Poly(ethylene oxide)-*b*-poly(L-lysine) Complexes with Retinoic Acid

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ABSTRACT: Properties of the complexes formed by poly(ethylene oxide)-*b*-poly(L-lysine)s (PEO−PLL) and *all-trans*-retinoic acid were investigated by differential scanning calorimetry, wide- and small-angle X-ray scattering, circular dichroism, dynamic light scattering, and atomic force microscopy. It was determined that for micellar solutions of complexed PEO−PLL the poly(L-lysine) blocks form an α-helix structure at pH values higher than 9 and that the coil−helix transition spans from pH 3.7 to 9.0. The high stability of the α-helix was attributed to a protective effect of the retinoate moieties and the PEO surrounding. Micelle structures were found to be of core−shell type. Each micelle has a compact core, which is formed by a smectic A-like poly(L-lysine) retinoate complex. The shell is formed by the PEO. When the pH value is lowered, the micelle sizes increases. The conformation of the noncomplexed PEO−PLL in the solid state was found to be a mixture of α-helix and *β*-sheet structures while that of the complexed polymers is a pure α-helix. The PEO blocks crystallize in the solid state of the pristine and the complexed polymers, but the degree of crystallinity is considerably lower in the complexed state. Such block copolymer complexes are considered to be useful for the development of new drug delivery formulations.

Introduction

Micelles formed by amphiphilic block copolymers have been the subject of intense research during the past decade. In addition to their interesting physicochemical properties, they are promising as carriers for hydrophobic drugs. A recent review was given by Eisenberg et al.[1] In an aqueous environment, the hydrophobic blocks of the copolymer form the core of the micelle while the hydrophilic blocks form the corona. The hydrophobic micelle core serves as a microenvironment for the incorporation of lipophilic drugs, while the corona serves as a stabilizing interface between the hydrophobic core and the external medium. It was shown that complexation of PEO−PLL with DNA as an opposite charged polyelectrolyte led to the formation of water-soluble complexes in aqueous milieu.[2] PEO−PLL block copolymer micelles were used to explore the feasibility of polymeric micelles as a novel vector system for genes and oligonucleotides.[3,4] Supramolecular association of a block copolymer consisting of PEO and poly(amino acid)s through hydrophobic or electrostatic interaction leads to the formation of core−shell type micelles in which drug molecules are hydrophobically or electrostatically included in the core of the particle which is surrounded by the PEO outer shell.[5] The proper micelle size of about 50 nm and the hydrophilicity of the outer-shell PEO seem to contribute to an extension of the period for which the drug circulates in the blood. Exceptionally high accumulation in a solid tumor were demonstrated for copolymer micelles with an entrapped anticancer drug (doxorubicin).[6,7] Recently, Kabanov et al. proposed a similar system, the complex of poly(ethylene oxide)-*g*-poly(ethylenimine) and biological active surfactants, as a novel drug delivery system.[8] Such complexes form "micellar microcontainers" with retinoic acid, a molecule which is highly optically active and is considered to be a surfactant from the physicochemical point of view.

Vitamin A and its analogues, in particular retinoic acid, are involved in the proliferation and differentiation of epithelial tissues and have continued to be used in the treatment of dermatological disorders such as acne, psoriasis, and hyperkeratosis.[9,10] A lot of interest has being focused on how to understand the role of retinoic acid in cell differentiation. This is done by investigating the binding properties of retinoids on specific proteins[11,12] and its role in malignant-tumor inhibition[13,14] and in its regulation of brain functions.[15] Natural retinoids need to be bound to specific retinoic binding proteins in order to ensure their protection, solubility, and transport by body fluids. Immobilization is a major problem in administering retinoic acid as a pharmacological agent. One way of achieving such immobilization and protection of retinoic acid is by binding it to a protein as occurs in nature. A successful example for mimicking nature's strategy was demonstrated by Zanotti[5] et al. when he cocrystallized transthyretin and retinoic acid. This, however, is a difficult and costly procedure. We have developed an easier and less expensive method for the required immobilization by the complexation of retinoic acid with synthetic cationic polyelectrolytes.[16,17] Further, we have shown that nanoparticles of complexes of synthetic poly(amino acid)s with retinoic acid contain an internal smectic A-like structure.[18]

We report here on the physicochemical characteristics of water-soluble complexes formed between PEO−PLL block copolymers and retinoic acid. The structures in the solid state and solution are discussed. Further, we

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discuss the dissociation of the complex when changing the pH.

