Ionic Strength-Dependent pK Shift in the Helix–Coil Transition of Grafted Poly(L-glutamic acid) Layers Analyzed by Electrokinetic and Ellipsometric Measurements

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Surface-bound layers of poly(L-glutamic acid) prepared by a recently described “grafting-from” method were analyzed with respect to electrical charging and structural alterations upon variation of pH and concentration of the background electrolyte in aqueous solutions. The microslit electrokinetic setup (MES) was utilized for the combined determination of zeta potential and surface conductivity on the basis of streaming potential and streaming current measurements at polypeptide layers in contact with aqueous electrolyte solutions of varied composition. In situ ellipsometry was applied at similar samples immersed in identical aqueous solutions to investigate the influence of the solution pH on the structure of the polypeptide layers. Zeta potential and Dukhin number versus pH plots revealed the dissociation behavior of the surface-bound polypeptides indicating a significant shift of the pK of their acidic side chains correlating with the concentration of the background electrolyte potassium chloride and the related variation of the Debye screening length. Surface conductivity data pointed at a more expanded structure of the polypeptide layer in the fully dissociated state as an increased ion conductance in this part of the interface was determined. The occurrence of a strong increase of the thickness and a corresponding decrease of the refractive index for the coil state of the layer strongly supports the findings of the electrokinetic measurements. This fully reversible “switching” of the layer structure was attributed to helix–coil transitions within the grafted polypeptides induced by the dissociation of carboxylic acid functions of the polypeptide side chains. The shift of the “switching pH” of the surface-bound poly(L-glutamic acid) layers at varied concentrations of the background electrolyte was interpreted as a result of the pH shift of the carboxylic acid groups of the polypeptide side chains. The observed patterns prove that the electrostatic interactions causing this shift occur within but not between the grafted chains.

Introduction

Surface modification by means of end-grafted polymer chains receives more and more attention in both application-oriented and fundamental research. Applications of the layered materials comprise affinity biosensors, nanomicroanalytical devices, chemical microreactors, protein-resistant surfaces, thin-film waveguides, optical data storage, and liquid displays.

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During the past decade, several efforts were undertaken to produce and study surface-grafted polypeptide layers.26,27 The unique precision of structural features and transitions in polypeptides can provide valuable options for the development of new functional materials when applied as surface-bound chains. Examples include layers with piezoelectric24 or nonlinear optical25 characteristics. Furthermore, surface-attached polypeptides can be utilized to control biomimetic mineralization processes.26

Interfacially confined polypeptide chains may exhibit altered stability of secondary structure elements at different environmental conditions as compared to polypeptides in solution. To study the relevance of such deviations for pH-induced structural transitions of poly(L-glutamic acid) chains grafted according to the method described in ref 1, we analyzed the dissociation and structural variations of layered substrates prepared accordingly. Since the degree of dissociation of the carboxylic acid of the polypeptide side chains controls the helix–coil transition in this system, the determination of the dissociation at varied pH and ionic strength of the adjacent solution had to receive special attention. For that reason, electrokinetic measurements were applied to follow the dissociation of functional groups at macroscopic thin films of the grafted polypeptide. The in-house developed microslit electrokinetic setup2,3 utilized for that purpose not only allows for the precise determination of the zeta potential at interfaces with significant surface conductivity but furthermore permits the simultaneous quantification of this surface conductivity for the comprehensive characterization of charge–structure interrelations. As the helix–coil transition within the grafted polypeptide is expected to manifest itself in a strong variation of the structure of the polymer layer, the layer thickness and the refractive index were determined by ellipsometry in parallel. In view of the impact of electrostatic forces on the helix–coil transition, we analyzed the pH-dependent zeta potential, surface conductivity, layer thickness, and refractive index of grafted poly(L-glutamic acid) layers while the spatial extension of electrostatic forces was modulated by variations of the ionic strength (i.e., concentration of the background electrolyte potassium chloride).

