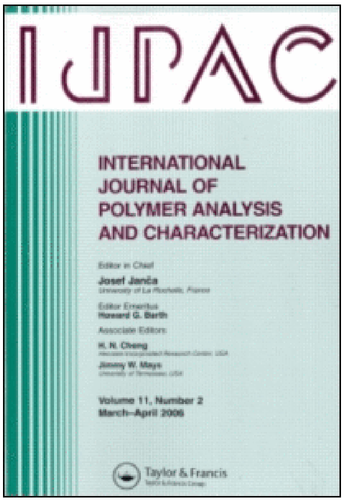

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Martin Müller[a]; Wuye Ouyang[a]; Bernd Keßler[a]
[a] Department of Surface Modification, Leibniz-Institute of Polymer Research Dresden e.V., Dresden, Germany

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Orientation of Polyelectrolyte Multilayers Containing Charged Polypeptides Determined by ATR-FTIR and AFM

Martin Müller, Wuye Ouyang, and Bernd Keßler
Leibniz-Institute of Polymer Research Dresden e.V., Department of Surface Modification, Dresden, Germany

Abstract: We report on factors influencing the orientation of polyelectrolyte multilayers (PEM) containing charged polypeptides. As factors, the presence and absence of mechanical unidirectional texturization, the conformation, and the molecular weight will be outlined in four typical samples. For that, the charged cationic homopolypeptide poly(L-lysine) (PLL) was used, whose conformations can be changed from random coil to α-helix by salt type or pH value. PEM of PLL and poly(vinylsulfate) (PVS) were prepared by consecutive adsorption. Dichroic in situ attenuated total reflection infrared (ATR-IR) spectroscopy and atomic force microscopy (AFM) were used for orientation and surface topology characterization, respectively. Untexturized surfaces, the random coil conformation, and low molecular weight of PLL caused the formation of isotropically structured PEM films, and the α-helical conformation and the high molecular weight of PLL resulted in anisotropic PEM films. Polypeptide-containing PEM could be used as biomimetic coatings for specific interaction with α-helical proteins.

Keywords: AFM; ATR IR; IR dichroism; Nanostructure; Polyelectrolyte complex; Polyelectrolyte multilayer; Polymer orientation; Polypeptide conformation

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Address correspondence to Martin Müller, Leibniz-Institute of Polymer Research Dresden e. V., Department of Surface Modification, Hohe Strasse 6, 01069 Dresden, Germany. E-mail: mamuller@ipfdd.de

INTRODUCTION

Electrostatically self-assembled structures like polyelectrolyte complex (PEC) particles or polyelectrolyte multilayers (PEM) are of increasing interest since they can be generated with low experimental expense from aqueous solutions using low-cost commercial or natural polyelectrolytes (PEL). Such PEL complexes can be prepared by two methods: (i) mixing oppositely charged PEL in solution leads to dispersed PEC nanoparticles known from the work of Bungenberg de Jong,[1] Michaels et al.,[2] Kabanov and Zezin,[3] and Philipp et al.[4] and (ii) consecutively adsorbing those PEL at solid supports or template particles results in PEL multilayer (PEM) coatings known from the work of Decher et al.[5] and Donath et al.[6]

Herein we focus on PEMs that allow for defined nanoarchitectures and selective surface modification and are challenging objects for polyelectrolyte theory. Initially, PEMs were claimed to be composed of stratified individual layers. However, this model was revised and replaced by another one according to which commonly used flexible polyelectrolytes cause high entanglement, no distinct layering, and a low degree of order in the internal structure.[7,8] Nevertheless, the creation of PEMs consisting of stratified or lamellar arranged PEL layers is an interesting task. Macromolecular order in PEM can be obtained by several approaches. Besides PEM composed of layered silicates[9] (i), those composed of hydrophobic ionenes[10] have been used (ii). Recently, as a third concept, PEM composed of charged stiff α-helical polypeptides alternating with oppositely charged strong polyanions or polycations were introduced[11,12] (iii) and shall be reviewed and extended herein. Generally, PEMs consisting of charged polypeptides are subjects of fundamental structural studies[13,14,15] as well as of biomedical and pharmaceutical applications.[16,17,18,19] Furthermore, PEMs exposing polypeptides in both defined conformations and orientations might be a new strategy for biomimetic surface modification, as will be shown in future work.

