Perturbation of Nanoscale Structure of Polypeptide Multilayer Thin Films

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Multilayer thin films formed by sequential deposition of oppositely charged polypeptides on a charged surface are known from previous studies to comprise a mixture of types of secondary structure. Here, study of the perturbation of polypeptide film structure by deposition of poly(allylamine hydrochloride) (PAH) and poly(styrenesulfonate) (PSS) on the film surface has revealed differences in behavior attributable to physical properties of the peptides. The methods of analysis were circular dichroism spectroscopy (CD), ultraviolet spectroscopy (UVS), and quartz crystal microbalance (QCM). Films made of poly(L-lysine) (PLL) and poly(L-glutamic acid) (PLGA) with an average charge per monomer of about 1 were substantially more susceptible to perturbation of structure than films made of designed polypeptides with an average charge per monomer of about 0.5, despite preparation under identical conditions. PLL−PLGA films showed loss or gain of material and change in secondary structure content on perturbation, whether made of high molecular mass (ca. 90 kDa) or low molecular mass (ca. 14 kDa) polymers. By contrast, films made of very low molecular mass (ca. 3.5 kDa) designed polypeptides showed little change in secondary structure content. The data suggest that the penetrability of PSS or PAH into a film and therefore film density can depend substantially on the polypeptides of which it is made and the character of intermolecular interactions.

Introduction

A polyelectrolyte multilayer film of predetermined layer order can be made by alternate dipping of a charged substrate into dilute solutions of a polycation and a polyanion.[1,2] The process is known as layer-by-layer self-assembly (LbL). Mostly strong polyelectrolytes are used in LbL, with electrostatic interactions playing the dominant role in polymer adsorption. The result of the assembly process will usually be a thin, somewhat dense, isotropic film. Recently interest has grown in films formed from weak polyelectrolytes. The linear charge density of these polyions can vary substantially with pH, enabling hydrogen bonds and hydrophobic interactions to contribute more substantially to multilayer formation and stability. One can form a loose and anisotropic film of locally ordered structure. Buildup of material on the substrate during the film formation process will be linear or nonlinear, depending on the polyelectrolytes,[3−6] substrate,[7] and ionic strength.[8]

Several researchers have reported effects on multilayer film properties attributable to the nature of the polyelectrolyte in the outermost layer.[9−11] Xie and Granick,[9] for example, have investigated local electrostatic interactions within a quaternized poly(vinylpyridine)−PSS polyelectrolyte multilayer in which the weak polyelectrolyte poly(methacrylic acid) was embedded. The degree of ionization of the polymer in the film was found to be sensitive to the quantity and nature of strong polyelectrolyte deposited in the outermost layer. Boulmedais et al.[10] have reported on multilayers of the polypeptides PLL and PLGA. Deposition of $(PSS-PAH)_2$ on $(PLL-PLGA)_n$ resulted in "the total disappearance of the β sheets" within the film, according to analysis by Fourier transform infrared spectroscopy (FTIR). Diffusion of the strong polyelectrolyte PSS into the film and substitution of PLGA by PSS were suggested as mechanisms of loss of β structure.

Designed polypeptides have recently been introduced as a type of advanced material for the preparation of multilayer films.[6,12,13] Assembly of oppositely charged 32-mers containing the amino acid cysteine has enabled inherent reversible chemical cross-linking and strengthening of films under mild reaction conditions.[6,13] The chiral nature of polypeptides makes them and films made of them amenable to analysis by CD. References 13 and 14 discuss how the relative quantities of the various secondary structure types can be estimated by deconvo-

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lution of a CD spectrum. In short, the process assumes that the negative Cotton effects at 208 and 222 nm indicate α helix, the positive and negative bands at 197 and 216 nm indicate β sheet, and the negative band at 205 nm indicates random coil, as in the characteristic spectrum of the respective type of secondary structure.[15]

Here, we report results of a study of the susceptibility of the structure of a polypeptide film to perturbation by deposition of poly(allylamine hydrochloride) (PAH) and poly(styrenesulfonate) (PSS) on the film surface. The extent and character of change of film structure has been found to depend substantially on chemical and physical properties of the peptides, as judged by CD. Two classes of polypeptide have been analyzed: relatively long chains of PLL and PLGA, with an average charge per monomer of about 1, and relatively short chains of designed polypeptides, with an average charge per monomer of about 0.5.

