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Effect of Double-Tailed Surfactant Architecture on the Conformation, Self-Assembly, and Processing in Polypeptide−Surfactant Complexes

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This work describes the solid-state conformational and structural properties of self-assembled polypeptide−surfactant complexes with double-tailed surfactants. Polyl(1-lysine) was complexed with three dialkyl esters of phosphoric acid (i.e., phosphodiester surfactants), where the surfactant tail branching and length was varied to tune the supramolecular architecture in a facile way. After complexation with the branched surfactant bis(2-ethylhexyl) phosphate in an aqueous solution, the polypeptide chains adopted an α-helical conformation. These rod-like helices self-assembled into cylindrical phases with the amorphous alkyl tails pointing outward. In complexes with dioctyl phosphate and didodecyl phosphate, which have two linear n-octyl or n-dodecyl tails, respectively, the polypeptide formed antiparallel β-sheets separated by alkyl layers, resulting in well-ordered lamellar self-assemblies. By heating, it was possible to trigger a partial opening of the β-sheets and disruption of the lamellar phase. After repeated heating/cooling, all of these complexes also showed a glass transition between 37 and 50 °C. Organic solvent treatment and plasticization by overstoichiometric amount of surfactant led to structure modification in poly(1-lysine)−dioctyl phosphate complexes, PLL(diC8), (x = 1.0–3.0). Here, the α-helical PLL is surrounded by the surfactants and these bottle-brush-like chains self-assemble in a hexagonal cylindrical morphology. As x is increased, the materials are clearly plasticized and the degree of ordering is improved: The stiff α-helical backbones in a softened surfactant matrix give rise to thermotropic liquid-crystalline phases. The complexes were examined by Fourier transform infrared spectroscopy, small- and wide-angle X-ray scattering, transmission electron microscopy, differential scanning calorimetry, polarized optical microscopy, and circular dichroism.

Introduction

Self-assembly and supramolecular chemistry are powerful tools in creating new polymeric materials with tunable properties and functions.1−6 The secondary structures of polypeptides offer an additional level of structural control on the nanoscale when compared to conventional polymers.7,8 In this sense, polypeptides can be used as structural units beyond their biochemical context.9,10 Covalently modified polypeptides are extensively studied to achieve comb-like macromolecules. Covalent attachment of spacer-like alkyl chains to polypeptides has been reported to give rise to thermotropic liquid-crystalline phases where the rod-like α-helices act as calamitic mesogens.11,12 A particularly interesting polypeptide is poly(1-lysine) (PLL), which can adopt both α-helix and β-sheet secondary structures. Azobenzenemodified PLL forms a smectic A type liquid crystal where the polypeptide main chains are antiparallel β-sheets.13 Linking of mesogens in a side-on fashion, on the other hand, leads to α-helical PLL with simultaneous nematic ordering of the side chains.14

Contrary to complex synthetic procedures, the supramolecular approach offers an alternative way to prepare comb-like macromolecules by noncovalent interactions.15−17 Ionic bonds, in particular, have been used to bind amphiphilic side chains to polypeptides.18−22 The ionic interaction between low molecular weight surfactants and a charged polypeptide leads to a spontaneous precipitation of near-stoichiometric polypeptide−surfactant complexes when aqueous solutions of the constituents are combined. These complexes have been widely studied and depending on, for example, the starting materials, preparation method and temperature, the polypeptide backbone can adopt different secondary structures in the solid state. PLL complexes prepared from aqueous solutions,20,21 most typically show antiparallel β-sheets separated by surfactant layers, while organic solvent treatment (which swells the alkyl phase) can lead to α-helical PLL.18,21 Secondary structure formation can also be suppressed, as has been reported for hyperbranched PLL complexes with alkyl sulfates.23 The organization of short single-tailed surfactants in polypeptide−surfactant complexes is usually amorphous while longer surfactants can crystallize and also interdigitate.18,20

However, if polypeptide motifs are to be used as structural units in bulk polymeric state, their processing must be made easier. Polypeptide−surfactant complexes are typically brittle solid materials which are infusible until their degradation temperature. Depending on the secondary structure of the polypeptide backbone, the complexes can also be very difficult to dissolve.18 Previously, addition of hydrogen-bonding plasticizing surfactant to ionic polypeptide−surfactant complexes has been demonstrated to lead to shear-deformable well-ordered materials already at room temperature.21

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Until now, studies of polypeptide—surfactant complexes have concentrated mainly on single-tailed surfactants with only a few publications reporting on branched \(^{21,22}\) side chains, while studies with lipid side chains \(^{24-26}\) have concentrated mainly on the lipid phase transitions. In the present paper, we investigate the effect of surfactant branching and chain length on the secondary structure of the polypeptide and the self-assembled nanostructures. For this purpose, poly(\(L\)-lysine) was complexed with three phosphodiester surfactants \(^{27}\) with different architectures. In publications reporting on branched \(^{21,22}\) side chains, while studies concentrated mainly on single-tailed surfactants with only few side chains is expected to influence the secondary structure of the polypeptide and the overall self-assembly. The structures and temperature-dependent behavior of the complexes with various surfactant architectures were studied using Fourier transform infrared spectroscopy (FTIR), small- and wide-angle X-ray scattering (SAXS and WAXS), transmission electron microscopy (TEM), differential scanning calorimetry (DSC), and polarized optical microscopy (POM). For organic solvent treatment and further plasticization, we chose the aqueous precipitated poly(\(L\)-lysine)—dioctyl phosphate complex as a starting material due to the plasticization efficiency of the neat dioctyl phosphate surfactant and the properties of the ionic complex. The plasticized complexes were studied also by solid-state circular dichroism (CD).

