

To prepare the complex of poly(L-lysine) containing 20 per cent of ethyl and 80 per cent of octadecyl 'side chains' (PL-C₁₈ (20)-C₂), a mixture of 0.003 M ODSNa and 0.01 M ESNa solutions in water containing 35 volume per cent isopropanol was added to 0.05 M PLys HBr solution in water. The ratio of polypeptide chain units to ODSNa and OSNa in the mixture was 1:0.2:0.8, respectively. After stirring the mixture for one hour, the complex was isolated by centrifugation, washed with water and freeze-dried. Elemental analysis showed that the composition of the complex was essentially the same as that of the PL-C₁₈ complex (Table 4.1). This indicates that in the PLys HBr - ODSNa - OSNa water-isopropanol solutions, octadecyl sodium sulfate selectively interacts with poly(L-lysine) chains, forming the water-insoluble stoichiometric complex, while sodium ethyl sulfate and the unreacted polypeptide and/or water-soluble polypeptide - ethyl sulfate complex remain in the solution. The selective formation of the stoichiometric PL-

C₁₈ complex in these mixtures can be explained by differences in the hydrophobic driving forces for complexation with the longer and shorter chain surfactants.¹² The hydrophobic free energy contribution is considerably higher for octadecyl sulfate than for ethyl sulfate, thus making the polypeptide interaction with the longer chain surfactant more favorable than with the shorter chain surfactant. This effect correlates with the differences in the micellization behavior of the homologous surfactants in water, also driven by hydrophobic interactions: alkyl sulfates with chains longer than eight carbon atoms form micelles, while the lower homologs form considerably smaller aggregates.¹³

PL-C₈ was prepared by mixing equimolar quantities of 0.05 M of PLys HBr and 0.1 M OSNa solutions in water. The complex was isolated in the manner described for the PL-C₁₈ complex (*vide supra*). Elemental analysis showed that about 97 per cent of the polymer chain units were paired with surfactant ions (Table 4.2).

PL-C₁₈(20)-C₈ was prepared by adding a mixture of 0.003 M ODSNa and 0.01 M OSNa in water containing 35 volume per cent isopropanol to 0.05 M PLys HBr solution in water. The molar ratio of polypeptide chain units to ODSNa and OSNa in the mixture was 1:0.2:0.8, respectively. The complex was isolated in the manner described for the PL-C₁₈ complex (*vide supra*). Elemental analysis showed that 20 per cent of the polymer chain units were paired with octadecyl sulfate ions and about 75 per cent of the polymer chain units were paired with octyl sulfate ions¹⁴ (Table 4.3).

PL-C₁₈(10)-C₈ was prepared by adding a mixture of 0.003 M ODSNa and 0.02 M OSNa solutions in water containing 35 volume per cent isopropanol to 0.05 M PLys HBr aqueous solution. The molar ratio of polypeptide chain units to ODSNa and OSNa in the mixture was 1:0.1:0.9, respectively. The complex was isolated in the manner described for the PL-C₁₈ complex (*vide supra*). Elemental analysis showed that 10 per cent of the polymer chain units were paired with octadecyl sulfate ions and 85 per cent of the polymer chain units were paired with octyl sulfate ions¹⁴ (Table 4.4).

Surfactant Chain Organization at Room Temperature

Information about surfactant chain packing in the complexes is provided by wide angle X-ray diffraction (WAXD). The WAXD pattern of PL-C₁₈ is characterized by a sharp reflection corresponding to a Bragg spacing of 4.2 Å (Figure 4.2 a) (reflections with larger Bragg spacings on the WAXD patterns will be discussed below). The sharp reflection indicates that the surfactant chains are organized on a crystal lattice. A spacing of 4.2 Å has been previously assigned to the hexagonal packing of the alkyl chains in complexes of cross-linked polyacrylate anions and hexadecyltrimethyl ammonium cations¹⁵ and in complexes of poly(α ,L-glutamate) anions and octadecyl trimethylammonium cations,⁶ as well as for comb-like poly(alkyl methacrylate)s with twelve or more carbon atoms in the side chains.¹⁶

The WAXD pattern of the PL-C₈ complex is characterized by two reflections with Bragg spacings of 4.6 and 3.7 Å (Figure 4.2 b). These spacings are similar to those of the 010 and 100 reflections (4.3 - 4.4 and 3.9 - 4.0 Å, respectively) of the two-dimensional crystal lattices formed by alkane chains of ten or more carbon atoms in PALGs.¹⁷ These two-dimensional lattices are believed to resemble the subunit cell attained by the projection of the triclinic unit cell in crystals of low molecular weight n-alkanes. Reflections of greater breadth but with the same spacings have also been observed for the stoichiometric complex of poly(L-lysine) cations and dodecyl sulfate anions, and were assigned to short range order in the surfactant chain packing, based on the absence of thermal transitions of the complex observed by DSC.⁶

The WAXD pattern of PL-C₁₈(20)-C₈ is characterized by three reflections with Bragg spacings of 3.7, 4.2 and 4.6 Å (Figure 4.2 c). It is reasonable to assume that the 4.2 Å reflection corresponds to the hexagonal crystalline arrangement of the octadecyl chains, similar to that of the PL-C₁₈ complex, and the 3.7 and 4.6 Å reflections correspond to the two-dimensional alkane-type arrangement of the octyl chains, similar to that of the PL-C₈ complex.

The WAXD pattern of PL-C₁₈(10)-C₈ is identical to that of the PL-C₈ complex (Figure 4.2 b), suggesting that the packing of both the octadecyl and the octyl chains is similar to that in the PL-C₈ complex.