**Experimental Section**

**Materials.** Sample Carriers. The polypeptide layers for the electrokinetic measurements were prepared on polished glass carriers (20 × 10 × 3 mm³), which were purchased from Berliner Glas K GaA Herbert Kubatz GmbH & Co., Berlin, Germany. For the determination of the layer thickness and the refractive index by ellipsometry, the poly(L-glutamic acid) layers were prepared on thermally oxidized silicon wafers (20 × 15 mm²). An oxide layer thickness of 50.2 nm was determined by ellipsometry. The substrates were cleaned before use in freshly prepared H₂SO₄/H₂O₂ (3:2), excessively rinsed with water, and dried in a nitrogen stream.

For the reaction of the substrates with formic acid to remove the t-butyl-L-glutamate in tetrahydrofuran. After 24 h, the substrates were rinsed with tetrahydrofuran and treated with formic acid to remove the tert-butyl protecting groups. After 18 h, the substrates were removed, rinsed with deionized water, and dried in a nitrogen stream. From ATR-FTIR measurements, it is known that 97% of the protecting groups were removed from the polypeptide chains.1

**Methods.** Electrokinetic Measurements. The zeta potential ζ and the surface conductivity K' were obtained applying the recently developed microslit electrokinetic setup.2,3 The determination of ζ and K' is based on streaming potential and streaming current measurements on a streaming channel formed by two parallel sample carriers. The zeta potential can be obtained from the streaming potential and streaming current data by use of the Smoluchowski equations:

\[
\zeta (U_s) = \frac{\eta K_b}{\epsilon \rho_f} \frac{dU_s}{dp} \bigg|_{U_s=1}
\]

(1)

\[
\zeta (I_s) = \frac{\eta L}{\epsilon \rho_f b h} \frac{dl_s}{dp}
\]

(2)

where \(U_s\) is the streaming potential, \(I_s\) is the streaming current, \(p\) is the pressure drop across the streaming channel, \(\eta\) is the dynamic viscosity of the fluid, \(K_b\) is the specific electrical conductivity of the fluid, \(\epsilon_0\) is the permittivity of vacuum, and \(\epsilon_r\) is the dielectric constant of the fluid. L, b, and h are the length, width, and height of the channel. The conditions for the applicability of eq 1 are given by the Dukhin number Du.27 For rectangular streaming channels, Du is defined by the dimensionless ratio \(K'/\eta K_b\), that is, eq 1 can be used for the evaluation of the streaming potential measurements at high electrolyte concentrations and large channel heights. The Dukhin number can be calculated from the data of the streaming potential and streaming current measurements according to

\[
Du = \frac{1}{2} \left( \frac{dU_s}{dp} \right) \frac{L}{(dU_s/dp) b K_b} - 1
\]

(3)

Since the condition Du ≪ 1 was not fulfilled for the poly(L-glutamic acid) layers at low solution concentrations, only the streaming current data were evaluated for the calculation of the zeta potential.

To determine the surface conductivity of the layered substrates, streaming potential and streaming current measurements were performed at different channel heights. The surface conductivity was derived from the results of the (S)I-channel–height dependent streaming potential and streaming current data by a linear regression using eq 4:

\[
\frac{dI_s}{dp} \left( \frac{L}{2(dU_s/dp b K_b)} - 1 \right) = \frac{K_b}{2} \frac{K_b}{2}
\]

(4)

**Determination of the Layer Thickness and the Refractive Index.** For the determination of the thickness d and the refractive index...
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| aqueous KCl solution, \( n = 1.333 \) |
| PLGA layer, \( d = f(pH,c), n = f(pH,c) \) |
| SiO\(_2\), layer, \( d = 50.2 \text{ nm}, n = 1.4571 \) |
| silicon wafer, \( n = 3.8705 - 0.0168j \) |

**Figure 1.** Four-layer model used for the calculation of the thickness and the refractive index of poly(L-glutamic acid) layers. In the silicon wafer, the complex refractive index of the material was used.

**Figure 2.** Zeta potential vs solution pH for the grafted poly(L-glutamic acid) layers in 10\(^{-4}\) M, 10\(^{-3}\) M, and 10\(^{-2}\) M KCl solutions.

of the polypeptide layers prepared on silicon wafer surfaces, a single wavelength ellipsometer (EL X-02C, Dr. Riss Ellipsometerbau GmbH, Ratzeburg, Germany) with a He–Ne laser (632.8 nm) was applied. The measurements were performed in a commercial flow cell (Helma GmbH & Co. KG, Müllheim, Germany) at a constant angle of 68°. The layer thickness and the refractive index were obtained by using an optical four-layer model (Figure 1).

