Poly-(amino acid) polyelectrolyte films:Structure and interactions with proteins and lipids

Summary of Ph.D. Thesis

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Introduction

In this thesis, we present data concerning the structure and internal interactions of polypeptide multilayers. We also present the first studies on phospholipid bilayers incorporated into polypeptide multilayers. Due to their close resemblance to real biological systems, these bilayers on the cytoskeleton-like polypeptide films may serve as internal barriers, as carriers of hydrophobic proteins, which might have desirable biological functions (ion channels, transporters, etc.). Finally, preliminary data are presented on the possibility of biomedical use of such lipid bilayers in connection with polyelectrolyte films.

Aims of the Work

Since biocompatible, bio-functionalized surfaces have increasing importance in nano-technological approaches of several biomedical applications, we wanted to reveal basic properties of such systems, which might strongly affect their later applications. For this goal we aimed to study:

- The build-up and the structure of polyelectrolyte films made from polypeptides (poly-(L-glutamic acid), PGA and poly(L-lysine), PLL),
- The interaction of PGA/PLL films with another poly amino acid, poly-(L-aspartic acid) (PAA), and with a protein (human serum albumin).

In view of the extensively charged nature of the polyelectrolytes in these films, there is no chance for direct incorporation of non-polar, hydrophobic compounds into them. Therefore, our goal was:

- To create a phospholipid bilayer, to form an internal barrier for the water-soluble compounds, and a potential carrier of hydrophobic proteins in a polyelectrolyte multilayer.
- Finally, we wanted to examine the behavior of our model membrane system in a biological environment by checking the effect of the lipid bilayer on the activity of bio-functionalized PGA/PLL films.

Materials and Methods

Multilayer polyelectrolyte films were built by the layer-by-layer (LBL) consecutive adsorption method. In our case, the polyelectrolytes were brought into contact with the solid support, a

ZnSe internal reflection element, by being circulated with a peristaltic pump above the crystal. The build-up of the polyelectrolyte film started with circulating a polycation (the first layer was always poly(ethyleneimine), PEI). In the next step, a polyanion was allowed to adsorb and then a polycation again. This process was repeated until we have got the desired architecture: PEI-$(PGA/PLL)_6$.

To reveal the nature of the competition between PGA and PAA, PEI-$(PGA/PLL)_5$-PGA or PEI-$(PAA/PLL)_5$-PAA films were built; to these films PAA or PGA was added, respectively, and their penetration into the existing structure was followed by Fourier transform infrared (FTIR) spectroscopy in attenuated total reflection (ATR) mode. Since its FTIR spectra was found largely different on PGA/PLL and on PAA/PLL surfaces, human serum albumin (HSA) was used for “mapping” the surface of the three-component PGA/PAA-PLL films.

Lipid bilayer was created from DPPC (dipalmitoylphosphatidylcholine) liposomes by a heating-cooling cycle. Gramicidin A (GRA), considered as a test membrane polypetide was first incorporated into liposomoses, then with the same method as in the case of pure DPPC was the peptid-containing lipid bilayer created.

The surfaces of both the pure and the lipid bilayer-covered polyelectrolyte films were characterized by atomic force microscopy (AFM).

In the biological tests, we were using standard and bio-functionalized PGA in the PGA/PLL multilayers. The bio-functionalized PGA contained covalently bound α-melanocyte stimulating hormone (α-MSH). We have built the following architectures: (i) $(PLL/PGA)_5$-PLL; (ii) $(PLL/PGA)_5$-PLL/PGA-α-MSH; (iii) $(PLL/PGA)_5$-PLL/PGA-α-MSH/DPPC. Melanoma cells were grown until confluence on these surfaces, and their melanin production (supposed to be affected by the presence of α-MSH in the films) was measured by visible absorption spectroscopy.

Results and Discussion

PGA and PAA are very similar from chemical point of view, but when alternated with PLL layers they form differently structured films. PAA adopts α-helical structures, whereas PGA tends to form β-sheets with PLL.

The interaction of the second polyanion, PAA with a PEI-$(PGA/PLL)_5$-PGA film and that of PGA with a PEI-$(PAA/PLL)_5$-PAA film induced certain exchange between the two polyanions.