Thus, the WAXD data indicate that the arrangement of surfactant chains depends strongly on the composition of the complex. In the poly(L-lysine) complex with octyl 'side chains' only, and in the complex with 10 mole per cent octadecyl 'side chains', surfactant chains are organized on a two-dimensional alkane-type lattice. In the poly(L-lysine) complex with octadecyl 'side chains' only, surfactant chains crystallize on a hexagonal lattice. In the complex with 20 mole per cent of the octadecyl 'side chains', both types of the surfactant packing are observed.

An interesting observation about the conformational states of the 'side chains' can be made based on the FTIR spectra. In the PL-C₁₈ complex, the alkyl chains are in the all-trans conformation, as shown by the positions of the asymmetric and symmetric CH₂ stretching vibrations observed at 2919 cm⁻¹ and 2851 cm⁻¹, respectively¹⁸ (Figure 4.3 curve a). Similar positions of the CH₂ vibrational peaks are observed for the PL-C₁₈(20)-C₈ complex (Figure 4.3 curve b). This indicates that both octadecyl and octyl chains are in the all-trans conformations, despite the differences in their packing. In the PL-C₁₈(10)-C₈ and PL-C₈ complexes, the alkyl chains are in disordered conformations (asymmetric and symmetric CH₂ stretching vibrations observed at 2927 and 2856 cm⁻¹, respectively) (Figure 4.3 curves c and d).

Thermal Transitions

Figure 4.4 (curve a) shows a DSC thermogram of the PL-C₁₈ complex. The complex undergoes an endothermic first-order transition upon heating at 66 °C. No other transitions were observed in the temperature range 0 - 150 °C. This transition correlates with the disappearance of the 4.2 Å reflection on the WAXD pattern of the complex and the appearance of two reflections with Bragg spacings of 3.8 and 4.7 Å

(pattern not shown), similar to those of the PL-C₈ complex (Figure 4.2 b). These observations allow us to conclude that the transition corresponds to the transformation of hexagonal crystals of the octadecyl chains into a two-dimensional alkane-type arrangement, similar to that in the PL-C₈ complex.

For the PL-C₈ complex, no thermal transitions were observed by DSC, in the temperature range 0 - 100 °C. This correlates with the observation of disordered conformations of the surfactant chains by FTIR. It appears that the octyl 'side chains' are too short to crystallize at room temperature. The absence of 'side chain' crystallization has been reported previously for complexes of poly(α ,L-glutamate) anions with dodecyl and hexadecyl trimethylammonium cations,⁷ as well as for alkyl esters of poly(α ,L-glutamic acid) with chains shorter than ten carbon atoms.¹⁷ The presence of the relatively sharp reflections in the WAXD pattern of the complex can be attributed to short range two-dimensional positional order in the packing of octyl chains.

Figure 4.4 (curve b) shows a DSC thermogram of the PL-C₁₈(20)-C₈ complex. The complex undergoes an endothermic first order transition at 49 °C on heating. This transition correlates with the disappearance of the 4.2 Å reflection in the WAXD pattern of the complex and with a slight broadening of the 3.7 and 4.6 Å reflections (pattern not shown). The disappearance of the 4.2 Å reflection upon heating can be attributed to the transformation of hexagonal crystals of the octadecyl chains into a two-dimensional alkane-type arrangement, similar to that of the octyl chains in the PL-C₈ complex at room temperature. The two-dimensional order in packing of the octyl chains in the PL-C₁₈(20)-C₈ complex remains essentially unchanged. The fact that crystalline packing of the octadecyl chains in the PL-C₁₈(20)-C₈ complex is identical to that in the PL-C₁₈ complex, suggests that the octadecyl chains are arranged in blocks on the polypeptide chains.

PL-C₁₈(10)-C₈ does not undergo any thermal transitions in the temperature range -20 - 100 °C observed by DSC. This is consistent with the observation of the disordered

conformations of the surfactant chains by FTIR. The fact that the octadecyl sulfate chains do not form hexagonal crystallites in this complex (unlike in the PL-C₁₈ and PL-C₁₈(20)-C₈ complexes), suggests a random distribution of the octyl and octadecyl 'side chains' along the polypeptide chains. This conclusion is supported by the observation that the WAXD patterns of the PL-C₁₈(10)-C₈ and PL-C₈ complexes are identical.

The minimum octadecyl chain content required for crystallization in poly(γ -methyl L-glutamate-co-stearyl-L-glutamate)s with randomly distributed alkyl chains, determined based on the observation of the crystal melting transitions by DSC for a series of copolymers, is about 35 mole per cent.⁹ The considerably lower content of octadecyl chains required for crystallization in the complexes reported here (ca. 20 mole per cent) indicates the preference of the electrostatically bound 'side chains' of different lengths to separate into individual blocks. It is plausible that separation of octadecyl and octyl sulfates into individual blocks occurs in the process of their complexation with the polypeptide in water solutions, considering that no special annealing of the complexes in the solid state has been performed. One of the driving forces for phase-separation is the hydrophobic interaction of alkyl chains in water, which also promotes complexation. Screening of the alkyl chains from water would be more effective if surfactants with similar chain lengths are arranged in blocks. Arrangement of the octadecyl chains into blocks of sufficient size allows their crystallization in the solid state.

Thermal transitions of the PL-C₁₈ and PL-C₁₈(20)-C₈ complexes are characterized by relatively small differences in the transition half-width, enthalpy and entropy (Table 4.5). This allows us to conclude that the crystal sizes of the octadecyl chains in these two complexes are similar.

