Secondary Structure of Polypeptide Multilayer Films: An Example of Locally Ordered Polyelectrolyte Multilayers

F. Boulmedais,1,3 P. Schwinté,1 C. Gergely,1 J.-C. Voegel,4,5 and P. Schafalt1

Institut Charles Sadron (CNRS), 6 Rue Boussingault, 67083 Strasbourg Cedex, France, and INSERM Unité 424, UFR d’odontologie, 11 rue Humann, 67085 Strasbourg Cedex, France

Received December 4, 2001

Introduction

The alternate dipping of a charged surface in polyanion and polycation solutions leads to the step step construction of so-called “polyelectrolyte multilayers”.1 Such films can present a layered structure where each polyelectrolyte layer interpenetrates the neighboring ones. Moreover, most of the polycation/polyanion systems used in these constructions lead to films that are locally amorphous so that the polyelectrolyte multilayers were qualified as “fuzzy” structures.2 It could however for certain applications become also of interest to construct films presenting locally internal ordered structures. To this end, it is possible to incorporate in these films molecules already presenting such structures. Polyelectrolyte multilayers are indeed known to preserve, for example, the secondary structure of proteins,3 the helical structure of DNA,4 or polyl(lysine).5,6 Local order can also be generated in polyelectrolyte multilayers by specific interactions between the polycations and polyanions. For example, Cooper et al.7 reported multilayer films containing R-helical structures resulting from the interaction between polyl(lysine) and the Congo Red dye. Serizawa et al.8 exploited stereocomplex assembly to obtain a film growth that implies formation of double-stranded helical structures between two stereoregular poly(methyl methacrylate)s. Recently, Arys et al.9 reported the formation of ordered polyelectrolyte multilayers by using a lyotropic ionone and a strong polyelectrolyte. They concluded that, for their system, “the ability of the polycation and polyanion to give rise to structured complexes is one of the reasons explaining the appearance of order into the multilayer”.

In this Note, we investigate if this statement extends to the poly(lysine) /poly(l-lysine) (PGA/PLL) system. We selected these polypeptides because when they are mixed at room temperature in an aqueous 0.01 M NaF solution, at pH 4 and 7, a β-pleated sheet structure is found with 1:1 stoichiometry. These structures are obtained although PLL possesses a random coil conformation under the same conditions and PGA has a random coil (Richard) conformation at pH 7 (at pH 4).10 We thus worked in conditions where the PGA/PLL complexes in solution are expected to form β-sheets. Studies realized by Cooper et al.11 already suggest that PGA/PLL multilayers form a mixture of R-helices and β-sheets. Cheng and Corn12 also studied the buildup of PGA/PLL films by infrared spectroscopy without analyzing precisely the content of the amide I band. However, this band, at first sight, does not contain a large β-sheet contribution.

To investigate the statement of Arys et al.9, we compared the secondary structure of PLL and PGA when they compose a multilayer film and when they are mixed in solution. Therefore, “in situ” Fourier transform infrared (FTIR) spectroscopy was used to investigate the secondary structure of the polypeptides in multilayer films (FTIR in attenuated total reflection mode, ATR mode) and in solution (FTIR in transmission mode). The analysis was mainly focused on the amide I band.

Experimental Methods

All the experiments were performed at pH 8.4 where PLL and PGA adopt mainly random coil conformations in solution. The buffer medium used was prepared at pH 8.4 from 2-(N-morpholino)ethanesulfonic acid (Mes, 25 mM), Tris(hydroxymethylaminomethane) (Tris, 25 mM), purchased from Sigma, and sodium chloride (NaCl, 0.1 M). Either Millipore filtered water (Milli Q-plus system) or deuterium oxide (99.9% D) from Aldrich was used as solvent. Poly(lysine) sodium salt (MW > 54800) (PGA) and poly(lysine) hydrobromide salt (MW > 32600) (PLL) were both purchased from Sigma. Poly(ethyleneimine) (MW > 750000) (PEI), poly(sodium 4-styrenesulfonate) (MW > 70000) (PSS), and poly(allylamine hydrochloride) (MW > 50000–65000) (PAH) were purchased from Aldrich.

The (PGA-PLL)n multilayers were deposited on a PEI−(PSS−PAH)n precursor film in order to decrease the possible influence of substrate surface properties that were used for the optical waveguide lightmode spectroscopy (OWLS), i.e., SiO2 (TiO2)2O, and for the Fourier transform infrared spectroscopy in attenuated total reflection mode (FTIR-ATR) experiments, i.e., ZnSe.