**Results and Discussion**

**Zeta Potential versus Solution pH.** The pH-dependent charging of the end-grafted polypeptide layers was studied by streaming current experiments in 10\(^{-4}\) M, 10\(^{-3}\) M, and 10\(^{-2}\) M aqueous KCl solutions (Figure 2). The zeta potential versus pH plots and the position of the isoelectric points at pH = 2.6 ± 0.1 indicated that the charge formation of the layered samples is mainly determined by the dissociation of the carboxylic acid groups of the poly(L-glutamic acid) chains. Above the isoelectric point (pH > 2.6), the magnitude of the negative zeta potential increases with the degree of ionization of the carboxylic acid groups at increasing pH values until a zeta potential plateau is reached in the basic region corresponding to full dissociation of the carboxylic acid. The decrease of the absolute zeta potential values with increased KCl solution concentration is a well-known feature of electrokinetic experiments and can be attributed to the “compression” of the diffuse layer, that is, at higher electrolyte (KCl) solution concentrations a higher fraction of surface charge is compensated within the hydrodynamically immobile layer. The positive zeta potentials at pH values below the isoelectric point can be ascribed to the effect of unsymmetrical ion adsorption,28 which interferes—depending on the number of dissociable surface groups—with the charge formation by the dissociation of these groups. In line with

this consideration, a shift of the isoelectric points to higher values was observed at incomplete removal of the protecting groups.

**Evaluation of the Surface Conductivity Data.** To quantify the accumulation of mobile charge carriers within the polypeptide layers, the surface conductivity was determined in 10\(^{-4}\) M, 10\(^{-3}\) M, and 10\(^{-2}\) M KCl solutions at pH = 6.0 and pH = 9.0. The corresponding data are given in Table 1.

The experimentally determined surface conductivity can be attributed to ions located in the diffuse part of the double layer (\( K^{\alpha} \)) and to ions of the inner part of the double layer (\( K^{\omega} \)):

\[
K^{\omega} = K^{\alpha} + K^{\omega}\text{d}
\]

The contribution of the charge carriers in the diffuse layer to the surface conductivity can be estimated according to the equation of Bikerman:

\[
K^{\omega}\text{d} = \frac{2\pi F^2 c}{RT} \left[ D_0 \left( e^{-F\zeta/RT} - 1 \right) \left( 1 + \frac{3m_i}{z^2} \right) + D_\infty \left( e^{-F\zeta/RT} - 1 \right) \left( 1 + \frac{3m_i}{z^2} \right) \right]
\]

where \( z \) is the valency of the ions, \( F \) is the Faraday constant, \( c \) is the solution concentration, \( R \) is the gas constant, \( T \) is the absolute temperature, \( k^1 \) is the Debye radius, \( D_\infty \) are the diffusivities of the ions, and \( m_i = (RT/F)^2(2e^\epsilon/3\pi)D_\infty \) describes the relative contribution of the electroosmosis to \( K^{\omega}\text{d} \). Using this equation, the contribution of the hydrodynamic immobile layer to \( K^{\omega} \) can be calculated from \( K^{\alpha} \) and \( K^{\omega}\text{d} \) according to eq 5.

pH and electrolyte concentration dependent data of \( K^{\alpha} \) were determined in the wide range between 4.5 nS (10\(^{-4}\) M KCl at pH 6) and 210 nS (10\(^{-2}\) M KCl at pH 9). In comparison to these \( K^{\alpha} \) data, very low values were calculated for the conductivity of the diffuse part of the double layer \( K^{\omega}\text{d} \). Thus, the hydrodynamically mobile charge \( (K^{\omega}) \) reflected by the zeta potential contributes in all the analyzed cases less than 4.2% to \( K^{\alpha} \), that is, a vast majority of the excess charge carriers at the interface is located within the hydrodynamically stagnant volume phase of the grafted polypeptide layers. Differences in the magnitude of the conductivity of this inner layer can be related to the number of ions entrapped in the polypeptide layers.