Experimental Section

Materials. Crystalline *all-trans*-retinoic acid (tretinoin, vitamin A acid) powder, ϵ-(benzyloxycarbonyl)-L-lysine, bis-(trichloromethyl) carbonate (triphosgene), anisole, and petrol ether were purchased from Fluka and used without further purification. Trifluoroacetic acid, triethylamine, and methanesulfonic acid were purchased from Aldrich and used as received. Tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF) were doubly distilled by normal methods.[19] α-Methoxy-ω-aminopoly(ethylene oxide) (PEO; $M_w = 5000$, $M_w/M_n = 1.10$, functionality of amino groups, 0.96) was purchased from Sigma. The polymer was dissolved in benzene (1.0 g/mL), followed by freeze-drying to obtain the sample used for the block copolymer synthesis.

Synthesis of Block Copolymers. 1. *N*-Carboxyanhydride of ϵ-(Benzyloxycarbonyl)-L-lysine. The synthesis of *N*-carboxyanhydride of ϵ-(benzyloxycarbonyl)-L-lysine (Lys(Z)-NCA) was carried out by the Fuchs−Farthing method using triphosgene.[20] A suspension of 4 g (14.3 mmol) of Lys(Z) in 100 mL of THF was heated to 40 °C in an argon atmosphere. A solution of 1.7 g (5.7 mmol) of triphosgene dissolved in 10 mL of THF was added dropwise to the stirred reaction mixture. When the reaction mixture started to become transparent, a stream of dry argon was bubbled through the solution to remove HCl. After the reaction was complete (approximately 45 min) the solvent was evaporated under reduced pressure to give a colorless oily residue which crystallized upon cooling. For further purification the obtained Lys(Z)−NCA was recrystallized three times from a mixture of THF/petrol ether and dried immediately at room temperature in a vacuum. The yield was 3.9 g (90%). The composition of Lys(Z)−NCA was determined by 400 MHz ^{1}H NMR (Bruker DPX-400) in $CDCl_3$.

2. Poly(ethylene oxide)−Poly[ϵ-(benzyloxycarbonyl)-L-lysine] Block Copolymer (PEO−P(Lys(Z)). A total of 1 g (3.26 mmol) of Lys(Z)−NCA was dissolved in 10 mL of DMF, and the solution was degassed. Then 0.83 g (0.17 mmol) of α-methoxy-ω-aminopoly(ethylene oxide) was dissolved in 10 mL of THF and added to the solution of Lys(Z)−NCA. The reaction mixture was stirred for 72 h at 40 °C under a dry nitrogen atmosphere. Then the solvent was evaporated under reduced pressure, and the residue obtained was diluted with 10 mL of CH_2Cl_2 and then precipitated into 500 mL of diethyl ether. The yield was 1.34 g (78%). The composition of PEO−P(Lys(Z)) was determined by 400 MHz ^{1}H NMR (Bruker DPX-400) in DMSO-d_6. This polymer was used for the synthesis of PEO−PLL18.

3. Poly(ethylene oxide)−Poly(L-lysine) Block Copolymer (PEO−P(Lys)). A total of 0.66 g of PEO−P(Lys-Z) was dissolved in 6 mL of trifluoroacetic acid and stirred for 1 h. Then 6 mL of anisole and 4.5 mL of methanesulfonic acid were added to the reaction mixture. After stirring for an additional 1.5 h the solution was diluted with 30 mL of distilled water and extracted with 30 mL portions of diethyl ether (total 300 mL) in order to remove the anisole and the excess acids. The water phase was neutralized with triethylamine and dialyzed with distilled water using a Spectrapor dialysis membrane (molecular weight cutoff = 1000). The PEO−P(Lys) was obtained as a white powder after lyophilization. The yield was 0.42 g (85%). The ^{1}H NMR measurement (Bruker DPX-400) in D_2O was carried out to determine the composition. For PEO−P(Lys), the peak intensity ratio of methylene protons of PEO (OCH_2CH_2: $\delta = 3.7$ ppm) and ϵ-methylene protons of P(Lys) ($(CH_2)_3CH_2NH_3$: $\delta = 3.0$ ppm) was measured to calculate the polymerization degree (DP), which was determined to be 18. Both calculation for PEO−P(Lys-(Z)) and PEO−P(Lys) gave the same DP value of lysine units (DP = 19), indicating that a loss of lysine units by main-chain cleavage during the deprotection was negligible. The unimodal distribution of the PEO−P(Lys(Z)) was confirmed by SEC with $CHCl_3$ as described earlier.[5] This polymer with a poly(L-lysine) block length of 18 is shortened as PEO−PLL18, and the analogously synthesized polymer with a poly(L-lysine) block length of 30 is shortened as PEO−PLL30.