The five-layered $(PGA\text{-}PLL)_5$ and $(PAA\text{-}PLL)_5$ films were the closest to the 'mixed' films; their infrared spectra were used in a linear combination fitting. For $(PGA\text{-}PLL)_5$-PGA+PAA-PLL film, the infrared spectrum could have been fitted well with 79% $(PGA\text{-}PLL)_5$ and 21% $(PAA\text{-}PLL)_5$. Surprisingly, in $(PAA\text{-}PLL)_5$-PAA+PGA-PLL film there were 49% $(PAA\text{-}PLL)_5$ and 51% $(PGA\text{-}PLL)_5$ found. Here, the incorporation of PGA was much higher than that of PAA in a symmetric situation.

If we put human serum albumin (HSA) on PEI-$(PGA/PLL)_6$ film, there was a strong adsorption of the protein on the surface. This HSA infrared spectrum was very similar to that of measured in solution. In the case of the PEI-$(PAA/PLL)_6$ film, the adsorption of the HSA was very different. Here, there were considerable changes in the film+protein infrared spectrum. At the first glance, these changes were more similar to PAA than to HSA, but we found differences as well, which indicated the contribution of the protein to this infrared spectrum.

It was not clear whether in the PGA↔PAA competition only the terminal layer was involved, or the interior of the film was also affected. Since the infrared spectrum of HSA was very different on pure $(PGA\text{-}PLL)_5$ and $(PAA\text{-}PLL)_5$ films there was a chance that the infrared spectrum of HSA adsorbed onto mixed films can be decomposed to component spectra corresponding to pure PAA/PLL and PGA/PLL surface domains. To test, the amide I region of the

HSA adsorbed onto a $(PGA/PLL)_5$-PGA+PAA-PLL film spectra was fitted HSA_{PAA} and HSA_{PGA} failed to reproduce the $HSA_{PAA+PGA}$ spectrum in the amide I region. $HSA_{PAA+PGA}$ exhibits a band characteristic of PGA/PLL complexes at around 1610 cm^{-1}, which was missing from the HSA_{PGA} and HSA_{PAA} spectra. This may indicate additional polyelectrolyte film rearrangement upon the adsorption of HSA to $(PAA/PLL)_5$-PAA+PGA-PLL involving mostly PGA.

By circulating DPPC liposomes above the polyelectrolyte film, and going above the gel→liquid crystalline phase-transition temperature, the liposomes were 'melted' into a continuous bilayer, thus more lipid molecules were in the depth accessible for the evanescent light, and this was apparent from the increased infrared absorption. The presence of DPPC on the film surface was evident by observing the characteristic $\nu_{sym}CH_2$ band at around 2850 cm^{-1}, the $\nu_{as}CH_2$ band at around 2920 cm^{-1}, and the corresponding $\nu_{sym}CH_3$ and $\nu_{as}CH_3$ modes at around 2872 and 2958 cm^{-1}, respectively.

First, we tested the barrier properties of DPPC against the polyelectrolyte molecules. Originally, we were working in the exponential growth domain of the PGA/PLL films. Exponential growth assumes the free diffusion of at least one of the components in the interior of the polyelectrolyte film. When a lipid bilayer was embedded into the polyelectrolyte film, we observed a re-starting of the growth of the polyelectrolyte film on the top of the lipid bilayer.

This effect we interpret as a proof that the DPPC bilayer does not allow the diffusion between the two polyelectrolyte-film compartments it is separating. Its characteristic infrared band at around 1630 cm^{-1} evidenced the incorporation of GRA, a lipid-soluble polypeptide into the DPPC bilayer. The amount of the incorporated GRA was much smaller than expected by the stochiometry applied at the preparation of the GRA-containing liposomes (this point merits further studies). Therefore, we could not separate temperature-dependent GRA-related alterations from structural changes of the PGA/PLL film even by SVD analysis. Nevertheless, the data show that it is possible to incorporate GRA into DPPC bilayers, and GRA molecules remain stably embedded in the lipid environment.

In the biological test, the melanin production of the melanoma cells stimulated by interacting with α-MSH was followed by measuring the absorption of the total cell suspension at 405 nm, at a wavelength characteristic of melanin. The purpose of such a test is to reveal, whether polyelectrolyte films can be used as local, programmed releasers of biologically active compounds. The experiments described in the thesis represent only the first trials; the data are integral type, actually the accumulation of the melanin was followed. We observed some stimulating effect of the DPPC on the melanin production. The mechanism of this induction is still unclear, but based on these observations further experiments can be designed.