Comparison of the melting temperatures of the complexes and the analogous alkyl polyglutamates provides indirect information about the relative sizes of the octadecyl chain crystals in the complexes and in their covalent analogs. The melting temperature and enthalpy of the PL-C₁₈ crystallites are similar to those of the poly(γ -octadecyl α ,L-

glutamate) (PG-C₁₈) (Table 4.5). However, a decrease in the octadecyl chain content from 100 per cent to about 50 per cent in poly(γ -octadecyl α ,L-glutamate-co-methyl L-glutamate) (PG-C₁₈(52)-C₁) results in a decrease in the melting temperature from 62 to 35 °C. In the complexes studied herein, the five-fold decrease in the content of crystallizable 'side chains' (from PL-C₁₈ to PL-C₁₈(20)-C₈) leads to only a decrease in the melting temperature from 66 to 49 °C.

Supramolecular Structures

The structures of the complexes were investigated by small angle X-ray diffraction (SAXD). The SAXD patterns of all of the complexes consisted of one reflection of high intensity and multiple reflections of low intensity (Table 4.6). The ratio of the Bragg spacings of these reflections suggests lamellar structures for all of the complexes (Table 4.6). The long periods of the lamellae are independent of temperature in the range 20 - 100 °C. Lamellar organization was reported previously for stoichiometric complexes of synthetic polypeptides and oppositely charged surfactants, with lamellae believed to consist of alternating layers of polypeptide chains separated by layers of surfactant molecules.⁶⁻⁸

Based on the values of the lamellar long periods, we conclude that two types of lamellar organization are present, depending on the composition of the complex. Lamellae with a long period of 40-42 Å are formed by the complexes with hexagonal crystalline arrangement of the 'side chains' (PL-C₁₈ and PL-C₁₈(20)-C₈, respectively), and lamellae with a long period of 35 Å are formed by the complexes with 'side chains' in the disordered conformation (PL-C₁₈(10)-C₈ and PL-C₈).

Poly(L-lysine) chains in all complexes are predominantly in the β -sheet conformation, as shown by the positions of the amide I and amide II vibrations in the FTIR spectra¹⁹ (observed at 1628 - 1630 and 1532 - 1534 cm⁻¹, respectively, spectra not shown). The thickness of the poly(L-lysine) layers in the β -sheet conformation is about

16 Å.²⁰ The length of fully extended octadecyl chains is about 21 Å. Thus, the experimental value of the long period of the PL-C₁₈ lamellae (42 Å) suggests that the surfactant tails bound to the polypeptide chains lying in adjacent layers should be either interdigitated and perpendicular to the lamellar surface, or tilted with respect to the layers without being interdigitated. The fact that the long periods of the PL-C₁₈ and PL-C₁₈(20)-C₈ lamellae are nearly identical, and the crystalline packing of the octadecyl chains is the same in both complexes, suggests that the octadecyl chains in both complexes are interdigitated (Figure 4.5 a and b); tilted octadecyl chains in the PL-C₁₈(20)-C₈ lamellae would result in large unfavorable gaps between the blocks of the longer and shorter chains.

The length of the fully extended octyl chains is about 9 Å. Based on the value of the lamellar long period of 35 Å and on the thickness of the poly(L-lysine) layers of about 16 Å,²⁰ we conclude that surfactant chains in the complex are not interdigitated (Figure 4.5 d). The observation that the long periods of the PL-C₈ and PL-C₁₈(10)-C₈ lamellae are identical, confirms our conclusion about the random distribution of the octyl and octadecyl chains in the PL-C₁₈(10)-C₈ complex.

Conclusions

We have shown that the solid state structures of the water-insoluble complexes formed by poly(L-lysine) cations and alkyl sulfate anions can be controlled by altering the composition of the complex.

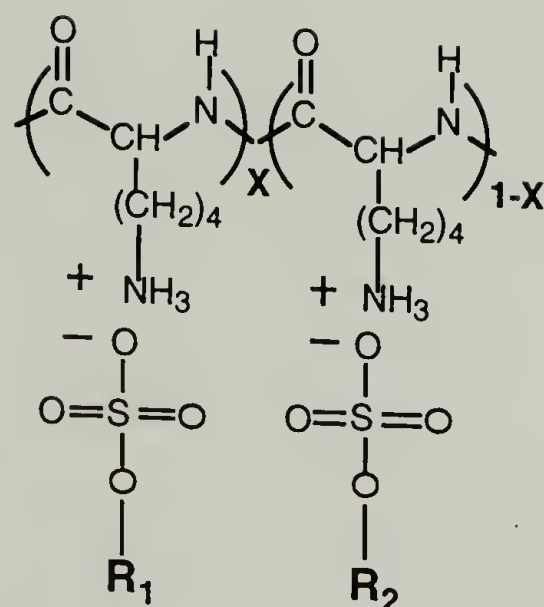
Organization of the surfactant chains in the complexes is governed by the surfactant chain length and the complex composition. In complexes with a single surfactant, octadecyl chains crystallize on a hexagonal lattice, and octyl chains organize on a two-dimensional alkane-type lattice with short range order. In the complexes with mixed surfactants, incorporation of 20 mole per cent octadecyl sulfate and about 75 mole per cent octyl sulfate results in the formation of blocks of the longer chain surfactant with

hexagonal crystalline packing in a 'sea' of octyl sulfate chains in a two-dimensional alkane-type arrangement. Decreasing the octadecyl sulfate content in the complexes to 10 mole per cent results in a random distribution of the surfactant chains with short-range two-dimensional ordered packing of both surfactants.