### Experimental Section

**Materials.** Poly(\(L\)-lysine hydrobromide) (PLLHBr, Sigma, 18800 g mol\(^{-1}\); PDI 1.5), sodium hydroxide (NaOH, Riedel-de Haën, \(\geq 99\%\)), \(n\)-propanol (Merck, \(\geq 99.5\%\)), silver nitrate (AgNO\(_3\), Fluka, \(\geq 99.0\%\)), and chloroform (CHCl\(_3\), Riedel-de Haën, \(\geq 99.0\%\)) were used as received. Bis(2-ethylhexyl) phosphate (diC\(_{2,6}\)) (Aldrich, \(\geq 97\%\)) was purified using a previously reported method \(^{28}\) with slight modifications (see Supporting Information). Water was purified with Millipore Milli-Q Ultrapure Water Purification System with a 0.22 \(\mu\)m membrane filter.

Dioctyl phosphate (diC\(_8\)) was synthesized as follows. Octanol (8 g, 61.5 mmol) and phosphorus oxychloride (2.7 g, 17.9 mmol) were dissolved in dry benzene (150 mL) and refluxed at 90 \(^\circ\)C for 48 h. The solvent was then removed by rotary evaporation, producing a yellow oil. The crude product was purified by recrystallizing twice from 50 mL of hexane, producing a colorless liquid. Yield: 4.1 g (73\%).

Disodioctyl phosphate (diC\(_{12}\)) was synthesized with the same procedure (dodecanol 8 g, 4.30 mmol, phosphorus oxychloride 1.86 g, 1.23 mmol), producing a white powder. Yield: 3.0 g (76\%). For detailed analysis of the surfactants, see Supporting Information.

The surfactants diC\(_{2,6}\), diC\(_8\), and diC\(_{12}\) were converted into sodium salts (Na-diC\(_{2,6}\), Na-diC\(_8\), and Na-diC\(_{12}\)) by dissolving in water with an equimolar amount of NaOH and a small amount of \(n\)-propanol. After vigorous stirring of at least 24 h, the solutions were evaporated on a hot plate (60 \(^\circ\)C) and the salts were dried under vacuum at room temperature for 48 h. The complexes were denoted as PLL(diC\(_{2,6}\))\(_{1.0}\), PLL(diC\(_8\))\(_{1.0}\), and PLL(diC\(_{12}\))\(_{1.0}\) based on the nominal compositions (see Figure 1a) that were confirmed by elemental analysis (see Supporting Information). For organic solvent treatment and addition of overstoichiometric amounts of diC\(_8\) surfactant to the stoichiometric PLL(diC\(_8\))\(_{1.0}\) complex, PLL(diC\(_8\))\(_{1.0}\) and diC\(_8\) were separately dissolved in CHCl\(_3\) as 0.5 and 1.0 wt % solutions, respectively. The PLL(diC\(_8\))\(_{1.0}\) solution was cleared after gentle heating to 45 \(^\circ\)C, cooled back to room temperature and combined with the diC\(_8\) solution in molar ratios 1.0, 1.05, 1.1, and 1.2 to prepare PLL(diC\(_8\)) complexes with \(x = 1.0, 1.5, 2.0, 3.0\), respectively, as denoted according to their nominal compositions (see Figure 1b). After several hours of mixing, the solutions were evaporated in air and further dried under vacuum at room temperature.

**FTIR Measurements.** The ionic complexes prepared from aqueous solution were mixed with KBr and pressed to form pellets. PLL(diC\(_8\)) complexes \((x = 1.0, 1.5, 2.0, 3.0)\) prepared from CHCl\(_3\) were drop cast on silicon wafers and dried under vacuum prior to measurements. Transmission spectra were recorded with a Nicolet 380 FT-IR spectrometer by averaging 64 spectra with 2 cm\(^{-1}\) resolution. A Linkam TMS94 temperature controller and a Linkam heating stage were used to control the sample temperature. A heating rate of 10 \(^\circ\)C/min was used and the temperature was allowed to stabilize for 3 min before measurements. The spectra have been smoothed with EZ Omnics 7.2 software Automatic smooth, which uses the Savitzky–Golay algorithm.

**SAXS Measurements.** Bulk samples were sealed between Kapton or Mylar films. The measurements were performed with a rotating anode Bruker Microstar microfocus X-ray source (Cu K\(\alpha\) radiation, \(\lambda = 1.54\) \(\AA\)) with Montel Optics. The beam was further collimated with four sets of slits, resulting in a beam area of about 1 \(\times\) 1 mm at the sample position. Scattering intensities were measured using a Bruker AXS 2D area detector. The sample-to-detector distance was 45 or 60 cm, depending on the experimental setup. The magnitude of the scattering vector is given by \(q = (4\pi/\lambda)\sin \theta\), where \(2\theta\) is the scattering angle. A custom-built externally controlled heating oven was used for temperature control.

**WAXS Measurements.** WAXS measurements were performed with Bruker AXS D8 Advance diffractometer. Complexes PLL(diC\(_{2,6}\))\(_{1.0}\) and PLL(diC\(_{12}\))\(_{1.0}\) were measured in transmission mode and PLL-
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Room Temperature Behavior of Ionic Complexes. When a solution of PLL-HBr was combined with solutions of sodium bis(2-ethylhexyl) phosphate (Na-diC2/6), sodium dioctyl phosphate (Na-diC8), and sodium didodecyl phosphate (Na-diC12), respectively, a white precipitate was immediately formed in all cases. This precipitation takes place due to the formation of the ionic complexes, which were shown by elemental analysis to have a stoichiometric complexation ratio within the limits of error (see Supporting Information). These complexes are therefore denoted after their nominal compositions as PLL(diC2/6)\textsubscript{1.0}, PLL(diC8)\textsubscript{1.0}, and PLL(diC12)\textsubscript{1.0} (see Figure 1a).

