Stoichiometric Complexes of Synthetic Polypeptides and Oppositely Charged Surfactants in Organic Solvents and in the Solid State

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ABSTRACT: Properties of the stoichiometric complex formed by poly(L-lysine) cations and dodecyl sulfate anions in organic solvents and in the solid state were investigated via viscometry, ^{1}H NMR, infrared, circular dichroism, and X-ray diffraction techniques. In dilute solutions in chloroform with up to 10 vol % of trifluoroacetic acid, the complex neither forms interchain aggregates nor dissociates to yield free surfactant. In chloroform solutions containing small amounts of trifluoroacetic acid (1−2 vol %), the polypeptide chains are in an α-helical conformation, while a transition to a disordered form occurs at higher trifluoroacetic acid contents (4−6 vol %). The helix−coil transition of the poly(L-lysine) chains is accompanied by a decrease in the ^{1}H spin−lattice relaxation times of the polypeptide chain segments, while the relaxation times of the surfactant chains remain essentially unchanged. In chloroform−trifluoroacetic acid mixtures, the α-helical conformation of the poly(L-lysine) chains is stabilized by increasing temperature. Polypeptide chains in the solid complex can adopt either α-helical or β-sheet conformations, depending on the trifluoroacetic acid content of the chloroform solution used for film casting. The solid complex is organized into a lamellar structure consisting of alternating layers of poly(L-lysine) chains and double layers of surfactant, arranged tail to tail.

Introduction

Complexes consisting of polyelectrolytes and oppositely charged surfactants form spontaneously if aqueous solutions of the two components are mixed.[1−3] The driving forces for the formation of such complexes are the electrostatic attraction between the oppositely charged polymer chain units and the surfactant ions and hydrophobic interactions of the surfactant chains in water. Depending on the polymer to surfactant ratio in aqueous solution, the complexes formed are either stoichiometric or nonstoichiometric. Nonstoichiometric complexes containing excesses of either polymer chain units or surfactant molecules are generally soluble in water. The formation and structure of such complexes in water have been studied in detail.[1−3] The interest in water-soluble polyelectrolyte−surfactant complexes is driven in part by their potential industrial applications, e.g., stabilization of colloidal suspensions, solubilization of organic compounds in water, viscosity modification of aqueous solutions, etc.

If equimolar amounts of charged polymer chain units and surfactant molecules are mixed in water, stoichiometric complexes are formed. Such complexes are insoluble in water. Stoichiometric complexes consisting of hydrophobically modified polyelectrolytes and double-chain surfactants[4,5] as well as complexes formed by conventional synthetic polyelectrolytes and single-chain amphiphiles[6] can be dissolved in some common organic solvents of low polarity ($\epsilon = 4-10$) without dissociation. In relatively polar solvents, e.g., *N*,*N*-dimethylformamide, dimethyl sulfoxide, and aliphatic alcohols, polyelectrolyte−surfactant complexes are believed to dissociate.[7] Complexation of polymers with oppositely charged low molecular weight compounds may result in stiffening of the polymer chains due to steric repulsion and dipole−dipole interactions between the amphiphile binding sites and thus may lead to the formation of main chain liquid crystals in organic solvents.[8,9]

In the solid state, complexes of slightly cross-linked[10,11] or linear[12−15] polyelectrolytes with oppositely charged single-chain surfactants self-organize into lamellar structures, with lamellae consisting of alternating layers of polymer chains separated by layers of surfactant molecules. If the surfactant chains are long enough, they may crystallize.[15,16]

Complexation of polyelectrolytes with oppositely charged surfactants at the air−water interface leads to the formation of unusually stable mono- and multilayers which can be transferred to solid substrates.[17−19] Formation of multilayers of controlled thickness from bolaform amphiphiles and oppositely charged polyelectrolytes assembled via alternating adsorption of the two components from water solutions on charged surfaces has also been reported.[20]

The simplicity of the synthesis of stoichiometric polyelectrolyte−surfactant complexes and the wide variety of available polyelectrolytes and surfactants have excited recent interest in this area. The ready variability of the components provides opportunities for tailoring the microstructures of such complexes and for control of macroscopic properties.