EXPERIMENTAL SECTION

Polyelectrolytes

Poly(L-lysine) (PLL) with M_w of 25.700 and 205.000 g/mol was supplied by Sigma-Aldrich (Steinheim, Germany). Poly(vinylsulfate) (PVS, $M_w = 162.000$ g/mol) was from Polysciences (Warrington, USA), and sodium perchlorate ($NaClO_4$) was from Merck (Darmstadt, Germany). PLL and PVS solutions ($c_{PEL} = 0.01$ M) were prepared either at pH = 6 or pH = 11 or in the presence of 1 M $NaClO_4$.

Surfaces

The silicon substrates were cleaned and texturized by mechanical treatment[5] as previously reported.

Polyelectrolyte Multilayers (PEMs)

Multilayers of oppositely charged polyelectrolytes (PEMs) were fabricated on the Si-IRE surfaces in the sample compartment of the attenuated total reflection infrared(ATR-IR) sorption cell (IPF Dresden) by consecutive adsorption/rinsing cycles, according to the stream coating procedure described by Müller et al.[20] using manual injection. The PEMs were dried by a gentle N_2 stream above the Si-IRE in the sample compartment. PEM-5 resulted in brownish films of a thickness around 50–100 nm (empirical determination by the film color).

ATR-FTIR Spectroscopy

The monitoring of consecutive deposition of the polyelectrolytes and the characterization of the deposited PEMs was performed by in situ ATR-FTIR spectroscopy using the single-beam sample reference (SBSR) concept[21] to obtain well-compensated ATR-FTIR spectra, as described by Müller et al.[20] Dichroic measurements and data analysis were performed according to a methodology reported by Müller et al.[12] IR light was polarized by a wire grid polarizer (SPECAC, UK). The ATR-FTIR attachment was operated on the IFS 55 Equinox spectrometer (Bruker-Optics GmbH, Leipzig) equipped with globar source and mercurium cadmium telluride (MCT) detector. Typically polycation, rinsing (1 M $NaClO_4$), and polyanion solutions were stepwise injected by syringes in the in situ measuring cell (M. M., IPF Dresden), each step having a residence time of 15 min. After each rinsing step p- and s-polarized ATR-FTIR spectra were recorded, for which 200 scans were accumulated. To check for undesired time-dependent variations, the polarized spectra (p, s) were recorded in the sequence p-s-p and the first p-polarized spectrum was compared with the second one. No spectral differences should appear between the two p-polarized spectra. Either peak intensities or integrated band areas were used for the dichroic ratio determination of the amide bands.

Orientation Analysis

For the quantitative analysis of dichroic ATR-IR data, a formalism was applied developed by Zbinden[22] and Fringeli et al.[23] for polymer and smaller liquid crystalline compounds and adapted to assembled α-helical polypeptide systems.[12] In Equation (1), the experimental dichroic ratio measured in the ATR mode R_y^{ATR} of A_p and A_s, which are the integrated absorbances of a given band measured with p- and with s-polarized light, is given.

$$R_y^{ATR} = \frac{A_p}{A_s} \tag{1}$$

For simplification R_y^{ATR}, the dichroic ratio measured in ATR mode, can be converted by Equation (2) into a dichroic ratio R^T, which would have been measured in transmission mode, knowing the amplitudes of the relative electric field components (E_x, E_y, E_z) of the evanescent wave.

$$R^T = R_y^{ATR} \cdot \frac{E_y^2}{(E_x^2 + E_z^2)} \tag{2}$$

Based on a cone model from this R^T value, an order parameter S can be calculated knowing the angle θ between the transition dipole moment M and the molecular main axis (e.g., the helical axis for polypeptides) according to

$$S = \frac{(1 - R^T)}{(2R^T + 1)} \cdot \frac{2}{(3\cos^2\theta - 1)} \tag{3}$$

Generally, the values of $S = 0$ are obtained for no order, $S = 1$ for high order or parallel arrangements, and $S = -1/2$ for vertical arrangements with respect to a texture or a physical drawing direction. Since in a macromolecule R^T values can be determined for lots of vibration bands, a molecular model can be built based on their various relative transition dipole moment angles. Finally the above introduced order parameter S is directly related to the cone opening angle γ by

$$\gamma_0 = \arccos\left(\sqrt{\frac{2}{3}S + \frac{1}{3}}\right) \tag{4}$$

Accordingly, high values of γ correspond to low-order parameters S or to a low degree of unidirectional alignment of the polymer rods. For polypeptides, the θ values for the Amide I and Amide II bands and further for the Amide A band (around 3300 cm^{-1}) are known from the literature,[24,25] according to which $\theta_{\text{AMIDE A}} = 28°$, $\theta_{\text{AMIDE I}} = 38°$, $\theta_{\text{AMIDE II}} = 73°$.