The room temperature behavior of the solid-state complexes is discussed, starting from PLL(diC2/6)\textsubscript{1.0}, where the corresponding RII correspond to a well-defined \( \alpha \)-helical secondary structure because only 3.6 amino acid monomers are available for helix formation per turn in an \( \alpha \)-helix. A broad WAXS reflection at \( q_1^\alpha = 0.247 \ \text{Å}^{-1} \) in the SAXS regime and another one at \( 3q_1^\alpha \) in the WAXS regime, indicating that self-assembled order takes place. In addition, there is a broad reflection near 0.42 Å\(^{-1} \), which is close to the expected reflection \( \sqrt{3}q_1^\alpha \) for hexagonal cylindrical self-assembly of the \( \alpha \)-helical chains. However, its large width suggests that it is, in fact, a combination of several smaller-peaks. There is also pronounced scattering at about 0.29 Å\(^{-1} \). To clarify the scattering data, Lorentzian peaks were fitted to the SAXS pattern (see Supporting Information) to reveal reflections at 0.247, 0.297, and 0.422 Å\(^{-1} \). Based on this, we suggest that a hexagonal cylindrical self-assembly, as evidenced by the reflections at \( q_1^H = 0.247 \ \text{Å}^{-1} \), \( \sqrt{3}q_1^H = 0.422 \ \text{Å}^{-1} \) and \( 3q_1^H \) (WAXS regime), coexists with a tetragonal cylindrical self-assembly where \( q_1^T = 0.297 \ \text{Å}^{-1} \) and \( 2q_1^T = 0.422 \ \text{Å}^{-1} \). Such a coexistence suggests also a poor overall order with only small local self-assembled domains. In other words, the morphology could not be imaged with TEM.

The maximum length of a hexyl chain (7 Å) and the diameter of a PLL \( \alpha \)-helix (15 Å) suggest that the diameter of a single bottle-brush-like PLL(diC2/6)\textsubscript{1.0} chain is about 29 Å. The hexagonal cylindrical self-assembly has similar 29 Å spacing between adjacent helices, so the alkyl tails pointing out from different helices are not interpenetrated in the hexagonal phase. In the tetragonal cylindrical self-assembly, the lattice spacing is 22 Å, so here the helices are packed considerably closer to each other. This inhibits the surfactants attached to neighboring helices must be interpenetrated or stacked on top of each other in the direction of the helical axis, which is plausible because only 3.6 amino acid monomers are available for complexation per turn in an \( \alpha \)-helix. A broad WAXS reflection at 1.44 Å\(^{-1} \) (Figure 3, corresponding to 4.4 Å) furthermore shows that the alkyl tails are amorphous, which is also confirmed by FTIR absorptions of PLL(diC2/6)\textsubscript{1.0} at 2932 and 2861 cm\(^{-1} \) (see Supporting Information). This is not surprising considering the highly branched molecular structure of the diC2/6 surfactant. The structure of PLL(diC2/6)\textsubscript{1.0} at room temperature is schematically illustrated in Figure 5.

To investigate the effect of side-chain branching in more detail, we prepared PLL(diC8)\textsubscript{1.0} which has the same alkyl volume but linear side chains. Here, FTIR absorptions (Figure 2) at 1695 (amide I), 1628 (amide I), and 1530 cm\(^{-1} \) (amide II) reveal that the PLL backbone forms antiparallel \( \beta \)-sheets. The shoulder at about 1658 cm\(^{-1} \) suggests that also a small amount of \( \alpha \)-helical or random coil conformation is present in PLL(diC8)\textsubscript{1.0}. In other words, we can effectively change the morphology of the surfactant. SANS (Figure 3) shows reflections \( q^n \) (0.174 Å\(^{-1} \)) and \( 2q^n \), which are further accompanied in the WAXS region with reflections \( 4q^n \) and \( 5q^n \). These reflections demon-
strate that a well-ordered lamellar self-assembly with a period of 36 Å is formed. Also, in TEM (Figure 4a), a regular lamellar self-assembly is observed and the period, as determined from the SAXS reflections, corresponds well to the estimated thickness of β-sheets (15 Å) surrounded by tail-to-tail arranged double layers of diC8 (11 Å). In WAXS (Figure 3), a reflection centered at 1.48 Å⁻¹ (corresponding to 4.2 Å) is observed, indicating an amorphous alkyl phase with a trace of crystalline alkyl tails. The FTIR absorptions at 2926 and 2855 cm⁻¹ (see Supporting Information) are located at lower (i.e., more crystalline) wave numbers than in PLL(diC2/6)₁₀ but can still be assigned to mostly noncrystalline alkyl chains. The structure of PLL(diC8)₁₀ at room temperature is schematically illustrated in Figure 5.