All of the complexes adopt lamellar structures consisting of alternating layers of polypeptide chains in the β -sheet conformation and layers of surfactants. The lamellar structures are governed by the surfactant organization. The complex with the hexagonal crystalline arrangement of surfactant chains (PL-C₁₈) forms lamellae with interdigitated surfactant chains. The complex with the two-dimensional alkane-type organization of surfactant chains (PL-C₈) forms lamellae with surfactant chains arranged tail to tail. The PL-C₁₈(20)-C₈ complex with the blocky distribution of surfactant chains forms lamellae with interdigitated octadecyl chains and octyl chains arranged tail to tail. The long period of these lamellae is similar to that of the PL-C₁₈ complex. The complex with randomly distributed surfactant chains (PL-C₁₈(10)-C₈) forms lamellae with the long period identical to that of the PL-C₈ complex.

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$$\text{R}_1 = \text{C}_{18}\text{H}_{37}$$

$$\text{R}_2 = \text{C}_8\text{H}_{17} \text{ or } \text{C}_2\text{H}_5$$

$$\text{X} = 0 \text{ (PL-C}_8\text{)}$$

$$0.1 \text{ (PL-C}_{18}(10)\text{-C}_8\text{)}$$

$$0.2 \text{ (PL-C}_{18}(20)\text{-C}_8 \text{ or PL-C}_{18}(20)\text{-C}_2\text{)}$$

$$1.0 \text{ (PL-C}_{18}\text{)}$$

Figure 4.1 Chemical structures of the complexes formed by poly(L-lysine) and mixed alkyl sulfates.

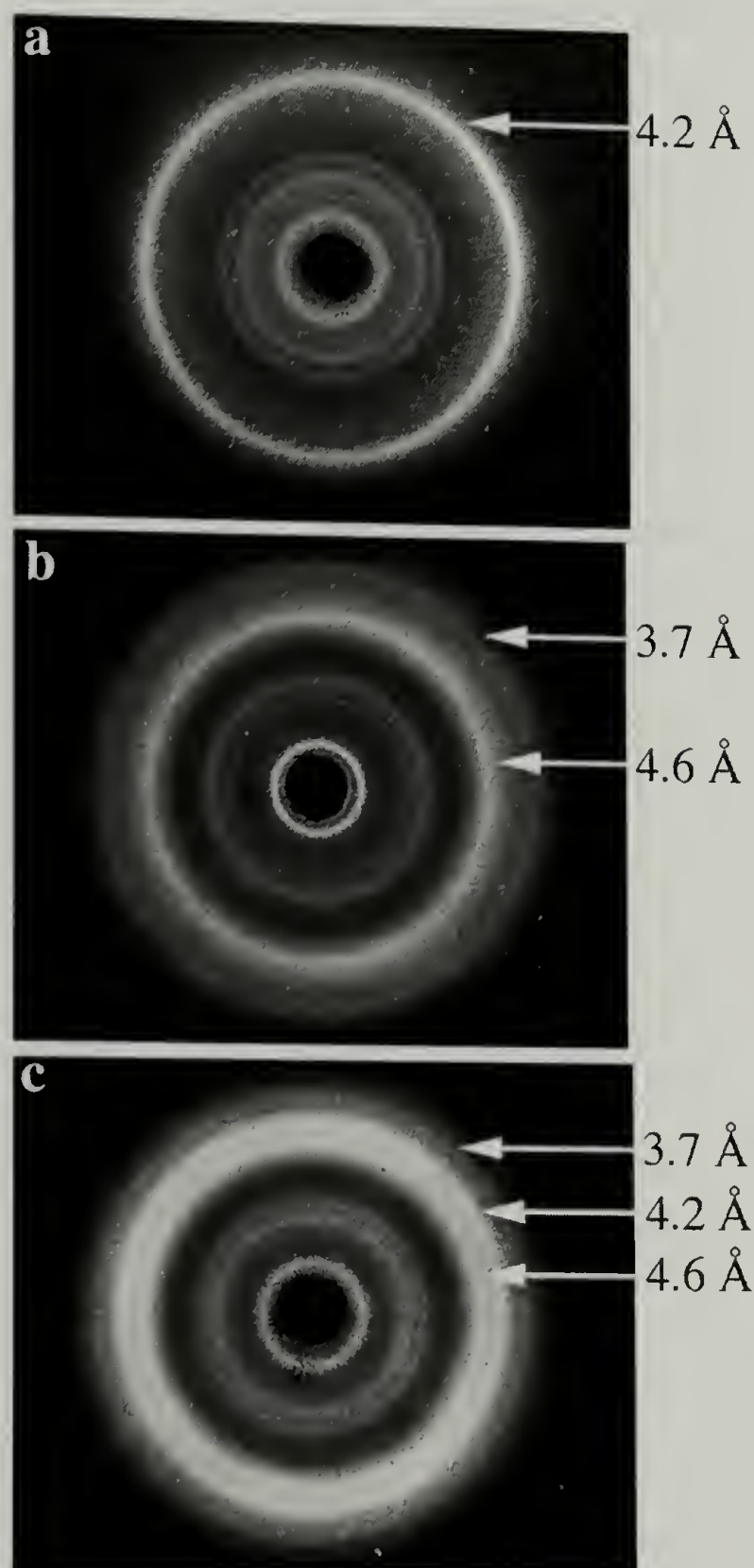


Figure 4.2 Wide angle X-ray diffraction patterns of PL-C₁₈ (a), PL-C₈ (b) and PL-C₁₈(20)-C₈ (c) complexes at room temperatures.