One possible mechanism could be, that DPPC provides “better” environment for α-MSH, which therefore can interact more effectively with the cell membrane.

Summary

Following layer-by-layer build-up of poly-(L-glutamic acid)/poly-(L-lysine) (PGA/PLL) multilayers, we could demonstrate the gradual formation of a strong β secondary structure in the polyelectrolyte films. When the chemically very similar poly-(L-aspartic acid) (PAA) was used as polyanion, the secondary structure of the polypeptide film was markedly different, it contained sizable amount of α-helix, and random structure as well. PGA and PAA could substitute each other in the polyelectrolyte films, but the structural consequences of the substitutions were not symmetrical: While PAA could be incorporated without large effects, the incorporation of PGA into an existing PAA/PLL film caused major structural rearrangement. The different effects can be related to the longer side chain in the glutamic acid, which results loosened polyelectrolyte film structure.

We could create a lipid bilayer on the surface of PGA/PLL polyelectrolyte films. In addition, these lipid bilayers could be

covered with another polyelectrolyte layers, thus the lipid double layer was embedded into the polyelectrolyte architecture. This system may provide a tool to incorporate lipid-soluble, hydrophobic compounds into the highly charged polyelectrolyte films, which would be important for practical applications. Moreover, such a lipid double layer may be considered as a new model membrane system, where protein-lipid and protein-membrane interactions can be studied. The underlying polypeptide polyelectrolytes provide a large-scale protein-like surface, thus they can mimic the cytoskeleton, the protein network, which stabilizes the cell membrane.

Finally, we made a first attempt to check, whether such artificial lipid bilayers can affect the activity of bio-functionalized polypeptide surfaces. PGA/PLL films, functionalized by a covalently bound melanocyte-stimulating hormone **(**α-MSH) were covered with lipid double layers. The melanoma cells coming in contact with these surfaces produced somewhat more melanin when the polyelectrolyte architecture contained the lipid double layer as well. These preliminary experiments show, that upon optimization of the lipid composition, and the polyelectrolyte architecture for each intended application, such systems might have practical use in solving biomedical problems.

Papers related to the thesis

1. **A.-M. Pilbat**, V. Ball, P. Schaaf, J. Voegel, B. Szalontai
   Partial Poly(glutamic acid) ↔ Poly(aspartic acid) Exchange in Layer-by-Layer Polyelectrolyte Films. Structural Alterations in the Three-Component Architectures.
   Langmuir 22, 5753-5759 (2006).
2. **A.-M. Pilbat**, Z. Szegletes, Z. Kota, V. Ball, P. Schaaf, J. Voegel, B. Szalontai
   Phospholipid Bilayers as Biomembrane-like Barriers in Layer-by-Layer Polyelectrolyte Films.
   Langmuir 23, 8236-8242 (2007).

Conference proceedings:

Foszfolipid kettősrétegek, mint membránmodellek polielektrolit filmekben. **A.-M. Pilbat**, Z. Szegletes, Z. Kota, B. Szalontai. Nanobiológia miniszimpózium (**2006** nov. 10, Pécs)

Structure and Dynamics of Phospholipid Bilayers Incorporated into Layer-by-Layer Polyelectrolyte Films. A Fourier Transform Infrared Spectroscopic study. **A.-M. Pilbat**, Z. Szegletes, Z. Kota, V. Ball, P. Schaaf, J. Voegel, B. Szalontai. Regional Biophysics Conference (21-25 August, **2007**, Balatonfüred)

Phospholipid bilayers as biomembrane-like barriers in layer-by-layer polyelectrolyte films. **A.-M. Pilbat**, Z. Szegletes, Z. Kóta, V. Ball, P. Schaaf, J. Voegel, and B. Szalontai. 12th European Conference on the Spectroscopy of Biological Molecules,(1-6 September, **2007**, Paris, France)

Phospholipid bilayers as biomembrane-like barriers in layer-by-layer polyelectrolyte films. **A.-M. Pilbat**, Z. Szegletes, Z. Kota, V. Ball, P. Schaaf, J. Voegel, B. Szalontai. Polyelectrolytes, (16-19 June, **2008**, Coimbra, Portugal)

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