Complex Formation. For complex formation 0.1 g (0.33 mmol) of retinoic acid was dissolved in 40 mL of water at pH 9. One equivalent of PEO−PLL18 (97.3 mg) and PEO−PLL30 (149.7 mg) were each dissolved in 15 mL water (pH 9). The stoichiometry was calculated with respect to the amino functions. While stirring, the polymer solutions were slowly added to the solution of retinoic acid within a period of 15 min. The color of the retinoic acid solution changed from intense yellow to a light orange. No precipitation was observed for both polymers. The resulting micellar solutions were investigated by dynamic light scattering at pH 9. The average particle diameters at pH 9 are about 60 nm. Typical polydispersities were 0.25. Micellar solutions of complexes were cast on glass plates to form films of the complexes in their solid state. The two-dimensional geometry of the films was stabilized by a glass frame, which was mounted on top of the glass sheet in the same way as in the procedure described earlier.[21]

Methods. Differential scanning calorimetry (DSC) measurements were performed on a Netsch DSC 200. The samples were examined at a scanning rate of 10 K/min by applying one cooling and two heating scans. The anisotropic optical properties of complex films were examined by using a polarization microscope (Orthoplan-pol, Leitz) with an angle of 90° between polarizer and analyzer. Wide-angle X-ray scattering (WAXS) measurements were carried out with a Nonius PDS120 powder diffractometer by transmission geometry. A FR590 generator was used as the source for Cu Kα radiation, monochromatization of the primary beam was achieved by means of a curved Ge crystal, and the scattered radiation was measured with a Nonius CPS120 position-sensitive detector. The resolution of this detector in 2θ is 0.018°. Small-angle X-ray scattering (SAXS) measurements were carried out with an X-ray vacuum camera with pinhole collimation (Anton Paar, Austria, model A-8054) equipped with image plates (type BAS III, Fuji). The image plates were read with a MAC Science dip-scanner IPR-420 and IP reader DIPR-420. The dynamic light scattering measurements were carried out with a submicron particle sizer, model 370 (Nicomp). The scanning force microscopy was performed with a Nano Scope III a microscope (Digital Instruments, Santa Barbara, CA) operating in tapping mode. The instrument was equipped with a 10×10 μm E-scanner and commercial silicon tips (model TESP, force constant 50 N/m, resonance frequency 300 kHz, tip radius smaller than 20 nm). The samples were prepared by letting droplets of diluted aqueous solutions (0.01% w/w) dry on freshly cleaved muscovite mica surfaces at room temperature. IR spectroscopy was performed on a Bio Rad 6000 FT-IR spectrometer.

Results and Discussion

Crystallinity. Figure 2a,b shows DSC traces of the copolymers and of their complexes with retinoic acid in the solid state. It can be seen there that the two noncomplexed polymers undergo first-order transitions upon heating with maxima at 56 ± 1 °C (dashed lines). Upon cooling, the transitions were found at 35 ± 2 °C. The DSC traces of the complexes differ significantly from those of the pristine polymers. A weaker transition is present in the curve of PEO−PLL18 retinoate upon heating at 47 and at 23 °C upon cooling (Figure 2a, solid lines). In the curves of PEO−PLL30 retinoate a very weak transition at 38 °C was detected upon heating, while it was not found upon cooling (Figure 2b, solid lines). No other transitions were observed in the temperature range 0−150 °C. Homopolymers of poly(ethylene oxide) are known to form lamellar crystals with melt transitions that depend significantly on their molecular weights. As an example, the melting points of poly(ethylene oxide)s with narrow molecular weight distributions are 48, 58, and 64 °C for molecular weights of 1500, 3000, and 6000 g/mol, respectively.[22]

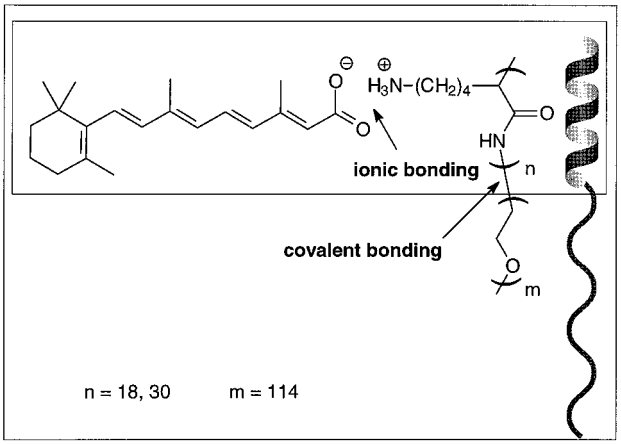

Figure 1. Molecular structure of a poly(ethylene oxide)-*b*-poly(L-lysine) retinoate complex and a sketch of the polymeric backbone with an α-helical poly(L-lysine) block.