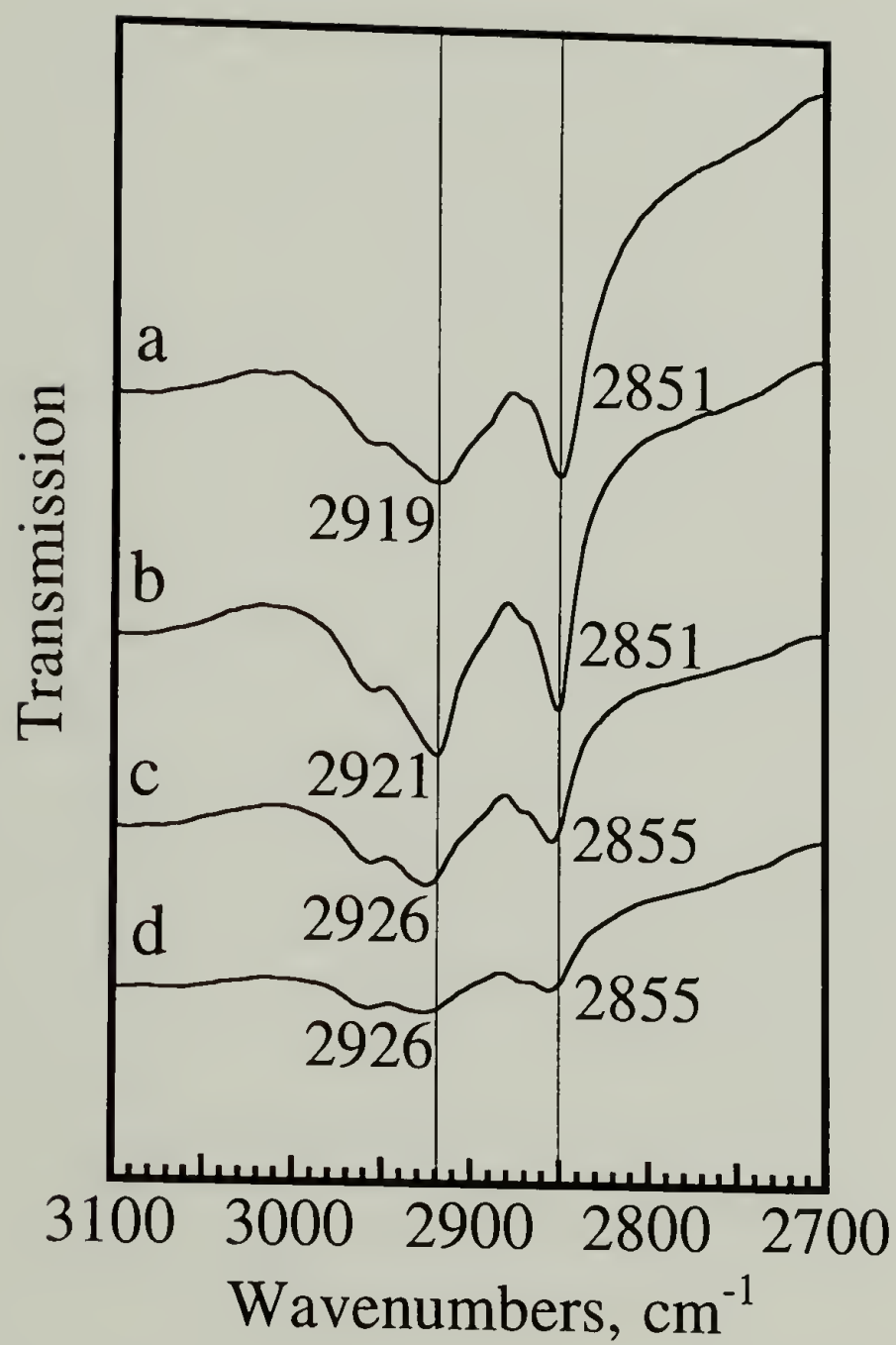


Figure 4.3 FTIR spectra of the PL-C₁₈ (a), PL-C₁₈(20)-C₈ (b), PL-C₁₈(10)-C₈ (c) and PL-C₈ (d) complexes.