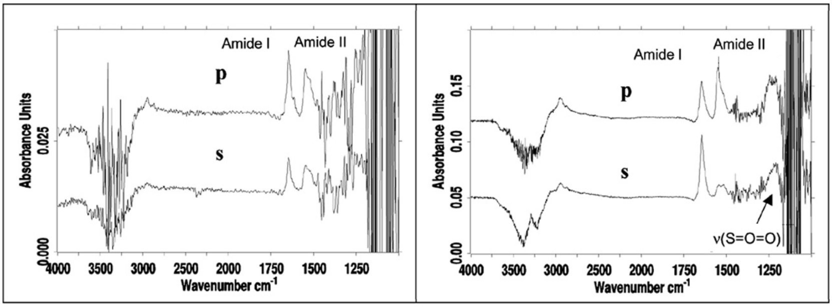
RESULTS AND DISCUSSION

In the following we partly review former spectroscopic results on oriented PEM and present new microscopic results on factors influencing their macromoleular order at surfaces. As factors of the presence and absence of mechanical unidirectional texturization, the conformation and the molecular weight (M_w) will be outlined in four typical samples (A, B, C, D). For that, the charged cationic homopolypeptide poly(L-lysine) (PLL) and the strong polyanion PVS was used. The α-helical conformation of PLL was either induced by the presence of perchlorate anions or by $pH = 11$, while the random coil conformation was obtained in the absence of perchlorate anions at $pH = 6$, as we have proved by circular dichroism (CD) spectroscopy.[11,12] In the following, first the results from ATR-FTIR dichroism on the molecular orientation of PEM-PLL/PVS and, second, AFM results on the nano- and microscopic level are presented.

Dichroic ATR-FTIR Characterization

Access to PLL orientation within PEM can be obtained via dichroic ATR-FTIR spectroscopy, as shown in Figure 1. The p- and s-polarized spectra of the PEM-5 of PLL/PVS in the presence of $NaClO_4$ are shown for PLL with $M_w = 25.700$ g/mol (further denoted as PLL-25.700, Figure 1(a)) and with 205.000 g/mol (further denoted as PLL-205.000, Figure 1(b)).[26]

Qualitatively, for PLL-205.000 a high dichroic effect was observed, i.e., the ratios with respect to the amide I and the amide II bands were differing very much. This was not the case for PLL-25.700, where the R_y^{ATR} value of Amide I was about the same of Amide II. Furthermore, we recorded dichroic ATR-FTIR spectra for PEM-5 of PLL-205.000/PVS PVS with PLL being in the random conformation and for PEM-5 of α-PLL-205.000/PVS (ATR-FTIR spectra not shown). The dichroic ratios of the samples arbitrarily denoted as A, B, C, and D are summarized in Table I. Generally, for IR spectra on PEM in contact with water, the order parameters S were based on the amide II band, since the amide I



(a) (b)

Figure 1. p- and s-polarized ATR-FTIR spectra of the PEM-5 of PLL (25.700 g/mol (a) and 205.000 g/mol (b)) and PVS in the presence of 1 M $NaClO_4$. (From Müller et al.[26] with kind permission of Wiley-VCH.)

Table I. Dichroic ratio R^T based on the amide II band, order parameter S, and opening angle γ of four samples of PEM-5 of PLL/PVS deposited onto silicon supports varied by M_w, conformation, and surface texture

	A[12]	B[26]	C	D[26]
Substrate PLL	Untexturized	Texturized	Texturized	Texturized
conformation	α-helical	α-helical	random coil	α-helical
PLL M_w/[g/Mol]	205.000	205.000	205.000	25.700
R^T	2.20 ± 0.10	4.47 ± 0.20	1.17 ± 0.20	1.74 ± 0.10
S	0.29 ± 0.04	0.75 ± 0.03	−0.26 ± 0.15	0.10 ± 0.04
γ	43° ± 2°	24° ± 2°	66° ± 7°	51° ± 2°

The given errors are based on uncertainties due to amide II band integration.

band might be slightly interfered by the δ(OH) band of water due to incomplete spectral compensation.