It was shown above that complexation of PLL with double-tailed surfactants with eight carbons per tail can lead to either cylindrical self-assembly of α-helices or to predominantly lamellar self-assembly of β-sheets when the surfactant is branched or linear, respectively. This encouraged us to study also how the length of linear double tails affects the conformation and self-assembly. Therefore, PLL(diC12)₁₀ with two dodecyl side chains was prepared. FTIR absorptions (Figure 2) at 1693 (amide I), 1628 (amide I), and 1531 cm⁻¹ (amide II) show that the PLL backbone forms purely antiparallel β-sheets. SAXS reflections q₁ (0.131 Å⁻¹), q₂, weak 3q₁, and 4q₁, which are continued in the WAXS region with reflections 5q₁, 6q₁, and 7q₁ (Figure 3), clearly show that PLL(diC12)₁₀ has a lamellar self-assembly with a period of 48 Å. This period corresponds to the estimated thickness of β-sheets (15 Å) surrounded by tail-to-tail arranged double layers of dodecyl chains (15 Å). The lamellar morphology was again verified by TEM (Figure 4b). A sharp WAXS reflection at 1.53 Å⁻¹ (Figure 3, corresponding to 4.1 Å) indicates substantial crystallization of the side chains, which is confirmed by the FTIR alkyl absorptions at 2919 and 2850 cm⁻¹ (see Supporting Information). The observed period suggests that crystallization takes place in a tuning-fork type configuration where the two dodecyl chains are parallel to each other, instead of in a V-shaped configuration observed for pure diC12 in the acid form. Interestingly, the potassium salt of diC12 has been observed to prevent crystallization of the alkyl phase efficiently. Removal of the branching of the side chain, as in the structural isomer PLL(diC8)₁₀ which is prepared from an aqueous solution, although usually polyamide−surfactant complexes prepared from an aqueous solution adopt the β-sheet secondary structure. Previously, hexagonal cylindrical self-assembly of α-helical polylysine−surfactant complex chains has been observed only after organic solvent treatment which swells the alkyl phase. We suggest that the bulky surfactant diC2/6 cannot fit efficiently between PLL β-sheets due to its shape, thus forcing the backbone to adopt an α-helical secondary structure where each surfactant is more easily accommodated in the available space around the polypeptide backbone. Branching of the diC2/6 surfactant prevents crystallization of the alkyl phase efficiently. Removal of the branching of the side chain, as in the structural isomer PLL(diC8)₁₀, leads to a completely different self-assembly pathway, where antiparallel β-sheets are separated by alkyl tail layers. This kind of lamellar self-assembly is common with single-tailed surfactants. By changing the length of the linear alkyl tails of the surfactant we can tune the lamellar periodicity as well as the crystallization of the alkyl tails, as shown by the complexes PLL(diC8)₁₀ and PLL(diC12)₁₀.

Modification by Thermal Treatment. To make full use of the potential of polyamide−surfactant complexes, the polypep-
Earlier work on PLL and lipids.24 and open the way for thermal transitions, as encouraged by the alkyl phase and the polypeptide conformation more balanced complexes, which is expected to make the interplay between surfactants effectively increases the alkyl volume of the ionic thermally triggered transitions in the solid state before the micrographs of PLL(diC12)1.0 at 30 and 120 °C indicates melting of the dodecyl tails. This melting is here prevented by the bulky nature of the diC2/6 surfactant, which sterically prevents PLL from adopting a secondary structure should be controllable by external stimuli or external conditions. We have previously reported a partial thermally triggered transformation from α-helix to β-sheet in a polypeptide-surfactant complex with a branched single-tailed surfactant.21 However, in polypeptide–surfactant complexes of PLL and single-tailed linear20 or branched21 surfactants, as prepared from water, the native conformation of the polypeptide is usually β-sheet. Generally, it is known that the β-sheet secondary structure in polypeptides in nature (e.g., silk) promotes mechanical and thermal stability. Also, in the case of polypeptide–surfactant complexes, the β-sheet secondary structure was reported to be extremely stable. Such complexes could not, for example, be dissolved without strong acid, which breaks the multiple intermolecular hydrogen bonds.18,21 In addition, the existence of β-sheets usually inhibits thermally triggered transitions in the solid state before the materials are degraded. Here, the double-tailed nature of the surfactants effectively increases the alkyl volume of the ionic complexes, which is expected to make the interplay between the alkyl phase and the polypeptide conformation more balanced and open the way for thermal transitions, as encouraged by earlier work on PLL and lipids.24

Of the ionic complexes prepared from aqueous solution, PLL(diC12)1.0 has the largest number of carbon atoms per surfactant and its overall crystallinity is higher than those of PLL(diC2/6)1.0 and PLL(diC8)1.0. During thermal treatment of PLL(diC12)1.0, we observe many changes: In the first heating cycle in DSC (Figure 6c), no exothermic processes are observed during the first cooling, which also indicates that the alkyl tails do not crystallize in a significant amount. Although the dodecyl tails in PLL(diC12)1.0 are crystalline at room temperature before any thermal treatments, the mixed structure observed after heating does not allow fast recrystallization of the alkyl tails. On second heating in DSC, no melting peak is anymore observed for PLL(diC12)1.0 but a glass transition is now observed at 37 °C. PLL(diC8)1.0, which has an essentially similar structure as PLL(diC12)1.0 but with shorter and less crystalline side chains at room temperature, exhibits comparable thermal behavior (see Supporting Information for temperature-dependent measurements). However, based on visual inspection of the FTIR amide bands, the disruption of β-sheets does not proceed here as far as in PLL(diC12)1.0. Furthermore, the lamellar SAXS reflections do not vanish completely. A glass transition is observed at a slightly higher temperature, 50 °C.

In PLL(diC2/6)1.0, thermal treatment does not lead to significant structural changes (see Supporting Information). In SAXS, a small gradual change is observed during heating, and we suggest that the mixed hexagonal and tetragonal cylindrical phase observed at room temperature shifts toward tetragonal cylindrical self-assembly during heating (see Supporting Information for detailed peak fitting). However, the SAXS reflections are quite broad, which indicates that thermal treatment also results in an overall deterioration of order in the material. A glass transition is observed at 49 °C.