First, the PEI−(PSS−PAH)n−(PGA-PLL)n film buildup process was followed by OWLS, an optical technique that allows access to the optical film thickness d and its optical mass Δnd (Δn represents the difference between the refractive index of the film and the refractive index of the solution in contact with the

---

film). Details about the experimental setup and the analysis method can be found in ref. 13.

The “in situ” FTIR experiments (for polypeptides in multilayer films or for polypeptides in solutions) were performed in deuterated Mes-Tris buffer. Spectra of polypeptide solutions were obtained by FTIR spectroscopy in transmission mode. Spectra of the PEI–(PSS–PAH)–(PGA–PLL)7 multilayer measured by means of FTIR-ATR spectroscopy. During the buildup, the film was continuously in contact with the buffer solution and was never dried. Analysis of raw spectra obtained in ATR mode were performed by subtracting from PEI–(PSS–PAH)–(PGA–PLL) or PEI–(PSS–PAH)–(PGA–PLL)n–PGA spectra the contribution of the PEI–(PSS–PAH) spectrum. Moreover, all raw infrared spectra were Fourier smoothed as described by Schwinté et al.3

The PEI, PSS, PAH, PLL, and PGA polyelectrolytes were used at 5 mg/mL concentration in Mes-Tris buffer, except the PGA/PLL complexes in solution (2.5 mg/mL), which were obtained by mixing PLL (5 mg/mL) and PGA (5 mg/mL) solutions at a 1:1 mass ratio.

Results and Discussion

Figure 1 shows the evolution of the optical mass \( \Delta n.d \) with the number of deposited layers. One observes a slightly superlinear increase for both \( \Delta n.d \) and \( d \) with the number of deposited (PGA–PLL) layers. This is in accordance with the findings of Cheng and Corn.12 The mean thickness is about 5.2 nm per bilayer over the seven initial deposited bilayers.

A typical FTIR-ATR spectrum of PEI–(PSS–PAH)–(PGA–PLL)7, obtained after withdrawal of PEI–(PSS–PAH)2 precursor film contribution, is given in Figure 2. This spectrum, which represents the amide I region (1600–1700 cm\(^{-1}\)) of (PGA–PLL)7, is compared to the PGA or PLL alone in solution and also the one corresponding to PLL complexes in solution. Strong differences appear between the spectra of the free polypeptides in solution and the spectrum corresponding to the PGA/PLL complexes formed in solution, showing the influence of PGA/PLL interactions. In particular, the appearance of a strong peak centered around 1610 cm\(^{-1}\) and a smaller one centered around 1680 cm\(^{-1}\) denotes the formation of antiparallel \( \beta \)-sheet structures in the complexes, in accordance with data from literature.10, 14 These two frequencies (\( \beta \)-sheet/\( \beta \)-sheet) correspond to two different vibration modes of the carbonyl groups of the peptide bonds involved in the antiparallel chain pleated sheets.14 The great similarity between the spectra for the (PGA–PLL)7 film and for the PGA/PLL complexes in solution indicates that the secondary structure of the polypeptides is close in both situations.

The decomposition of the amide I band allowed us to extract more detailed information about the contributions of the different secondary polypeptide structures to this band. The precise decomposition procedures can be found in ref 3. The frequencies of the different bands forming the spectra are first determined by means of the second derivative of the Fourier smoothed spectrum. There is thus no arbitrary decomposition into a preset number of bands. Once the number of component bands is defined, the spectrum of amide I band is fitted by using component frequency, width, and intensity (proportional to the area under the peak) as fitting parameters. All component bands are assumed to be Gaussian. The correspondence of each component band with a given secondary structure was established by comparing the frequency of its maximum to the value given in the literature.15, 16 We define the relative contribution of each component by the ratio of component band intensity over total amide I band intensity which is evaluated by the ratios of the areas under the peaks. The decomposition of the PLL and PGA spectra, obtained in solution at pH 8.4, reveals a total or partial absence of R-helix or \( \beta \)-sheet structure contributions. Spectra of a PLL solution at pH 13 and of a PGA solution at pH 4 were also taken. They have a significant R-helices content but no \( \beta \)-sheets. The precise relative contributions of the different components of the amide I band are gathered in Table 1. These results are in agreement with the secondary structures of the PLL and PGA in solutions described in the literature.17–20

We then followed the evolution of the relative contribution of different bands composing the amide I band of the (PGA–PLL)n films during their buildup (Figure 3). The adsorption of PGA on a PEI–(PSS–PAH)2 film leads to a significant contribution of R-helices whereas the \( \beta \)-sheet