Polyelectrolyte−surfactant complexes can be viewed as a new class of comb-shaped polymers, in which every polymer chain unit has an electrostatically bound "side chain". Such compounds self-assemble into a variety of supramolecular structures, depending on the chemical properties of the components.[10−14,16,18,19,21−25] Assembly through noncovalent interactions may have advantages over that involving covalent bonds, e.g., selective control of the interactions of the segments by changing the solvent or temperature, leading to adaptive rearrangement of the structure. The amphiphilic properties of water-insoluble polyelectrolyte−surfactant complexes make these compounds promising as materials for molecular composites, separation membranes, solubilization, and compatibilization.

Most work in this area has addressed complexes formed by conventional synthetic polyelectrolytes and

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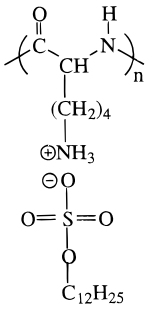
Chart 1

PLD



low molecular weight surfactants. However, biopolymers, which can adopt highly ordered structures with a variety of architectures, 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 nonaqueous media can be found in the literature.[22,23,26] Copolymers consisting of a block of poly(α,L-glutamic acid) and two blocks of poly(styrenesulfonate) complexed with alkyltrimethylammonium surfactants were found to undergo a pH-sensitive helix−coil transition at the air−water interface.[22] 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.[23]

We have recently reported on the solid-state properties of the stoichiometric complexes formed by sodium poly(α,L-glutamate) and the oppositely charged surfactants dodecyl-, cetyl-, and octadecyltrimethylammonium bromides.[16,21] The complexes are soluble in chloroform, as well as in solvents of higher polarity, e.g., benzyl alcohol, methanol, and dimethylformamide. We have shown that the polymer chains in the complexes adopt α-helical conformations in the solid state, similar to that of the alkyl esters of poly(α,L-glutamic acid) (PALGs). These complexes are organized in lamellar structures like those of comblike polymers and complexes of conventional synthetic polyelectrolytes and oppositely charged surfactants, with lamellae consisting of alternating layers of the polypeptide chains separated by bimolecular layers of surfactant. In the complexes studied, two types of surfactant organization were observed: shorter chains consisting of 12 or 16 carbon atoms are extended but positionally disordered, while the longer chains consisting of 18 methylene groups crystallize on a hexagonal lattice.[16] The surfactant chains are aligned perpendicular to the lamellar surfaces and are significantly interdigitated.

In this work, 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. We refer to this complex as PLD. The chemical structure of the complex is presented in Chart 1. We compare the behavior of the complex with that of its covalent analogs−poly(L-lysine)s bearing covalently attached acyl side chains. 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.

Preparation of the PLD Complex. 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 1 h, the resulting white precipitate was isolated by centrifugation, washed several times with water to remove low molecular weight salts, and dried in vacuum at 45 °C for at least 36 h. 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_{found} < 0.1%.

Sample Preparation. Solutions of the PLD complex in organic solvents were prepared by dissolution with stirring for 12−24 h 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). ^{1}H 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 vol % 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 wt %. For FTIR measurements of the PLD powder, KBr pellets were prepared.

Measurements. ^{1}H 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°−τ−90° pulse sequence and a delay time of 10 s. The accuracy of T_1 determination for different samples was estimated to be ±15% for the polypeptide signals and ±5% for the surfactant resonances. 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 Cu Kα (λ = 1.5418 Å) radiation was used.

Results and Discussion

Stability of the Complex. We have recently reported the properties of the stoichiometric complexes consisting of poly(α,L-glutamate) anions and alkyltrimethylammonium cations (PGX).[16,21] We have observed that these complexes undergo irreversible changes in properties upon storage, with formation of small amounts (5−10%) of unbound crystalline surfactants detected via X-ray and DSC analysis. Formation of crystalline surfactant was promoted by storage of samples in air,

Table 1. Solubility of PLD and PGX Complexes in Organic Solvents[a]

solvent	ϵ	PLD solubility in the mixture with 2−3 vol % TFA	PGX solubility
isooctane	∼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	∼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 %. [d] The ϵ value is reported for 1-hexanol.

by casting of films from chloroform solutions, and by heating of samples above room temperature. Similar changes in properties occur upon storage of complexes of polyacrylate anions and alkyltrimethylammonium cations.[27] We assume that formation of unbound surfactant occurs via hydrolysis of these complexes.