To summarize, the β-sheets in PLL(diC12)1.0 and PLL(diC8)1.0 are partially opened and the well-defined lamellar self-assemblies are transformed into mixed structures by thermal treatment. Unlike in the natural polypeptides or polypeptide–surfactant complexes with single-tailed surfactants, here simple heating in the solid state is enough to break the β-sheets. This is suggested to be due to a number of reasons, including packing and steric considerations during thermal motion of the double-tailed side chains. The β-sheets are more efficiently disrupted and the structure changed in PLL(diC12)1.0 with longer side chains due to the thermal energy in the molten state. In PLL(diC6/2)1.0 with branched side chains, the α-helical secondary structure of PLL is stable even after heating to 160 °C. The partial α-helix to β-sheet transformation that has previously21 been observed in a polypeptide–surfactant complex during heating is here prevented by the bulky nature of the diC2/6 surfactant, which sterically prevents PLL from adopting a β-sheet conformation. In all the complexes, the side chains are mostly amorphous after the first heating cycle and a glass at about 80 °C. But most interestingly, there are changes in the PLL conformation during heating, as shown by FTIR (Figure 6a). From 120 °C onward, a new amide I peak is observed at 1660 cm⁻¹ and the amide II band shifts from 1531 to 1543 cm⁻¹, indicating that a large amount of the PLL chains have changed from antiparallel β-sheet to α-helical secondary structure. The existence of some amount of random coil conformation cannot be excluded. In other words, we are able to disrupt a significant amount of the β-sheets by simple heating in the solid state. The well-ordered lamellar self-assembly of PLL(diC12)1.0 with β-sheet secondary structure observed at room temperature is thus converted into a mixed structure containing many conformations. The coexistence of several conformations prevents formation of any regular overall structure after heating.

After heating of PLL(diC12)1.0, the FTIR alkyl absorptions (see Supporting Information) shift back toward lower wave numbers only slightly, suggesting that the surfactant alkyl chains are on average more amorphous than before heating. In DSC (Figure 6c), no exothermic processes are observed during the first cooling, which also indicates that the alkyl tails do not crystallize in a significant amount. Although the dodecyl tails in PLL(diC12)1.0 are crystalline at room temperature before any thermal treatments, the mixed structure observed after heating does not allow fast recrystallization of the alkyl tails. On second heating in DSC, no melting peak is anymore observed for PLL(diC12)1.0 but a glass transition is now observed at 37 °C.

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transition can be observed at moderate temperatures. Previously, a glass transition has been observed in a solid-state polypeptide—surfactant complex with a branched single-tailed surfactant. Based on the observations above, we suggest that the glass transitions originate here from the vitrification of the side-chain alkyl-containing architectures and not from relaxations of the polypeptide backbone.

Modification by Solvent Treatment and Plasticization by Added Surfactants. In general, polypeptide—surfactant complexes are known to be infusible solids and, therefore, difficult to process. The ionic complexes discussed above are all solid, powder-like materials even when the neat surfactant is a liquid at room temperature, as in the case of the diC2/6 or diC8 surfactants. Although we have demonstrated that thermal treatment is one way to modify the structures, the ionic complexes prepared from aqueous solutions are hard materials that do not easily flow, even at elevated temperatures. Previously, addition of hydrogen-bonding plasticizing surfactant to ionic polypeptide—surfactant complexes was used to prepare well-ordered materials that are shear-deformable already at room temperature. In addition to the plasticization possibilities, we were interested to see how organic solvent treatment could be used to control the conformation and self-assembly of the complexes.

For solvent treatment and plasticization of the materials by double-tailed surfactants, we chose the PLL(diC8)1.0 complex and neat diC8 surfactant for two reasons: (a) diC8 is a liquid at room temperature so it can be used to efficiently plasticize the complexes and (b) the complex PLL(diC8)1.0 has been shown above to exist in both \( \alpha \)-helical and \( \beta \)-sheet conformations, which could make several interesting solvent-treated and plasticized structures possible.

First, to study the effects of solvent treatment, PLL(diC8)1.0 prepared from an aqueous solution was dissolved in CHCl3. Although \( \beta \)-sheet-forming complexes usually cannot be dissolved without strong acid, we could here use the thermal properties of PLL(diC8)1.0 and break the \( \beta \)-sheets by careful heating to 45 °C, which led to immediate and permanent clearing of the solution. After drying under vacuum, FTIR (Figure 7a) shows a dominating amide I peak at 1658 cm\(^{-1} \), a smaller one at 1626 cm\(^{-1} \), and an amide II peak at 1545 cm\(^{-1} \), indicating that the conformation is changed predominantly to \( \alpha \)-helical with only a small contribution from antiparallel \( \beta \)-sheets. Due to the preparation from organic solvent, good quality films could be prepared by drop casting, which also made solid-state CD studies possible. Indeed, the CD spectrum (Figure 4b) shows a single broad negative peak at 222 nm, indicating that the solvent-treated complex has a mixture of conformations. The conformational change from \( \beta \)-sheet (as prepared from aqueous solution) to mostly \( \alpha \)-helical (after organic solvent treatment) is also reflected in the overall self-assembly: In SAXS (Figure 8), two broad reflections \( q^* (0.212 \text{ Å}^{-1}) \) and \( \sqrt{3} q^* \) are observed, corresponding to a poorly organized hexagonal cylindrical phase with a distance of 34 Å between adjacent cylinders. In other words, we can effectively change the conformation and structure of PLL(diC8)1.0 ionic complex by dissolution in organic solvent, which swells the alkyl phase and thus makes the \( \alpha \)-helical conformation preferred. However, as the organic solvent is removed, the conformation stays \( \alpha \)-helical. This leads to a very sparse packing of the alkyl tails and their low density within the trapped glassy structure of the material.