From the listed order parameters and opening angles of the four PEM samples, different orientation levels could be qualitatively estimated. At first, sample A, which was deposited from high molecular weight α-helical PLL-205.000 solutions and PVS on untexturized substrate, resulted in a moderate order parameter (S = 0.29), while the analogous deposition on the texturized substrate for sample B resulted in a remarkably high order parameter (S = 0.75) and thus a high degree of alignment of the PLL rods. Hence, we conclude that the macromolecular systems enabling (lamellar) self-organization like PEM consisting of stiff α-helical polypeptides obviously need a further directing field for evident anisotropy effects on the microscopic level. Furthermore, changing the conformation of PLL in PEM-5 of PLL/PVS, from α-helix to random coil, by conserving M_w resulted in a negative order parameter (S = −0.26), meaning that there is no or a rather vertical orientation of a molecular axis with respect to texturization direction. However, since a random coil polypeptide cannot have any defined molecular axis and considering the high error due to low amide II band integrals (measured in p- and s-polarization) we interprete this as a complete lack of orientation, concluding that in that sample PLL does not show any alignment in the nanogrooves. Finally, sample D, for which the molecular weight of PLL was reduced to 25.700 g/mol, a low-order parameter (S = 0.10) was also found compared to PLL-205.000 g/mol (B), both being in the α-helical state and deposited onto texturized substrates. Obviously, longer α-helical PLL-205.000 rods complexed by PVS could be aligned more effectively in the texturized grooves compared to the shorter α-helical PLL-25.700 rods. Given a groove width of around 50 nm[12] and assuming a higher degree of negative surface charge in the freshly scratched groove (valley) than in the undamaged silicon surface (peak), it is likely that the shorter PLL-25.700 rods are able to arrange freely in the groove, while the longer PLL-205.000 rods are forced to align in the direction of the groove upon adsorption. Recently, using eight different M_w of PLL, a significant increase of S with increasing M_w was obtained.[26] Obviously, the PLL rods with the higher polymerization degree DP and thus longer contour lengths L = DP x r (r: rise per residue for the right-handed α-helix[27]) showed the higher alignment in the surface grooves than the smaller ones, which were not influenced by the confined space.

Microscopic Characterization (AFM)

In the preceding section it was shown that the order parameter S of the four studied samples (A–D) was highly dependent on the three factors PLL conformation, M_w, and unidirectional substrate texturization. Hence, it was challenging to check these spectroscopic results by microscopy. In Figure 2, atomic force microscopy (AFM) images (topography mode) for samples A–D are shown. The AFM image of sample A, where α-helical PLL-205.000 was consecutively deposited alternating with PVS on the

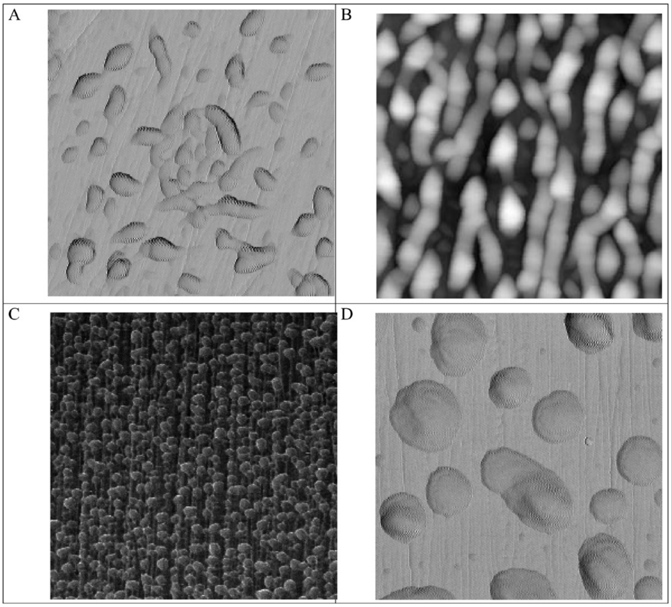


Figure 2. AFM images (2 × 2 μm, topography) of four different samples of PEM-5 of PLL/PVS: A, α-helical PLL-205.00, untexturized substrate; B, α-helical PLL-205.00, texturized substrate; C, random coiled PLL-205.00, texturized substrate; D, α-helical PLL-25.700, texturized substrate.