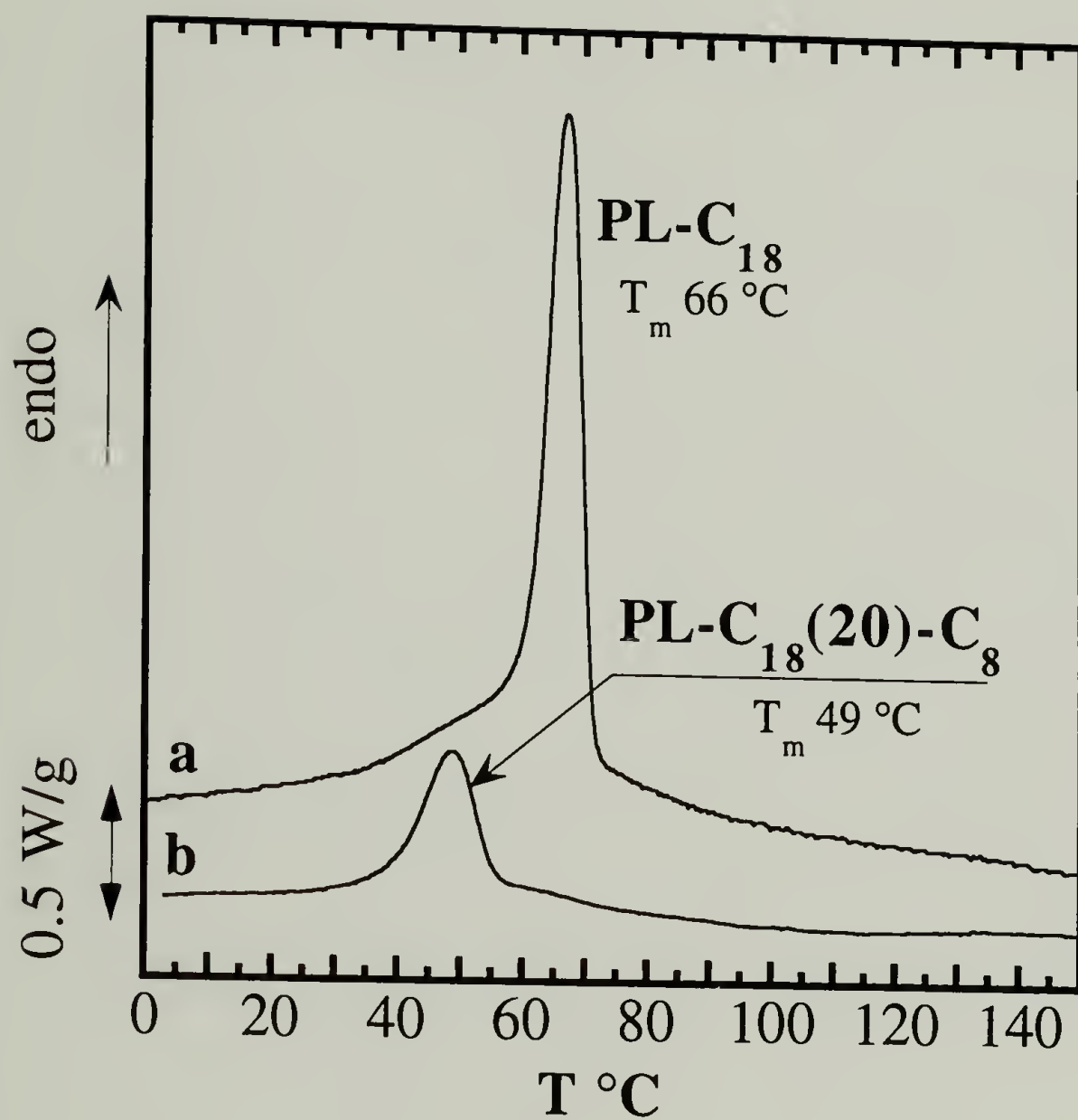


Figure 4.4 DSC thermograms of the PL-C₁₈ (a) and PL-C₁₈(20)-C₈ (b) complexes on heating.