For given background electrolyte concentrations, a strong increase of the surface conductivity with the solution pH was observed. In the 10\(^{-4}\) M KCl solution, the ratio of \( K^{\alpha} \) (pH = 9.0) to \( K^{\alpha} \) (pH = 6.0) was 35.1. For higher electrolyte concentrations, the corresponding values were 16.6 (10\(^{-3}\) M KCl) and 5.2 (10\(^{-2}\) M KCl), respectively. The

| Table 1. Experimentally Determined Zeta Potential and Surface Conductivity as well as Calculated Layer Parameters \( (K^{\omega}, K^{\omega}\text{d}) \) for the Surface-Grafted Poly(L-glutamic acid) in 10\(^{-4}\) M, 10\(^{-3}\) M, and 10\(^{-2}\) M KCl Solutions |
|---|---|---|---|---|---|
| solution | \( \zeta \) [mV] | \( K^{\alpha} \) [nS] | \( K^{\omega}\text{d} \) [nS] | \( K^{\omega} \) [nS] | \( K^{\omega}/K^{\omega}\text{d} \) [%] |
| 10\(^{-4}\) M KCl, pH 6.0 | -75.8 | 4.5 | 0.18 | 4.32 | 4.16 |
| 10\(^{-4}\) M KCl, pH 9.0 | -92.5 | 157.9 | 0.29 | 157.61 | 0.18 |
| 10\(^{-3}\) M KCl, pH 6.0 | -57.3 | 10.6 | 0.29 | 10.31 | 2.84 |
| 10\(^{-3}\) M KCl, pH 9.0 | -67.9 | 179.6 | 0.43 | 179.17 | 0.24 |
| 10\(^{-2}\) M KCl, pH 6.0 | -46.6 | 40.6 | 0.59 | 40.01 | 1.47 |
| 10\(^{-2}\) M KCl, pH 9.0 | -53.9 | 209.9 | 0.81 | 209.09 | 0.39 |


(29) Bikerman, J. J. Koloid-Z. 1935, 72, 100.
increase in the surface conductivity at the different solution concentrations is also indicated by the Dukhin number versus pH plots (Figure 3), which were obtained from streaming potential and streaming current data at channel heights of 50 \( \mu \)m. From the Du versus pH plots, it is obvious that the strongest increase of \( K_0 \) occurs at about pH 8.3 in the 10\(-^4\) M and at about pH 7.5 in the 10\(-^3\) M KCl solution. Although the surface conductivity is increased with the electrolyte concentration, the Dukhin number vanishes to zero at the solution concentration of 10\(-^2\) mol/L. Therefore, the accumulation of mobile charge carriers in the polypeptide layer cannot be derived from the Du versus pH plot.

To get independent information on the extension of hydrodynamic immobile layer, the layer thickness \( d \) and the refractive index \( n \) of the surface-grafted polypeptides were determined by ellipsometry at similar solution settings as compared to the electrokinetic experiments. However, because of the different cell geometries, the flow conditions and the corresponding wall shear stress were quite different in both experiments (see Appendix for details).

Depending on the pH and the electrolyte concentration of the solution, absolute values for \( d \) in the range between 25 and 74 nm were calculated on the basis of an optical four-layer model (Figure 1), whereas the corresponding values of the refractive index were in the range between 1.39 and 1.59. Since the ellipsometric measurements at the different KCl background concentrations were performed with different samples and the wall shear stress was quite different between the electrokinetic experiments, only the relative changes of the layer thickness \( (\Delta d / \Delta d_{\text{max}}) \) and the refractive index \( (\Delta n / \Delta n_{\text{max}}) \) are discussed below (Figure 4).