The solvent-treated PLL(diC8)1.0 is still a solid, brittle material (see Figure 8). To study plasticization by double-tailed surfactants, we combined diC8 surfactant with PLL(diC8)1.0 in CHCl3, followed by drying to obtain the complexes PLL(diC8)\( x \), where \( x = 1.5, 2.0, \) and 3.0 depict the nominal compositions (see Figure 1b). As \( x \) is increased, the appearance of the material (see Figure 8) changes radically from a slightly sticky solid substance (\( x = 1.5 \)) to a readily flowing liquid (\( x = 3.0 \)). No macrophase separation was observed in optical microscopy, and the absence of pronounced scattering at low \( q \) values in SAXS suggests that the plasticizing diC8 and the ionic complex are truly mixed on the molecular level. This is also reasonable considering the similarity of the plasticizing additional surfactants and the ionically bonded surfactants and their proposed hydrogen bonding. FTIR spectra (Figure 7a) of PLL(diC8)\( x \), reveal that the PLL conformation is now purely \( \alpha \)-helical with the amide I band at 1657–1656 cm\(^{-1} \) and the amide II band at 1547 cm\(^{-1} \). CD spectra (Figure 7b) verify this for \( x = 1.5 \) and 2.0, where the typical \( \alpha \)-helical negative bands are observed at 209 and 222 nm and a positive band at 192 nm. The CD spectrum of \( x = 3.0 \) has negative bands at 209 and 223 nm, the latter one being more intense, and a positive band...
at 196 nm. The conformation of $x = 3.0$ is therefore interpreted to be also $\alpha$-helical and the small differences compared to $x = 1.5$ and 2.0 spectra suggested to be due to the very small relative amount of polypeptide in the $x = 3.0$ complex.

The $\alpha$-helical conformation is also reflected in the self-assembly of PLL(diC8) complexes. According to SAXS (Figure 8), all PLL(diC8) complexes self-assemble in a hexagonal cylindrical morphology, but as $x$ is increased, the degree of ordering is clearly improved, as shown by several new and more narrow SAXS reflections. The distances between adjacent cylinders are 32, 31, and 32 Å in $x = 1.5$, 2.0, and 3.0, respectively, which indicates that the hexagonal cylindrical self-assembly is not swollen by addition of overstoichiometric amounts of diC8. Because no macrophase separation takes place either, we suggest that the nonionically bonded surfactants are incorporated in the existing structure, as previously reported for a system with single-tailed branched surfactant.21 In this respect, it is essential to notice that free dialkyl phosphates are able to form hydrogen bonds, acting as acceptors and donors. The only available hydrogen bonding sites here are the ionically bonded diC8 molecules and the amide groups of the PLL backbone. The only available hydrogen bonding sites here are the ionically bonded diC8 molecules and the amide groups of the PLL backbone. The only available hydrogen bonding sites here are the ionically bonded diC8 molecules and the amide groups of the PLL backbone. The only available hydrogen bonding sites here are the ionically bonded diC8 molecules and the amide groups of the PLL backbone.

Figure 9. Schematic illustration of the suggested surfactant packing in the solvent-treated and plasticized PLL(diC8) complexes. (a) $x = 1.0$ complex where all surfactants are ionically bonded. There is a lot of space available between the ionically bonded surfactants and the PLL-chains that are trapped mostly in the $\alpha$-helical state within hexagonal cylindrical self-assembly. (b) $x = 2.0$ complex where the plasticizing diC8 is hydrogen bonded to the ionically bonded diC8 in the perimeter of the bottle-brush-like cylinders. The $\alpha$-helical PLL is shown in green and the surfactants in yellow/orange. Only a short segment of the complex cylinder is shown here.

Figure 10. Scheme of conformational and structural control.

The present study shows the effect of surfactant branching and chain length on the conformation of the polypeptide backbone and self-assembled nanostructures of polypeptide—surfactant complexes with double-tailed surfactants. The double-tailed nature of the surfactants gives rise to new packing and steric considerations in polypeptide—surfactant complexes. The studied pathways are summarized in Figure 10.

The ionic complexes of PLL and surfactants diC2/6 (with two branched tails), diC8 (two linear octyl tails), and diC12 (two linear dodecyl tails) as prepared from aqueous solution showed different conformations and self-assemblies, depending on the branching of the surfactant. With the branched, bulky surfactant diC2/6, the PLL backbone adopts an $\alpha$-helical conformation and the complexed supramolecular PLL(diC2/6)$_{1.0}$ chains are suggested to self-assemble having poor overall order with local domains of hexagonal and tetragonal cylindrical morphologies. The branching of the diC2/6 surfactant does not allow crystallization of the alkyl phase. When complexed with linear double-tailed surfactants diC8 and diC12, PLL forms antiparallel $\beta$-sheets sandwiched between two alkyl tail layers in a lamellar self-assembly. The dodecyl tails of PLL(diC12)$_{1.0}$ are able to crystallize within this lamellar organization, while the side chains of PLL(diC8)$_{1.0}$ are amorphous with just a trace of crystallinity.

These ionic complexes, as prepared from an aqueous solution, have been further modified by two approaches: thermal treat-
treatment or organic solvent treatment and plasticization. The goal here has been to find pathways to control the conformation of the PLL backbone, which is related to the different self-assembled morphologies, as well as producing more easily processable polypeptide-based materials. Thermal treatment of the ionic complexes PLL(diC8)1.0 and PLL(diC12)1.0 triggers a partial opening of the β-sheets. This interesting phenomenon leads to mixed phases with many conformations, which quite efficiently prevents crystallization of the alkyl tails in the time scale of the experiments. The subsequent complexes exhibit glass transitions at moderate temperatures. Excess organic solvent treatment of PLL(diC8)1.0 changes the conformation and structure completely, namely, to hexagonal cylindrical self-assembly with α-helical conformation. Plasticization was accomplished by adding excess diC8 to PLL(diC8)1.0 in organic solvent to give PLL(diC8), complexes with nominally x = 1.5, 2.0, and 3.0. These complexes are clearly softened materials where the ordering of the hexagonal cylindrical self-assembly is improved as x is increased. The plasticizing diC8 surfactants are suggested to be hydrogen-bonded to the ionically bonded surfactants, thus making the perimeter of the bottle-brush-like chains more dense but keeping the lattice spacing of the self-assembly constant. The stiff α-helical PLL chains surrounded by plasticizing surfactants are liquid-crystalline materials. The composition of the complex determines the macroscopic flow properties and the exact degree of microscopic ordering: softer materials are able to arrange in a more perfect lattice.