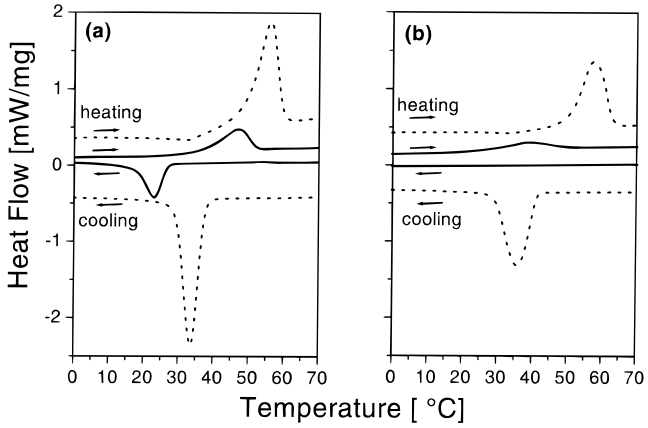

Figure 2. DSC heating and cooling traces of the copolymer PEO−PLL18 (a, dashed line) and its complex with retinoic acid (a, solid line). The heating and cooling traces of PEO−PLL30 and PEO−PLL30 retinoate are given in (b) (dashed and dotted lines, respectively).

No melt transition had been found earlier for the complex of a homopolymer of poly(L-lysine) and retinoic acid,[18] and the melting point of retinoic acid is 181 °C.[23] Therefore, we assign the transitions in the copolymers and in their complexes as due to the melting of the crystalline poly(ethylene oxide) segments. Compared to a poly(ethylene oxide) homopolymer with a molecular weight of 5000 g/mol, its copolymerization with a poly(L-lysine) block lowers the melting transition of poly(ethylene oxide) by about 5 °C. Here the melting point depression does not depend on the length of the lysine block. For the complexes the lowering of the melt transition temperature is much stronger and depends on the length of the lysine block (17 and 26 °C for PEO−PLL18 retinoate and PEO−PLL30 retinoate, respectively). We compared the measured enthalpies of the melt transitions with the perfect heat of fusion of PEO (203 J/g)[24] in order to estimate the degree of the bulk crystallinity. Taking into account the different amount of PEO in the compounds, we found the crystallinity of the PEO chains to be about 50% (PEO−PLL18), 45% (PEO−PLL30), 30% (PEO−PLL18 retinoate), and 10% (PEO−PLL30 retinoate). Compared to neat PEO whose crystallinity is in the range 70−80%,[24] the copolymerization with lysine reduces the degree of crystallinity by 20−30%. This is similar to the effect of blending PEO with PMMA.[24] In contrast to the copolymerization, the complexation reduces the degree of crystalline PEO even more strongly. Probably the complexed moieties disturb the formation of the extended chain conformation and the integral number of folded chain conformations, which are typical for the crystalline form of PEO.[25] The conclusion that crystalline PEO segments are the origin of the endothermic transitions was confirmed by wide-angle X-ray experiments. It can be seen in Figure 3 that the scattering intensities of the PEO reflections at scattering vectors of $s = 2.18$ and 2.65 nm^{-1} decrease in the line PEO−PLL18, PEO−PLL30, PEO−PLL18 retinoate, and PEO−PLL30 retinoate. This sequence of the intensities is in agreement with a decreasing amount of crystalline PEO segments in the samples derived by DSC.

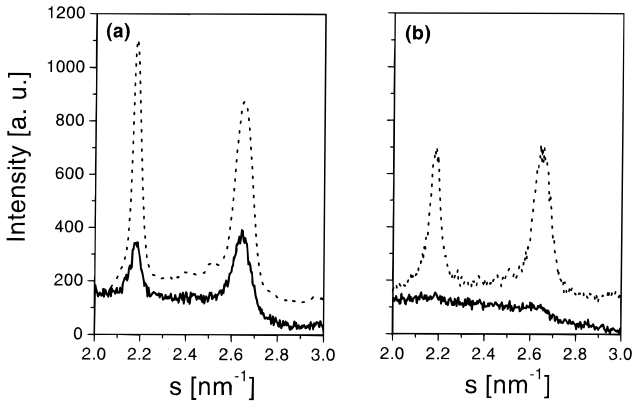

Figure 3. Wide-angle X-ray scattering in the region of the most intense reflections of PEO−PLL18 (dashed line) and PEO−PLL18 retinoate (solid line) are given in (a). The corresponding curves of PEO−PLL30 (dashed line) and PEO−PLL30 retinoate are given in (b).