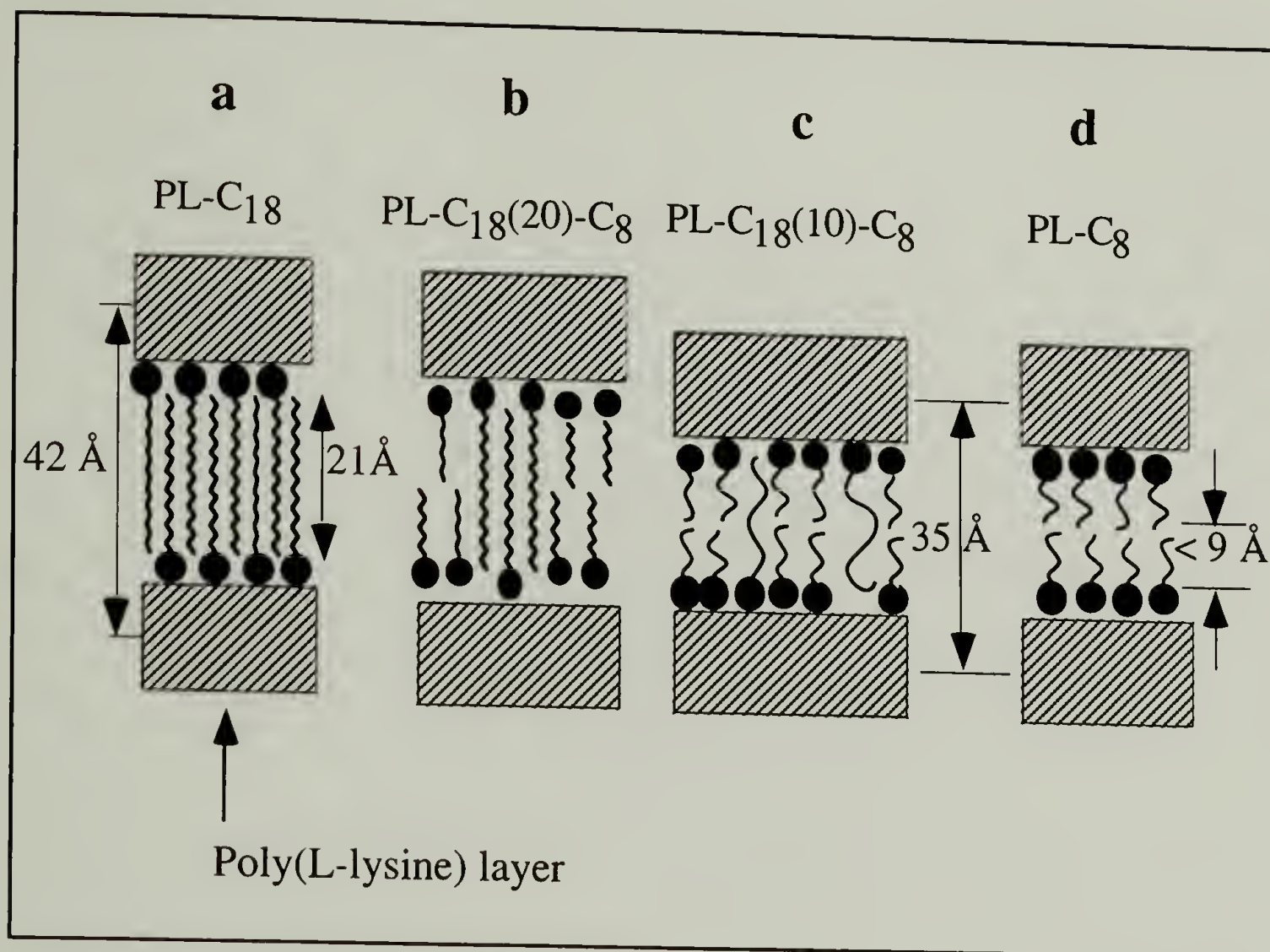


Figure 4.5 Proposed scheme of the arrangement of the surfactant molecules within the lamellae in the complexes formed by poly(L-lysine) and mixed alkyl sulfates.

Table 4.1
Elemental Analysis Results for the PL-C₁₈ and PL-C₁₈(20)-C₂ Complexes

Element	PL-C ₁₈		PL-C ₁₈ (20)-C ₂	
	Theory ^a	Exper.	Theory ^b	Exper.
C	60.25	59.43	44.94	59.49
H	10.46	10.43	8.16	10.41
N	5.86	5.81	9.36	5.81
S	6.69	6.57	10.70	6.02
Na	0	<0.1	0	<0.1
Br	0	<0.1	0	<0.1

^a[Lys]/[ODS]=1:1

^b[Lys]/[ODS]/[ES]=1:0.2:0.8

Table 4.2
Elemental Analysis Results for the PL-C8 Complex

Element	Theory ^a	Theory ^b	Exper.
C	49.56	49.43	47.9
H	8.88	8.83	9.00
N	8.28	8.38	8.05
S	9.47	9.29	9.12
Na	0	0	<0.1
Br	0	0.72	0.63

^a[Lys]/[OS]=1:1

^b[Lys]/[OS]=1:0.97

Table 4.3
Elemental Analysis Results for the PL-C₁₈(20)-C₈ Complex

Element	Theory ^a	Theory ^b	Exper.
C	52.60	51.70	51.70
H	9.04	9.17	9.02
N	7.67	7.30	7.31
S	8.77	8.30	8.18
Na	0	0	<0.1
Br	0	1.56	1.60

^a[Lys]/[ODS]/[OS]=1:0.2:0.8

^b[Lys]/[ODS]/[OS]=1:0.2:0.75

Table 4.4
Elemental Analysis Results for the PL-C₁₈(10)-C₈ Complex

Element	Theory ^a	Theory ^b	Exper.
C	51.14	50.69	50.59
H	8.81	9.01	8.95
N	7.95	8.10	7.83
S	9.09	8.80	8.89
Na	0	0	<0.1
Br	0	1.15	1.10

^a[Lys]/[ODS]/[OS]=1:0.1:0.9

^b[Lys]/[ODS]/[OS]=1:0.1:0.85

Table 4.5

DSC Data for the Complexes and the Analogous Alkyl Polyglutamates

Complex	T _m °C	ΔH _m	ΔS _m
		Kcal/Mol-C ₁₈ H ₃₇	cal/(Mol-C ₁₈ H ₃₇ °K)
PL-C ₁₈	66	8.1	24
PL-C ₁₈ (20)-C ₈	49	5.8	19
PG-C ₁₈ ^a	62 ¹⁶	7.3 ¹⁶	
PG-C ₁₈ (52)-C ₁ ^b	35 ⁴		

^apoly(γ-octadecyl α,L-glutamate) is abbreviated as PG-C₁₈.

^bpoly(γ-octadecyl α,L glutamate-co-methyl L-glutamate) with 52 per cent chain units with octadecy side chains and 48 per cent chain units with methyl side chains is abbreviated as PG-C₁₈(52)-C₁.

Table 4.6

X-Ray Diffraction Spacings (\AA) Related to the Lamellar Organization of the Complexes

PL-C ₁₈	PL-C ₁₈ (20)-C ₈	PL-C ₁₈ (10)-C ₈	PL-C ₈	<i>hkl</i>
42.0 vs	40.0 vs	35.0 vs	35.0 vs	100
-	-	16.8 vw	17.3 vw	200
14.4 vw	14.4 vw	12.1 vw	11.9 vw	300
-	-	-	-	400
8.3 vw	8.3 vw	7.0 vw	7.1 vw	500
7.0 vw	7.0 vw	-	-	600

Visual estimates of intensities are denoted as vs (very strong) and vw (very weak).

CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

Summary

In this work, we synthesized and studied novel water-insoluble complexes formed by synthetic polypeptides, sodium poly(α ,L-glutamate) and poly(L-lysine) hydrobromide, and oppositely charged low molecular weight surfactants with alkyl chains. Complexes of nearly stoichiometric compositions were prepared by mixing equimolar amounts of the components in water. The complexes were viewed as a new type of comb-shaped polymers, in which every polymer chain unit had an electrostatically bound 'side chain'. The goal of the research was to understand the influence of the electrostatically bound 'side chains' on properties of polypeptide chains (solubility and conformation) and the effect of polymer chains on organization of the complexed surfactants. For this purpose, we compared the behavior of the complexes to that of their covalent analogs, alkyl esters of poly(α ,L-glutamic acid) and acyl derivatives of poly(L-lysine).