We expect that these concepts will be relevant in designing new polypeptide materials where the polypeptide conformation gives rise to very precise supramolecular architectures with good long-range order. Furthermore, it is expected that the exact properties of the side chains can allow complete switching between different secondary structures and between crystalline and amorphous alkyl phases, especially in the case of novel surfactants with addressable functionalities in the tails.33

Acknowledgment. We thank Panu Hiekkataipale for his assistance in the SAXS experiments and Mari Granström for the several fruitful discussions.

Supporting Information Available. Details of the purification of diC2/6 surfactant and DSC, NMR, and MS characterization of the surfactants used are provided. Of the aqueous precipitated ionic complexes, elemental analysis, TGA curves, and FTIR spectra from the methylene group C—H stretching absorption range are presented. Measurements of the aqueous precipitated PLL(diC8)1.0 and PLL(diC2/6)1.0 as a function of temperature (SAXS, FTIR, DSC, and POM) as well as detailed SAXS curve fitting for PLL(diC2/6)1.0 are shown. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


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Effect of Double-Tailed Surfactant Architecture on the Conformation, Self-Assembly and Processing in Polypeptide–Surfactant Complexes

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Purification of the Branched diC2/6 Surfactant. Bis(2-ethylhexyl) phosphate (diC2/6, Aldrich, ≥ 97%) was dissolved in cyclohexane (1M) and partitioned against aqueous copper(II) hydroxide solution. After vigorous shaking and waiting for 1-2 hours, the blue/green cyclohexane layer was collected, added to 500 ml acetone with stirring and left at a lowered temperature overnight. The blue precipitate was filtered, washed with acetone and air dried. The precipitate was then dissolved in cyclohexane and partitioned against dilute HCl solution. After vigorous shaking, the organic layer lost the blue/green coloration. The organic layer was washed with water and the cyclohexane was removed by reduced pressure.

Surfactant Analysis. Melting points were obtained from DSC measurements (see the Experimental section of the article for details) as the peak temperature during a second heating scan. \(^1\)H-NMR (500 MHz), \(^{13}\)C-NMR (126 MHz), \(^{31}\)P-NMR (202 MHz), and 2D-NMR spectra were recorded on a Bruker DRX-500 instrument. Chemical shifts are reported in ppm on the δ scale from an internal standard of residual chloroform (7.26 ppm in \(^1\)H-NMR spectra and 77.0 ppm in \(^{13}\)C-NMR spectra). The purity of the products was determined by electrospray ionization mass spectra (ESI-TOF) using a Micromass LCT spectrometer.

Bis(2-ethylhexyl) phosphate (diC2/6):

\[ \text{C}_{16}\text{H}_{35}\text{O}_4\text{P} \]

Exact Mass: 322.23
Mol. Wt: 322.42
C, 59.60; H, 10.94; O, 19.85; P, 9.61

\(^1\)H NMR (500 MHz, CDCl\(_3\)): δ 11.69 (s, 1H, OH), 3.91 (m, 4H, OCH\(_2\)), 1.55 (m, 2H, OCH\(_2\)CH), 1.23-1.43 (m, 16H, CH\(_3\)(CH\(_2\))\(_3\) and CH\(_3\)CH\(_2\)CH), 0.88 (m, 12H, 4x CH\(_3\)).

\(^{13}\)C NMR (126 MHz, CDCl\(_3\)) 69.61 and 69.56 (C1 and C1’), 40.06 and 40.00 (C2 and C2’), 29.85 (C3), 28.84 (C4), 23.20 (C7), 22.91 (C5), 13.94 (C6), 10.80 (C8).

\(^{31}\)P NMR (202 MHz, CDCl\(_3\)) δ 1.85 and 1.86.

ESI-TOF-MS m/z 321.22 [M-H], calcd for C\(_{16}\)H\(_{35}\)O\(_4\)P 322.23.

For NMR spectra, see Figures S1-S3.
Diocyl phosphate (diC8):

Melting point 31 °C (lit. 29-30 °C)\(^1\)

\(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 9.72 (s, 1H, OH), 4.01 (2x t, 4H, J = 6.8 Hz, OCH\(_2\)), 1.67 (m, 4H, OCH\(_2\)CH\(_2\)), 1.22-1.38 (m, 20H, CH\(_3\)(CH\(_2\))\(_5\)), 0.87 (t, 6H, J = 6.9 Hz, CH\(_3\)).

\(^{13}\)C NMR (126 MHz, CDCl\(_3\)) 67.69 and 67.64 (C1 and C1'), 31.76 (C6), 30.21 and 30.15 (C2 and C2'), 29.15 (C5), 29.10 (C4), 25.42 (C3), 22.60 (C7), 14.01 (C8).

\(^{31}\)P NMR (202 MHz, CDCl\(_3\)) \(\delta\) 1.70.

ESI-TOF-MS m/z 321.22 [M-H], calcld for C\(_{16}\)H\(_{35}\)O\(_4\)P 322.23.

For NMR spectra, see Figures S4-S6.