Supramolecular Solid-State Structure. Small-angle X-ray scattering techniques were used to investigate the structure of the PEO−PLL polymers and their complexes on length scales in the range 1−50 nm. The small-angle scattering diagrams of the pristine PEO−PLL18 and PEO−PLL30 are essentially identical and show two Bragg peaks whose positions have a ratio of $1:3^{1/2}$ (not shown). This suggests a hexagonal arrangement of the poly(L-lysine) chains in a two-dimensional lattice with a center-to-center distance of 1.53 nm. This value is close to the chain spacing in poly(L-lysine) hydrochloride crystals (1.50 nm).[26]

In contrast to the pristine polymers, Bragg peaks with relative positions of 1:2 were found in the small-angle scattering diagrams of PEO−PLL18 retinoate and PEO−PLL30 retinoate, which indicate lamellar structures (see Figure 4a,b). The long periods are 3.37 ± 0.05 nm (PEO−PLL18 retinoate) and 3.32 ± 0.05 nm (PEO−PLL30 retinoate), which is slightly larger than that found for a complex of a poly(L-lysine) homopolymer (M_v = 15 000−30 000 g/mol) and retinoate, which is 3.10 nm.[18] This is attributed to a better packing of the latter as a result of the absence of packing constraints caused by PEO blocks. It can be seen in the scattering diagrams that the peaks of the PEO−PLL18 retinoate are broader than that of the PEO−PLL30 retinoate. The correlation lengths were 10 and 12 nm as determined from the width of the first-order reflection. When taking into account the relative shortness of the lysine chains, the high degree of mesomorphous ordering is surprising. Therefore, well-defined conformations of the poly(L-lysine) chains have to be taken into account.

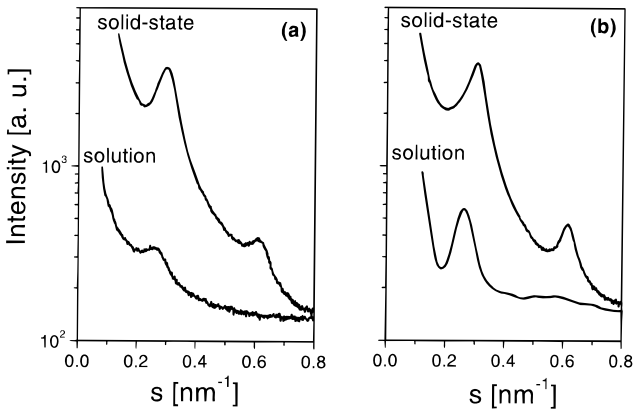

Figure 4. Small-angle X-ray scattering curves of PEO−PLL18 retinoate (a) and PEO−PLL30 retinoate (b). Curves are given for the materials in the solid state (upper curves) and for dispersion in aqueous solution (lower curves).

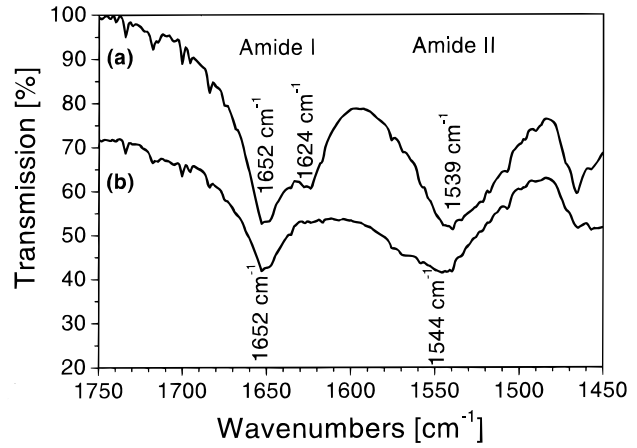

Figure 5. FTIR spectra of the PEO−PLL18 (a) and the PEO−PLL30 retinoate (b) in the amide region.