In contrast, the PLD complex described in this work 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. Stability will become an important consideration if new materials based on polymer−surfactant complexes are to be developed.

Solution Properties. Solubility in Organic Solvents. The solubility behavior of the PLD complex in organic solvents is quite different from that of the PGX complexes reported previously (Table 1) and from that of the stoichiometric complexes formed by conventional synthetic polyelectrolytes and oppositely charged surfactants.[6,7] 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 vol %) 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 dissociate[7]), 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, and aliphatic alcohols, and in mixtures of these solvents with TFA.[28,29]

Conformational Transitions in Chloroform−TFA Solutions: Effect of TFA. Addition of up to 10 vol % 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 1); 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 vol % TFA, are 0.12 and 0.66,

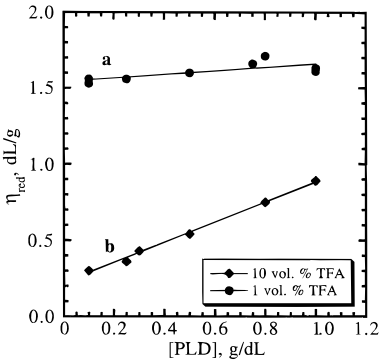


Figure 1. Concentration dependence of reduced viscosity of the PLD complex in chloroform solutions containing 1 vol % (a) and 10 vol % (b) trifluoroacetic acid.

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 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 ^{1}H NMR spectroscopy. Figure 2 (curves a and b) presents ^{1}H NMR spectra of the PLD complex in deuterated chloroform−TFA mixtures (1 and 10 vol % TFA, respectively). The ^{1}H NMR spectrum of SDS in deuterated methanol is presented for comparison (Figure 2 (curve c)). At low TFA contents, the α-CH[30] and NH resonances of the complex are observed at 4.00 and 8.15 ppm, respectively, consistent with hydrogen-bonded (α-helix or β-sheet) conformations of the polypeptide chains.[31] It is reasonable to assume that the poly(L-lysine) chains are in the α-helical conformation,[32] considering the absence of aggregation in dilute solutions and the known propensity of β-sheets to aggregate in solution. PALLs also adopt α-helical conformations in organic solvents, if no TFA is added.[28,29,33] Addition of TFA to chloroform solutions of the PLD complex results in a considerable narrowing of all resonances, an upfield shift of the NH resonances, and a downfield shift of the α-CH resonances of the polypeptide backbone (Figure 3).

Upon further addition of TFA at concentrations above 7.5 vol %, 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.[31] 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 vol % TFA (Figure 4).

Conformational transitions induced by TFA have been observed previously by optical rotary dispersion for poly(L-lysine) derivatives with covalently attached acyl side chains[29,33,34] and for other synthetic polypeptides in organic solvents.[35] For poly(L-lysine) derivatives in chloroform−TFA mixtures, the helix−coil transition is half complete at 20−30 vol % TFA; higher TFA contents are necessary to disrupt the helical forms of the PALLs with shorter side chains. The difference in the behavior of PALLs 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.