Didodecyl phosphate (diC12):

Melting point 60 °C (lit. 59.2-62.4 °C)\(^2\)

\(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 9.62 (s, 1H, OH), 4.01 (2x t, 4H, J = 6.7 Hz, OCH\(_2\)), 1.68 (m, 4H, OCH\(_2\)CH\(_2\)), 1.24-1.38 (m, 36H, CH\(_3\)(CH\(_2\))\(_9\)), 0.88 (t, 6H, J = 7.0 Hz, CH\(_3\)).

\(^{13}\)C NMR (126 MHz, CDCl\(_3\)) 67.74 and 67.69 (C1 and C1'), 31.92 (C10), 30.23 and 30.17 (C2 and C2'), 29.66, 29.64, 29.60, 29.54, 29.35 (C5-C9), 29.18 (C4), 25.45 (C3), 22.68 (C11), 14.08 (C12).

\(^{31}\)P NMR (202 MHz, CDCl\(_3\)) \(\delta\) 2.05.

ESI-TOF-MS m/z 433.20 [M-H], calcld for C\(_{24}\)H\(_{51}\)O\(_4\)P 434.35.

For NMR spectra, see Figures S7-S9.
Figure S1. $^1$H-NMR spectrum of diC2/6.

Figure S2. $^{13}$C-NMR spectrum of diC2/6.
Figure S3. $^{31}$P-NMR spectrum of diC2/6.

Figure S4. $^1$H-NMR spectrum of diC8.
Figure S5. $^{13}$C-NMR spectrum of diC8.

Figure S6. $^{31}$P-NMR spectrum of diC8.
Figure S7. $^1$H-NMR spectrum of diC12.

Figure S8. $^{13}$C-NMR spectrum of diC12.
Elemental Analysis. Elemental analysis was used to analyze the degree of complexation in the ionic complexes. The results are presented in Table S1.

### Table S1. Elemental analysis results and calculated degrees of complexation.

<table>
<thead>
<tr>
<th>Complex</th>
<th>C&lt;sub&gt;calc.&lt;/sub&gt; (%)</th>
<th>C&lt;sub&gt;found&lt;/sub&gt; (%)</th>
<th>N&lt;sub&gt;calc.&lt;/sub&gt; (%)</th>
<th>N&lt;sub&gt;found&lt;/sub&gt; (%)</th>
<th>Degree of complexation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLL(diC2/6)&lt;sub&gt;1.0&lt;/sub&gt;</td>
<td>58.6</td>
<td>57.9</td>
<td>6.2</td>
<td>6.1</td>
<td>101</td>
</tr>
<tr>
<td>PLL(diC8)&lt;sub&gt;1.0&lt;/sub&gt;</td>
<td>58.6</td>
<td>57.7</td>
<td>6.2</td>
<td>6.2</td>
<td>99</td>
</tr>
<tr>
<td>PLL(diC12)&lt;sub&gt;1.0&lt;/sub&gt;</td>
<td>64.0</td>
<td>61.7</td>
<td>5.0</td>
<td>4.8</td>
<td>99</td>
</tr>
</tbody>
</table>
Thermogravimetric Analysis (TGA). The decomposition temperature of the complexes was studied by TGA using a Mettler TG50 unit with a Mettler TC11 TA Processor. Bulk samples were heated in open aluminum cups from 30 to 450 °C with a rate of 5 °C/min and a nitrogen purge gas flow of 200 ml/min. The TGA curves are shown in Figure S10.

![TGA curves of PLL(diC2/6)1.0, PLL(diC8)1.0 and PLL(diC12)1.0.](Figure S10. TGA curves of PLL(diC2/6)1.0, PLL(diC8)1.0 and PLL(diC12)1.0.)

SAXS Curve Fitting. Lorentzian peaks were fitted to the SAXS curves of PLL(diC2/6)1.0 at room temperature and after the heating cycle and are shown in Figure S11. The exact locations of the Lorentzian peaks are 0.247, 0.297 and 0.422 Å⁻¹ before heating and 0.284 and 0.423 Å⁻¹ after heating.

![SAXS curves of PLL(diC2/6)1.0 a) at room temperature and b) at 30 °C after thermal treatment.](Figure S11. SAXS curves of PLL(diC2/6)1.0 a) at room temperature and b) at 30 °C after thermal treatment.)
Measurements of PLL(diC8)$_{1.0}$ and PLL(diC2/6)$_{1.0}$ as a Function of Temperature.

**Figure S12.** a) FTIR spectra, b) SAXS curves and c) DSC thermograms of PLL(diC8)$_{1.0}$ as a function of temperature. d) POM micrographs of PLL(diC8)$_{1.0}$ at 25 °C and 160 °C (inset). The scale bar is 100 μm.

**Figure S13.** a) FTIR spectra, b) SAXS curves and c) DSC thermograms of PLL(diC2/6)$_{1.0}$ as a function of temperature. d) POM micrographs of PLL(diC2/6)$_{1.0}$ at 30 °C and 160 °C (inset). The scale bar is 50 μm.
Surfactant Alkyl Tail Crystallinity by FTIR. The $-\text{CH}_2-$ asymmetric and symmetric C–H stretching absorptions located at 2933–2918 and 2863–2850 cm$^{-1}$, respectively, were used to study the degree of crystallinity of the surfactant alkyl tails as a supporting method to WAXS. The FTIR spectra and plots of the absorption band locations are presented in Figure S14.

**Figure S14.** FTIR spectra from the methylene group C–H stretching absorption range and plots of the absorption band locations in a)-b) PLL(diC12)$_{1.0}$, c)-d) PLL(diC8)$_{1.0}$ and e)-f) PLL(diC2/6)$_{1.0}$ during thermal treatment.
References for Supporting Information