We found that in the solid-state form of the noncomplexed polymers the poly(L-lysine) chains adopt mixtures of α-helix and β-conformations as shown by the position of the amide I and amide II vibrations in the FTIR spectrum[27] (see Figure 5). It can be seen that the amide I vibration of the α-helix is represented by a band at 1652 cm^{-1} and that of the β-sheet by a band at 1624 cm^{-1}. The individual amide II vibration bands overlap, but the band at 1539 cm^{-1} is strongly indicative of significant amounts of β-sheet conformations. It was found that the intensity of the bands and consequently the amount of α-helix and β-sheet conformations depend in a significant way on the preparation conditions such as solvent, temperature, and drying time. Therefore, we were not able to identify differences in the conformations between PEO−PLL18 and PEO−PLL30.

In contrast to the noncomplexed polymers, we found the positions of the amide I and amide II bands to be independent of the preparation conditions at 1652 and 1544 cm^{-1} for PEO−PLL18 retinoate and PEO−PLL30 retinoate (Figure 5b). This proves that the poly(L-lysine) chains strictly adopt an α-helix in the complexes. We suggest that the α-helix in the PEO−PLL retinoate complexes is energetically more stable than the β-sheet conformation, which is proably due to a better spherical arrangement of the retinoate moieties when bound to an α-helix. Tirrell et al. reported on the structure of poly(L-lysine) complexes with alkyl sulfates which form lamellar structures.[28] They found that the α-helical and β-sheet portions of the poly(L-lysine) chains in their complexes depend to a great extent on the conditions used for the preparation of the complexes from solution to the solid state. The exact reason for the adjustment of the different chain conformation is not clear yet, but we assume that the formation of interchain hydrogen bridges, which favors the β-sheet formation, is strongly suppressed for the two PEO−PLL retinoate complexes but not for the complexes of poly(-L-lysine) with alkyl sulfates.

Core−Shell Micelles. Block copolymers containing a PEO block as a water-soluble segment and a second, water-insoluble block are known to be suitable to form core−shell micelles whose cores can be loaded with hydrophobic drugs.[1] In expansion of this hydrophilic−hydrophobic concept, we converted the double hydrophilic PEO−PLL18 and PEO−PLL30 block copolymers into hydrophilic−hydrophobic ones by their complexation with retinoic acid, which resulted in micellar solutions of the complexes. The solutions were investigated by X-ray scattering in an aqueous medium in the concentration range 0.5−5% (w/w). The absence of reflections in the wide-angle scattering diagrams proved that no crystalline retinoic acid was formed during the complexation and that the PEO blocks are dissolved. In contrast to the wide-angle region, sharp reflections were found in the small-angle diagrams of the micelles (see Figure 4a,b) with Bragg spacings of 3.94 nm (PEO−PLL18 retinoate) and 3.83 nm (PEO−PLL30 retinoate). We interpret this as resulting from mesomorphous core−shell micelles. The core which contains the PLL-retinoate moieties is of a lamellar structure, and the shell is formed by PEO segments. The higher long period in the micelles compared to that of the complexes in the bulk material could be explained by the incorporation of water into the core. From the differences of the long periods between the solid-state complexes and the dispersed complexes the water content of the cores was estimated to be 17% (v/v) for PEO−PLL18 retinoate and 15% (v/v) for PEO−PLL30 retinoate. These values are close to the water uptake (18%) of a polyelectrolyte−fluorosurfactant complex, in which the water is predominantly located around the polymeric backbone.[29] The location of the incorporated water and its mobilizing effect on the molecular dynamics are given in a detailed solid-state NMR investigation on polyelectrolyte−surfactant complexes.[30]

From the widths of the reflections of the micellar solutions (Figure 4) we determined the correlation lengths of the mesophases within the cores to be 6 nm (PEO−PLL18 retinoate) and 10 nm (PEO−PLL30 retinoate). It is known that, in a helical poly(amino acid), the amino acid monomer expands over 0.15 nm.[31,32] In the case of poly(L-lysine)s (polymerization degree of lysine = 18 and 30) the helical end-to-end distances were calculated to be 2.7 and 4.5 nm. If we tentatively assume an α-helical conformation for the complexed PLL in the core of the micelles, the maximum lamellae stack size is about the same magnitude as the correlation lengths. Therefore, we assume that each micellar core contains a lamellar monodomain. Ten Brinke et al. have recently shown that a hierarchy of two different length scales (4.8 and 35.0 nm) can be produced when combining covalent bonding (block copolymer)s, proton transfer, and hydrogen bonding.[33] PEO−PLL18 retinoate and PEO−PLL30 retinoate in the solid state could be considered to be similar to their block copolymer complexes. But in addition to the lamellar structuring