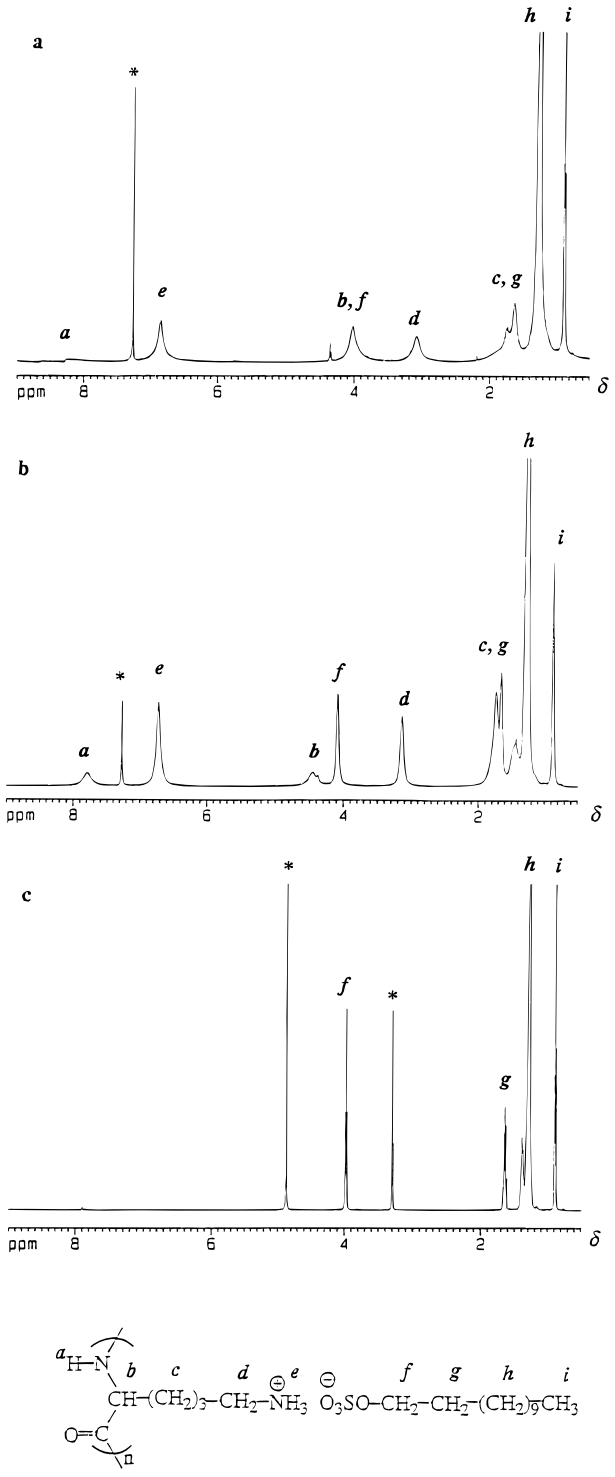


Figure 2. 500 MHz ^{1}H NMR spectra of PLD solutions in deuterated chloroform solutions containing 1 vol % (a) and 10 vol % (b) trifluoroacetic acid; concentration of PLD solutions is 10 mg/mL. (c) SDS solution in deuterated methanol; asterisks indicate signals due to solvents.

Additionally, the acyl side chains in PALLs are believed to be associated via hydrogen bonds,[34] 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 PALLs,[29,33] 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

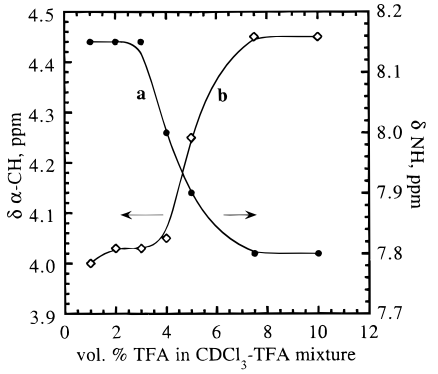


Figure 3. Dependence of the positions of NH (a) and α-CH (b) resonances of the PLD complex on the trifluoroacetic acid content in deuterated chloroform solutions; concentration of PLD solutions is 10 mg/mL.

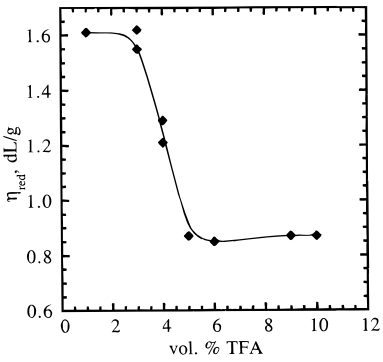


Figure 4. Dependence of reduced viscosity on the trifluoroacetic acid content in chloroform solutions of the PLD complex; concentration of PLD solutions is 10 mg/mL.