untexturized substrate, showed an isotropic granular overall structure with a small tendency to elongated objects. In contrast, the image of sample B shows an anisotropic overall picture, in which adsorbed wormlike objects have a preferential orientation parallel to the direction of the texturization (approximately the vertical axis of that page). This can be attributed to considerably aligned aggregated PLL/PVS complex moieties and clearly shows the importance of texturization as a mechanical director field for the α-PLL/PVS self-assembly. Furthermore, in the image of sample C, which is related to PLL-205.000 in the random coil state on the texturized substrate, an isotropic surface topology featuring copious small spherical dots but no elongated objects showed up. This is due to the dramatically decreased stiffness of the polypeptide not able to accommodate or mechanically interact anymore within the surface groove. Finally, the image of sample D on α-helical PLL-25.700/PVS on texturized substrate again shows isotropic surface morphology of unoriented larger sized dots (compared to A and C), which can be explained by the too short contour length of the PLL rods not able to orient significantly within the nanogrooves of the texturized substrate.

In principle, these AFM data confirm the dichroic ATR-FTIR data from above, since only for sample B was a very high order parameter in terms of orientation with respect to the unidirectional alignment obtained. The second high degree of anisotropy was found in sample A, where no texturization was applied, showing an elevated order parameter ($S = 0.29$) and a slight tendency to elongated but poorly oriented objects, while for sample C (random coil) and D (small M_w) strong evidence was found for purely isotropic surface topology.

Model

Finally, in Figure 3, a scheme for the internal structure of oriented PEM of PLL/PVS (sample B) is proposed. The layered lammellar

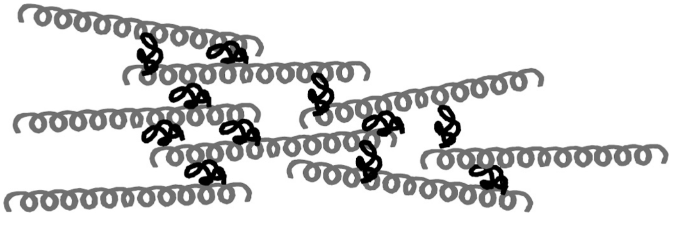


Figure 3. Scheme of the PEM phase consisting of oriented stiff charged α-helical PLL (grey) and coiled PVS (black): coiling of the non peptidic PVS by salt ('Screening') and α-helix induction of PLL by specific interaction or neutralization (pH). (From Müller et al.[12] with kind permission of ACS).

structure might be valid in the horizontal as well as in the vertical direction of the PEM.

Principally, PLL forms α-helical rods (grey) by specific interaction of bulky ClO_4^- anions with the polypeptide backbone in terms of inserting within the charged ammonium groups of PLL.[28] Obviously, charged α-helical polypeptides like PLL show an "anti-polyelectrolyte" behavior, since they stretch upon salt addition, while PVS shows classical polyelectrolyte behavior: it forms the coils (black) upon high salt addition (1 M $NaClO_4$) due to charge screening and reduced Debye length. Hence, anisotropic PEL supramolecular assemblies like sample B are suggested to consist of PLL α-helical rods, which are oriented in the case of both a texturized surface and high molecular weight by a mechanical director field, and PVS coils acting as a kind of flexible glue between the PLL rods.

CONCLUSION

- Four representative samples of PEM consisting of PLL in various conformations and molecular weights and PVS were deposited on the substrates with or without unidirectional surface texturization (parallel nanogrooves) and studied by dichroic ATR-FTIR spectroscopy.
- High-order parameters $S \geq 0.7$ were obtained only for texturized substrates, the α-helical conformation, and high molecular weights of PLL.
- AFM pictures of the same samples show only elongated anisotropically oriented worm-like structures for high molecular α-helical PLL on texturized substrates. Low molecular weight, random conformation, or absence of texturization revealed only unordered or isotropic surface topologies, which supports the spectroscopic results.
- Our experimental results show that even for highly organized systems, an additional macroscopic director field is required for nanoscopic self-assembly. This might be a conclusion that should be also considered for self-organizing systems based on block copolymers on surfaces.

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