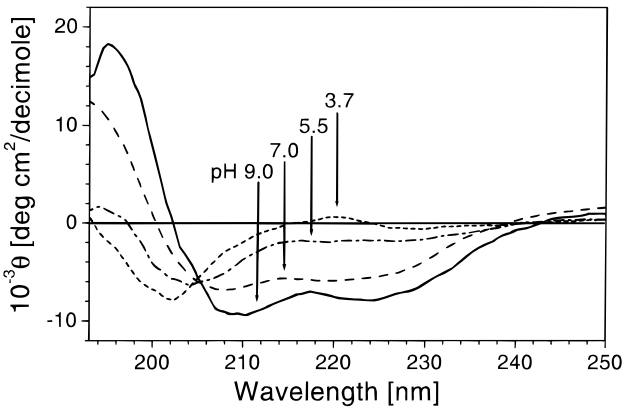

Figure 6. Circular dichroism spectra of PEO−PLL18 retinoate as a function of the pH value.

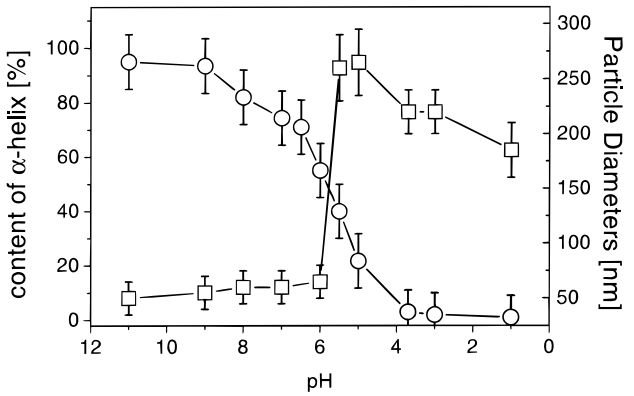

Figure 7. Variation of the content of the complex in an α-helical state (circles) and the particle diameters of the PEO−PLL18 retinoate (squares) depend on the pH value.

of the PLL retinoate, we could not identify a regular structure with a larger long period (10−50 nm), which would have been expected from a microphase separation of PEO and PLL retinoate.

Helix−Coil Transition. A detailed investigation of the helix−coil transition of the noncomplexed PEO−PLL18 has been reported by Kataoka et al.[34] By using circular dichroism, they have revealed that the PLL blocks of PEO−PLL18, which themselves cannot form an α-helix structure due to substantially lower molecular weight, form an α-helix structure in solution when they are bound to PEO. Their work shows clearly that the copolymerization with PEO has a stabilizing effect on the α-helical conformation of PLL. Bearing this in mind, we asked how the complexation of the PEO−PLL polymers affect the conformation of the PLL blocks. Circular dichroism was used as a sensitive method for the detection of the PLL chain conformation within the micelles. It was found that the shape of the circular dichroism spectra were neither dependent on the concentration of the complexes in a concentration range of 0.05−0.5% (w/w) nor does it depend on the storage time after preparation (1, 24, and 72 h). In addition, the spectra of PEO−PLL18 retinoate and PEO−PLL30 retinoate are identical within the range of experimental error. But, as expected, the spectra change with the variation of the pH value. This is shown in Figure 6. It can be seen that a right-handed α-helix structure, which is characterized by a maximum at 191 nm and two minima at 210 and 222 nm, is present at pH 9. A sketch of the polymer conformation is given in Figure 1. The circular dichroism spectra change gradually when lowering the pH and adopt the form of a random coil at pH 3.7, the spectrum of which is characterized by a minimum at about 201 nm and a maximum at about 220 nm. The crossover wavelength remained at 204 nm. It should be noted that this crossover wavelength agreed well with that of the pH-induced helix−coil transition for poly(L-lysine) and PEO−PLL reported in the literature.[34−36] The contributions of β-sheet structures to the spectra were not found to be present. We conclude that the PLL conformation in the PEO−PLL18 retinoate and PEO−PLL30 retinoate micelles adopts an α-helix for pH values higher than 9 and a random coil at a pH lower than 3.7, while between these limiting values a mixture of α-helical and random coil structures is present. According to Greenfield and Fasman,[36] the content of α-helices was estimated from the mean molar ellipticity at a wavelength of 222 nm. It can be seen in Figure 7 that the α-helix content decreases gradually from pH 9 and pH 7 from about 95% to 70%. This is followed by a steep decrease between pH 7 and pH 5 from 70 to 20% and approaches zero at about pH 3.7. The stability of the α-helix within the complexes is remarkable when bearing in mind that the helix−coil transition of poly(L-lysine) homopolymers is found at pH 10.3 and 25 °C where the polymer is 35% charged.[37] We assume that the α-helix of the complexes is stabilized, first by the PEO corona and second by protecting retinoate molecules. This process ends when the retinoate moieties become protonated. This assumption is supported by the pK_a of retinoic acid, which is in the range 6−8 and depends strongly on its surroundings, concentration, and microenvironment.[38,39]