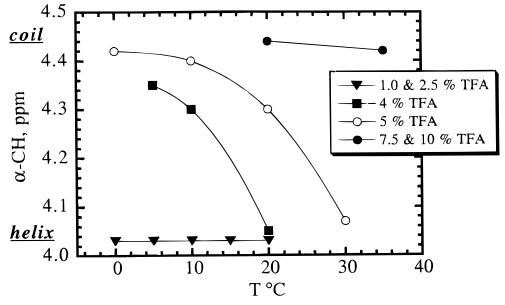


Figure 5. Dependence of the positions of the α-CH resonances of the PLD complex on temperature in deuterated chloroform solutions with 5 vol % trifluoroacetic acid; concentration of PLD solutions is 10 mg/mL.

dissociation of polyelectrolyte−surfactant complexes.[7] 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.

Conformational Transitions in Chloroform−TFA Solutions: Effect of Temperature. Additional control over the helix−coil transition of the PLD chains in chloroform−TFA mixtures is provided by temperature. At TFA contents ≤2.5% or ≥7.5% (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 5). In contrast, in $CDCl_3$−TFA mixtures with 4−5 vol % TFA, we observe a transition from disordered chains at low temperatures to helical chains at higher temperatures, as indicated by temper-

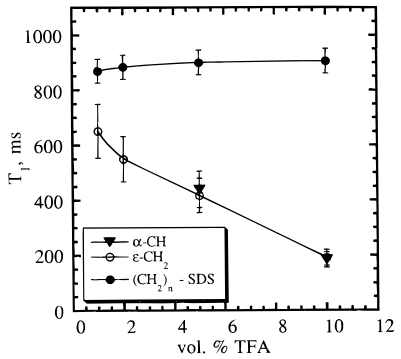


Figure 6. Dependence of the proton spin−lattice relaxation times (T_1) of the PLD complex on the trifluoroacetic acid content in deuterated chloroform solutions; concentration of PLD solutions is 10 mg/mL.

ature-dependent shifts in the α-CH resonance positions (Figure 5).[36]

These observations allow us to conclude that the α-helical conformation of the PLD chains is stabilized at higher temperatures in chloroform−TFA solutions, as has been observed for other synthetic polypeptides, e.g., PBLG[37] and poly(*δ*-*N*-carbobenzoxy-L-lysine).[38] The coil−helix transition induced by temperature is believed to be due to entropy gain by the solvent, arising from release of TFA from the polypeptide chains upon transition of the solvated coil to the helical form.

Effect of TFA on Segmental Mobility. 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 vol %, the relaxation times of the dodecyl sulfate methylene protons at about 1.26 ppm are larger than those of the polypeptide (Figure 6), 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 2).

An increase in the TFA content from 1 to 10 vol % does not affect the spin−lattice relaxation times of the surfactant protons (Figure 6), consistent with the suggestion 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 7).

Increasing the TFA concentration from 1 to 10 vol % gradually decreases the spin−lattice relaxation times of the polypeptide backbone protons (Figure 6), 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 $CDCl_3$−TFA mixtures.[39] In general, T_1s decrease with decreasing mobility, reach a minimum value, and then increase.[40] 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.[41]

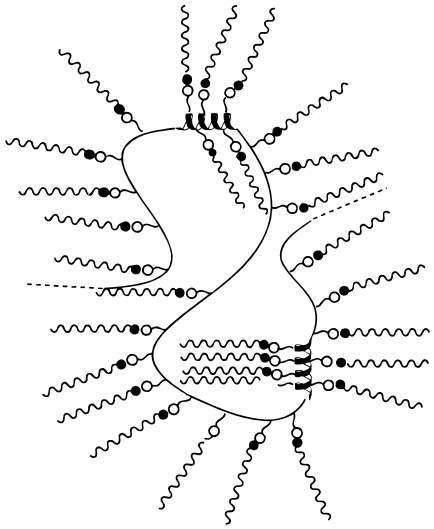


Figure 7. Schematic representation of the structure of the PLD complex in dilute chloroform solutions containing about 10 vol % trifluoroacetic acid.