The variations of the determined layer parameters (\( K_0 \), Du, \( d \), \( n \)) with the solution pH can be explained by charge-induced alterations of the structure of the polyl-glutamic acid) chains (Figure 5). At low pH values, the polypeptide chains are uncharged and adopt an \( \alpha \)-helical conformation, for which the average distance between the projections of the carboxylic groups on the helical axis is 0.15 nm.\(^{30}\) Consequently, a rather compact structure of the hydrodynamically immobile layer of the grafted polypeptide occurs. The low values of \( K_0 \) at pH 6 indicate that there is—in comparison to higher pH values—just a small amount of ions in the polypeptide layers sufficiently mobile to contribute to ion conduction. In addition, the overall mobility of the ions in more tightly packed polyelectrolyte layers can be expected to become substantially decreased because immobilized molecules distort the streamlines of the surface current and lengthen the effective path of the ions through the polymer layer.\(^{31,32}\)

At high pH, the carboxyl side groups of the grafted polyl-glutamic acid) chains are negatively charged and because of the electrostatic repulsion between the side groups their conformation is that of an extended coil. In

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Conclusions

On the basis of the combined determination of $\zeta$ and $K^+$ by microslit electrokinetic experiments and additional optical measurements, the pH induced conformational transition of surface-grafted poly(L-glutamic acid) layers were characterized in aqueous KCl solutions. At all KCl concentrations, an isoelectric point of $\text{IEP} = 2.6 \pm 0.1$ was derived from the zeta potential versus pH plots, confirming that the dissociation of carboxylic acid groups of the polypeptide chains is the main charge formation process at the analyzed interfaces. The hydrodynamically mobile charge reflected by the zeta potential contributed less than 4.5% to the total surface conductivity pointing at a rather extended stagnant, but ion-conducting, volume phase of the grafted polypeptide layers. Furthermore, a dramatic variation in the surface conductivity was observed in a narrow pH range of the solution. The data determined by ellipsometry impressively confirm this switching of the polypeptide layer by the variation of the layer thickness and the refractive index in the same pH range. Both the data obtained by the electrokinetic measurements ($K^+$, Du) and the data obtained by ellipsometry ($d$, $n$) permit to conclude that the helix–coil transition observed for poly(L-glutamic acid) in solution also occurs within the grafted polypeptide layers.

The transition point between the helical and the extended coil conformation strongly depends on the ionic strength of the solution. This shift of the transition point was attributed to the modulation of the apparent dissociation constant of the carboxylic acid groups with varied KCl solution concentration. In that line, the occurrence of the conformational transition at lower pH values for higher background electrolyte concentration is thought to be most likely a result of the weaker electrostatic interactions among neighboring ionized groups within any given grafted polypeptide chain.

Appendix

As mentioned in the Results and Discussion section, there was a slight difference between the pH value at which the largest changes in the surface conductivity were observed for the electrokinetic measurements in the slit channel and those measurements made in the optical channel. It was proposed that these differences were the result of the different surface shear stress conditions in the two geometries. The purpose of this appendix is to provide a detailed comparison of the flow and shear profiles for these cases.

In both geometries, the relatively low volume flow rates (1.7–7.0 mL/min in the electrokinetic and 10 mL/min in
the optical measurement) limited the Reynolds number to well below that associated with the transition to turbulence or a significant hydrodynamic entrance length. As such, a laminar flow solution to the flow profile in the channel cross section was deemed representative of the situation in the entire flow cell. Analytical solutions for the flow and shear conditions in the slit channel are well known; however, the irregular geometry associated with the optical cell required the implementation of a finite element solution (a first-order approximation could be obtained from the analytical solution to flow a trapezoidal channel presented by Shah and London; however, the convergence of the series solution tends to be slow compared with finite element methods). For both cases, the flow profiles and shear conditions were obtained using the in-house written BLOCS (bio-lab-on-a-chip simulation) code which has been independently verified elsewhere. The results of the flow profile computation as well as the dimensions of two channels are given in Figure 6. The much smaller cross sectional area in the slit channel geometry leads to the observed higher flow velocities. The shear stress, $\tau$, at the channel surface is given by eq 7

$$\tau = \eta \frac{dv}{dn}$$

where $\eta$ is the viscosity, $v$ is the velocity, and $n$ is a vector normal to the surface. In Figure 7, the shear rate at the sample surface is compared for the two flow cells. As can be seen, the shear stress in the slit channel is roughly 4 orders of magnitude larger than that for the optical channel. This higher shear rate likely resulted in the reorientation of the polypeptide chains and thus the shift in the pH level at which the largest changes in surface conductivity were observed as described above.

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