To characterize the size of the micelles, samples of micellar solutions were stored and analyzed by dynamic light scattering directly after the circular dichroism measurement. It was found that the mean particle size was dependent on the pH value. The particle sizes of PEO−PLL18 retinoate and PEO−PLL30 retinoate are essentially the same. At a high pH, where the α-helical conformation of the PLL is predominant and where the retinoic acid has a deprotonated form, the smallest particle sizes are observed. In the range form pH 11 to pH 6 the mean particle diameter increases slightly from 50 to 60 nm. Then at pH 5.5 the mean diameter increases to values higher than 250 nm, and it reduces constantly down to values of about 180 nm at a pH of 1. The pH dependency of the particle sizes was confirmed by AFM measurements. An example of PEO−PLL30 retinoate at pH 9 is shown in Figure 8. The core−shell morphology leads to significantly different amplitudes at the center of the micelles than those in their corona. Exact measurements of the core diameters are not possible on the basis of the AFM data due to the softness of the PEO, but the micellar sizes are relatively uniform and agree essentially with the correlation lengths determined by small-angle X-ray scattering. It is notable that the particle sizes increase strongly within a small range of variation of the pH from 6.0 to 5.5. This is exactly in the region where the content of α-helical conformation decreases most strongly. Our interpretation is that the retinoic acid is bound ionically to the PLL segments when the pH is higher than 6. This leads to small core−shell micelles with a compact core. At a pH of 5.5 the ionic bonds are broken, the micellar core dissolves, or the distinct core−shell structure becomes more diffuse in the sense that a broad core−shell transition develops. Because of the low cmc of retinoic acid of about 2×10^{-6} mol/L (pH 7) in aqueous

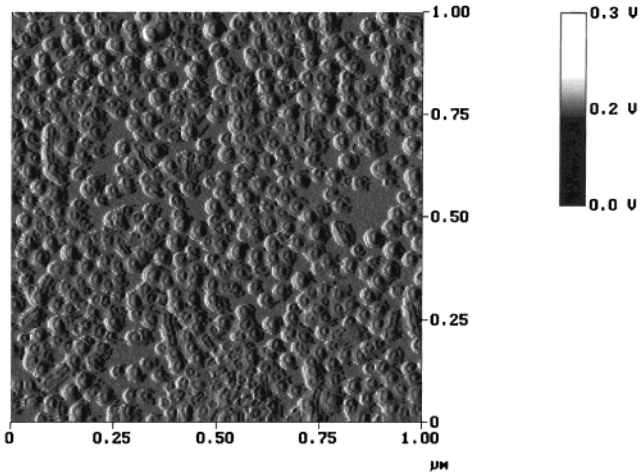

Figure 8. AFM amplitude picture of a PEO−PLL30 retinoate dispersion (pH 9) dried on mica.

environment, it is probable that the retinoic acid clusters within the micelles and do not diffuse into the aqueous surroundings. The micelle is the most lipophilic environment for the retinoic acid in its protonated form. On the other hand, the dissolution of the compact micellar core into a number of hydrophobic fragments could explain the strong increase in the particle sizes. It was found that the largest particle sizes are found around pH 5. When the pH is lowered, the particle sizes decrease significantly. This is proably due to a rearrangement of retinoic acid clusters due to the lowering of the pH.

Conclusion

In this study, we have found that complexes of poly(ethylene oxide)-*b*-poly(L-lysine) with retinoic acid with short poly(L-lysine) segments of 18 and 30 monomers form core shell micelles. The cores of the micelles contain a lamellar smectic A-like structure, formed by a poly(L-lysine) retinoate complex, which is surrounded by a corona of poly(ethylene oxide). Although the poly(L-lysine) chains are relatively short, they adopt an α-helical conformation to a pH as low as 9. This effective stabilization of the α-helix structure seems to be due to the formation of a protective surrounding coat of retinoate and a shell of poly(ethylene oxide). This novel type of supramolecular complex of the block copolymer with a particular conformation may have potential utility as pH-sensitive drug carrier system.

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