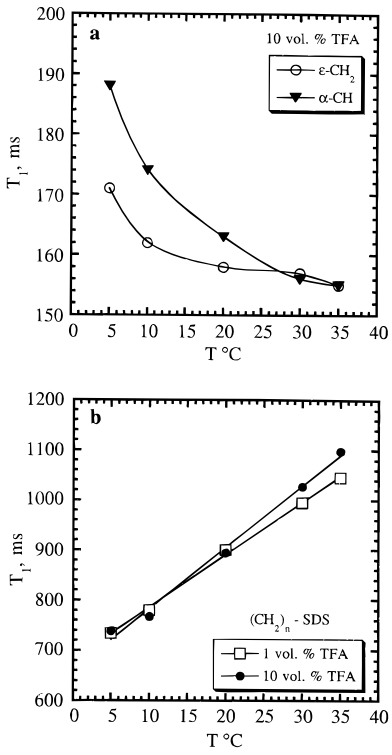


Figure 8. Temperature dependence of the proton spin−lattice relaxation times (T_1) of poly(L-lysine) (a) and dodecyl sulfate (b) chains in the PLD complex in deuterated chloroform−trifluoroacetic acid solutions with 1 and 10 vol % TFA; concentration of PLD solutions is 10 mg/mL.

At relatively high TFA contents (with the poly(L-lysine) chains in the disordered conformation in the temperature range 0−35° C), a decrease in T_1 of the polypeptide protons is observed with increasing temperature (Figure 8 a), suggesting that T_1 decreases as mobility is increased. This allows us to conclude that the decrease in T_1 of the poly(L-lysine) protons observed at the helix−coil transition (Figure 6) can be ascribed to an increase in segmental mobility, as observed for other synthetic polypeptides.[42]

At low TFA contents, when the polypeptide chains are predominantly α-helical in the temperature range 0−35

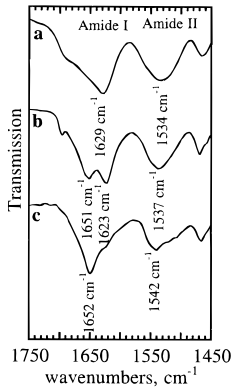


Figure 9. FTIR spectra of the PLD complex in the amide region: KBr pellet (a); films cast from chloroform solutions containing 1 vol % (b) and 10 vol % (c) trifluoroacetic acid.

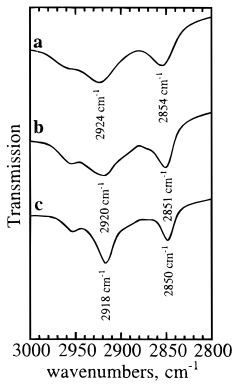


Figure 10. FTIR spectra of the PLD complex in the CH region: KBr pellet (a); films cast from chloroform solutions containing 1 vol % (b) and 10 vol % (c) trifluoroacetic acid.

°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 T_1 determination may be large compared to the small temperature effect.

Increasing temperature results in an increase in T_1s for the surfactant protons, regardless of TFA concentration (Figure 8 b), 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, as has been proposed for other synthetic polypeptides with covalently attached side chains.[29,33−35]

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 spectrum[43] (observed at 1629 and 1534 cm^{-1}, respectively, Figure 9 (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 lipids[23] and in the protonated form in the solid state.[43,44] However, covalent analogs of the PLD complex (PALLs) are α-helical in solid samples.[46]

Casting of films from chloroform−TFA mixtures results in the appearance of new amide I and amide II bands at 1652 and 1542 cm^{-1}, respectively (Figure 9 (curves b and c)), suggesting disruption of the β-sheet architecture.[43] If the films are cast from solutions with 10 vol % 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).[47] The amide I band at 1652 cm^{-1} is consistent with the α-helical geometry (Figure 9 (curve c)).

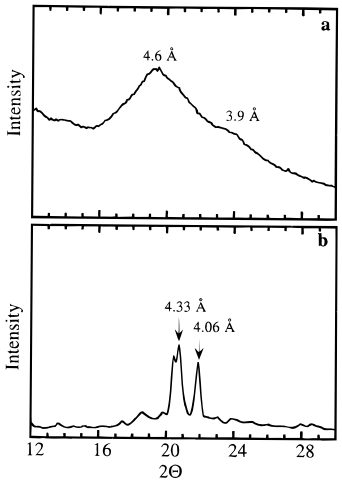


Figure 11. Wide-angle X-ray diffractometer traces of the PLD (a) and SDS (b) powders; for the SDS sample, only the signals of highest intensity are marked.