---

content remains negligible. The deposition of a PLL layer on the first PGA layer induces an important increase in the $\beta$-sheet content indicating that this structure is a result of the PGA/PLL interaction. It is interesting to note that this organization takes place despite the interactions of PGA with the underneath PAH layer. After deposition of the PGA–PLL$_2$ film, the secondary structure of the polypeptides, constituting the film, reaches a “steady state”. There is no longer evolution with the further film buildup process, which is in accordance with the results of Cheng and Corn.$^{12}$

The relative contribution of each component band to the entire amide I band in the “steady state” regime is summarized in Table 1, together with the same data relative to the PGA/PLL complexes in solution. The secondary structures of the polypeptides composing the multilayer or forming complexes in solution are very close as already foreseen from the raw spectra. In both cases, $\beta$-sheets constitute the secondary structure which mainly contribute to the amide I band, their total contribution being about 45–48%, whereas the contribution of the R-helices represents only 18%. Therefore, these films possess highly ordered secondary structures.

Finally, we also followed the evolution of the film structure once it was built, to test the structural stability of the films. The secondary structure of the film remains essentially unchanged over a time interval of about 6 h (data not shown). Only slight evolutions could be observed, in particular a small increase (about 1%) of the $\beta$-sheet content and a slight decrease of the random structure content.

To summarize, we have shown that it is possible to buildup polypeptide multilayer films whose amide I band spectra present significant secondary structure content such as $\beta$-sheets and R-helices. The polyelectrolytes used in this study form complexes as well in the multilayer architectures as in solution. Moreover, their secondary structures are similar. The multilayer polyelectrolyte film formation process should thus be very close to the formation of the polycation/polyanion complexes in solution as stated by Arys et al.$^{5}$ The constraints imposed by the presence of the surface should induce only marginal effects on the secondary structure of the film.

**Table 1. Relative Contents of the Different Secondary Structure Contributions in the Amide I Band of PGA and of PLL. Solutions at Different $pH$ Values, of PGA/PLL Complexes in Solution at $pH$ 8.4, and of a (PGA–PLL)$_7$ Multilayer Film Deposited on its PEI–(PSS/PAH)$_2$ Precursor Film also at $pH$ 8.4.$^{a}$**

<table>
<thead>
<tr>
<th></th>
<th>pH 8.4</th>
<th>R-helix</th>
<th>random</th>
<th>turn</th>
<th>$\beta$-sheet 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLL</td>
<td>8.4</td>
<td>1622</td>
<td>1643</td>
<td>1663</td>
<td>6%</td>
</tr>
<tr>
<td>PGA</td>
<td>8.4</td>
<td>1644</td>
<td>1664</td>
<td>62%</td>
<td>34%</td>
</tr>
<tr>
<td>PLL</td>
<td>13</td>
<td>1625</td>
<td>1640</td>
<td>1660</td>
<td>34%</td>
</tr>
<tr>
<td>PGA</td>
<td>4</td>
<td>1627</td>
<td>1642</td>
<td>1660</td>
<td>34%</td>
</tr>
<tr>
<td>PGA/PLL</td>
<td>8.4</td>
<td>1611</td>
<td>1624</td>
<td>1644</td>
<td>1662</td>
</tr>
<tr>
<td>complexes</td>
<td>8.4</td>
<td>1611</td>
<td>1626</td>
<td>1643</td>
<td>1661</td>
</tr>
<tr>
<td>(PGA–PLL)$_2$</td>
<td>8.4</td>
<td>1611</td>
<td>1626</td>
<td>1643</td>
<td>1661</td>
</tr>
<tr>
<td>multilayer</td>
<td>8.4</td>
<td>1611</td>
<td>1626</td>
<td>1643</td>
<td>1661</td>
</tr>
</tbody>
</table>

$^{a}$ The first line in each entry represents the maximum frequency in cm$^{-1}$ at which each component band appears. The second line corresponds to the value of its relative contribution to the amide I band (see text for definition).

**Figure 3. Evolution of the relative contribution of the five components of the amide I band during the buildup of a (PGA–PLL)$_7$ multilayer film at pH 8.4: (a) $\beta$-sheet 1, (b) R-helix, (c) random, (d) turn, and (e) $\beta$-sheet 2 contents. Each data point was labeled by the central frequency (cm$^{-1}$) at which this component band appears. The x axis is valid for parts a–e.**

**Acknowledgment.** This work was supported by CNRS and INSERM through the program “Ingénierie tissulaire”, by the EC through the SIMI program, and by the Ministère de la Recherche through the ACI “Technologie pour la santé”. We thank Dr. B. Szalontai for his help in the treatment of IR spectra. We also thank Dr. F. Cuisinier for his support in OWLS experiments. Finally we thank Dr. C. Picart, Dr. Ph. Lavalle, and Professor G. Decher for fruitful discussions concerning polyelectrolyte multilayers.