Investigation of the solution and solid state properties of the stoichiometric polypeptide - surfactant complexes has shown that there are many similarities in their behavior to that of their covalent analogs.

First, the water-insoluble complexes can be soluble in organic solvents, similar to polypeptides with covalently attached side chains.¹ However, due to the presence of polar groups in every chain unit, the range of the solvents for the complexes is greatly reduced, compared to that of their covalent analogs. Dilute solution studies of the poly(L-lysine) - dodecyl sulfate complex in chloroform - trifluoroacetic acid mixtures indicated that the complex does not dissociate into individual components.

Second, the polypeptide chains in the complexes can adopt ordered secondary structures, similar to other synthetic polypeptides,¹ despite the presence of dipole - dipole

interactions. Poly(α ,L-glutamate) chains in the complexes adopt α -helical conformation in the solid state at room temperature. Poly(L-lysine) chains in the complexes adopt either the β -sheet conformation in the solid state (as isolated after synthesis) or the α -helical conformation (in solid films cast from chloroform - trifluoroacetic acid solutions).

Third, the transitions from ordered to disordered conformations of the polypeptide chains in the complexes can be controlled by temperature and/or solvents capable of breaking hydrogen bonds, similar to other synthetic polypeptides.¹ Poly(α ,L-glutamate)-based complexes undergo a reversible helix - coil transition in the solid state at elevated temperatures. The poly(L-lysine) - dodecyl sulfate complex undergoes a helix - coil transformation in chloroform - trifluoroacetic acid solutions upon increase of trifluoroacetic acid content. In both cases, the stability of the ordered conformations of the polypeptides in the complexes is significantly lower than that in their covalent analogs, probably due to the presence of dipole - dipole interactions in the complexes, which destabilize ordered state.

Organization of the surfactant chains in the solid complexes is governed by the surfactant chain length. Shorter chains (eight - sixteen carbon atoms) are typically packed with a short range order, while the longer chains (eighteen carbon atoms) crystallize on a hexagonal lattice. The minimum chain length required for crystallization in complexes is higher than that in polypeptides with covalently attached side chains (ten for poly(γ -alkyl α ,L-glutamates)²), and the surfactant crystallites in the complexes are significantly smaller than those of the corresponding uncomplexed surfactants.

In complexes with mixtures of surfactants with two different chain lengths, surfactant organization depends on the composition. In the poly(L-lysine) complex with 20 mole per cent octadecyl sulfate and about 75 mole per cent octyl sulfate, the longer chain surfactant is organized in blocks with hexagonal crystalline order. In the complex with only 10 molar per cent octadecyl sulfate and about 85 mole per cent octyl sulfate, all 'side chains' are distributed randomly and packed with short range order.

All complexes studied are organized in lamellar structures consisting of alternating layers of polypeptide chains separated by layers of surfactants, similar to complexes of conventional synthetic polyelectrolytes and oppositely charged surfactants.³⁻⁹ The lamellar structures are not subservient to the state of surfactant chains, as in the alkyl esters of poly(α ,L-glutamic acid), where hexagonal packing of α -helices is observed if the side chains are crystalline and lamellar packing dominates only if side chains are disordered.²

Future Work

Stoichiometric polypeptide - surfactant complexes such as those studied herein possess several attractive features. First, they combine the amphiphilic properties of small molecules with the highly ordered secondary structures of polypeptides. Second, the simple synthesis of these complexes provides a means to easily change their components and to tune the resulting structures to specific technological applications. To utilize the potential of the complexes, we propose the following studies.

We suggest, first, an investigation of concentrated solutions of the stoichiometric polypeptide - surfactant complexes in organic solvents. This study is motivated by the following considerations. Rod-like polypeptides with covalently attached flexible side chains are known to form liquid crystalline solutions;¹ however, the concentrated solution properties of rod-like polypeptides with electrostatically attached 'side chains' are unknown. If liquid crystalline mesophases are found in such complexes, a wide variety of phases with adjustable properties (e.g., transition temperatures) can be generated by changing the components of the complexes. A promising candidate for study is the complex of poly(L-lysine) with a double chain surfactant, sodium bis(2-ethylhexyl) sulfosuccinate (aerosol OT), which has been shown to adopt an α -helical conformation in the solid state, according to our preliminary infrared spectroscopic experiments, and is soluble in such solvents as chloroform and hexane.

We propose, second, an investigation of the complexes formed by synthetic polypeptides and oppositely charged small molecules with mesogenic groups. Complexes of conventional synthetic polyelectrolytes and oppositely charged mesogen-containing amphiphiles have been shown to form liquid crystalline mesophases in solutions and in melts.¹⁰⁻¹² Such a study would elucidate the interplay of two factors: ordering of the 'side chains' and the tendency of polypeptide chains to adopt ordered secondary structures. This research would be expected to answer a question of what kinds of new structures can be formed if both the polymer chains and the side chains are capable of forming ordered mesophases.

In the third part of research, we suggest an investigation of blends of polypeptide - surfactant complexes with flexible chain polymers. Rod-like polymer chains are commonly used to reinforce flexible chain polymer matrices.¹³ Rod-like and flexible polymer chains are generally macroscopically phase-separated,¹⁴ but flexible side chains covalently attached to rod-like polymers are known to promote miscibility.¹³ Owing to their amphiphilic properties, rod-like polypeptides with electrostatically attached flexible 'side chains' may be promising candidates for blends with a variety of flexible chain polymers and molecular composites.

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