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"[48] (Figure 10 (curve a)). In PLD films cast from chloroform solutions containing 1 vol % TFA, the CH vibrations are shifted to 2920 and 2851 cm^{-1} (Figure 10 (curve b)), indicating that the surfactant alkyl chains become progressively more "solid-like", and in the films cast from solutions with 10 vol % 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[48] (Figure 10 (curve c)).

The WAXD pattern of the PLD powder (Figure 11 (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[49] formed by alkane chains of 10 or more methylene groups in PALGs.[50] 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 pure crystalline SDS is presented for com-

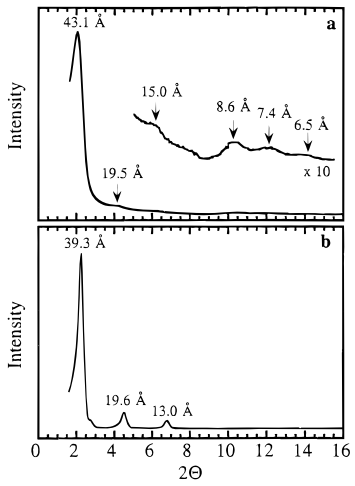


Figure 12. Small-angle X-ray diffractometer traces of the PLD (a) and SDS (b) powders.

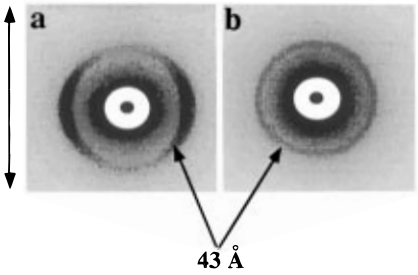


Figure 13. SAXD patterns of the PLD films cast from chloroform solutions containing 1 vol % (a) and 10 vol % (b) trifluoroacetic acid; X-ray beam was parallel to the plane of the films; arrow indicates the orientation of the film.

parison (Figure 11 (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.[51] 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 12 (curve a)), indicating a lamellar structure with a long period spacing of 43 Å. The SAXD pattern of the SDS powder is presented for comparison (Figure 12 (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 vol % 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 vol % 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 13 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 13b). The long period spacing of the lamellae does not depend on the conformation of the polypeptide chains.

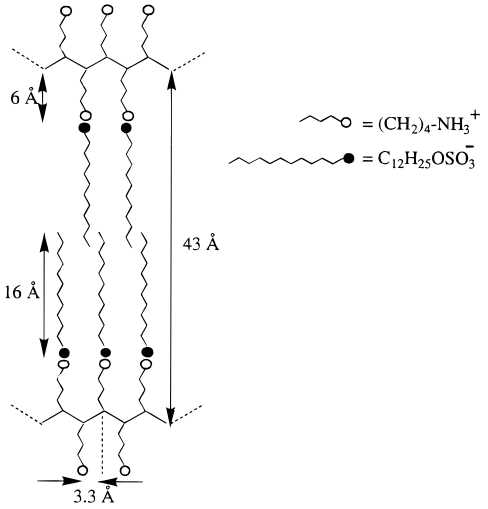


Figure 14. Schematic representation of the lamellar structure of the PLD complex in the β-sheet conformation.

In films cast from either solvent mixture, the lamellar aggregates of the PLD complex are likely to consist of layers of polypeptide 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,[44] if the tetramethylene side chains are fully extended. Thus, the value of the long period spacing (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. The possibility of a small tilt cannot be entirely excluded. A schematic illustration of the structure is presented in Figure 14.

The proposed 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,[51,52] with the hydrocarbon chains fully extended and slightly tilted (tilt angle 79°[52]) with respect to the layer plane.

Concluding Remarks

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 vol % TFA to chloroform solutions does not cause either dissociation or interchain aggregation of the complex. At low TFA contents (<5 vol %), 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 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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