Materials and Methods

Polymers. Lyophilized PLL hydrobromide salt (molecular mass 14.6, 48.1, 84, and 222 kDa) and PLGA sodium salt (molecular mass 13.6, 50.3, 84.6, and 97.8 kDa), from Sigma, Inc. (St. Louis, MO), were used without further purification. The designed polypeptides were synthesized as described previously on the Advanced ChemTech Apex 396 instrument at Louisiana Tech.[6,13] The amino acid sequences were as follows

positive, KVKGKCKVKVKGKCKVKVKGKCKVKVKGKCKY,

negative, EVEGECEVEVEGECEVEVEGECEVEVEGECEY,

where K, E, V, G, C, and Y represent, respectively, lysine, glutamic acid, valine, glycine, cysteine, and tyrosine. PAH (70 kDa) and PSS (70 kDa) were from Sigma−Aldrich, Inc., and were used without further purification. All films were prepared at pH 7.4 in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and 0.15 M NaCl. Disulfide cross-linking of designed polypeptide films was achieved by exposure overnight to 20% (v/v) dimethyl sulfoxide (DMSO), 1 μM $MnCl_2$, and 10 mM Tris-HCl saturated with air, pH 7.5.

Multilayer Formation. Substrates were quartz microscope slides or QCM quartz resonators, both negatively charged. Multilayer films were formed by alternate immersion of a substrate into buffered aqueous solution of 1 mg/mL positive peptide or negative peptide for 15 min per adsorption step. The process resulted in the deposition of polyelectrolyte on both sides of the substrate. After each adsorption step, the substrate was rinsed three times with ultrapure deionized (18.2 MΩ·cm) water (Milli-Q System, Millipore) and dried with gaseous nitrogen at room temperature for measurement by optical spectroscopy or QCM.

Optical Spectroscopy. Quartz microscope slides for CD and UVS were from Electron Microscopy Sciences (USA). Plates were cut into 10 × 25 mm^2 pieces for separate experiments. Slides were cleaned in 1% SDS at 80 °C with agitation for 30 min, immersed into 1% NaOH in ethanol/H_2O (60/40, v/v) for 2 h, rinsed with ultrapure deionized water, and dried with a stream of nitrogen gas.

CD can be used to determine structural properties of chiral molecules by measuring the difference in absorption of right- and left-circularly polarized light. In the far-UV region, 180−260 nm, the spectrum of a polypeptide is particularly sensitive to conformation, in solution or in a multilayer film.[14,16−19] Spectra were recorded using a Jasco J-810 spectropolarimeter (Japan) with instrument settings of 100 mdeg sensitivity, 1 nm bandwidth, 1 s response time, 1 nm data pitch, and 100 nm·min^{-1} scan rate. A total of 50−80 scans were accumulated and averaged in each experiment. The sample quartz microscope slide was placed in a sample holder and fixed; the area exposed to the beam was constant (∼80 mm^2). Unmodified substrate was used to collect the baseline spectrum, which was subtracted from the corresponding sample spectra. Far-UV CD spectra were deconvoluted into contributions from α helix, β sheet, β turn, and random coil using the CDPro software suite (program CONTINLL).[20] Background information on CD and deconvolution of peptide spectra can be found in refs 13, 14, 19, and 20 and references cited therein. UVS enables determination of the optical mass of a polypeptide multilayer on a substrate. Absorption spectra were recorded on a Shimadzu UV-1650 PC UV−visible spectrophotometer (Japan) in the range 190−300 nm. Film assembly and film perturbation were monitored by CD and UVS.

QCM. Polypeptide multilayer film formation and perturbation were monitored by QCM by use of an Agilent 53131A 225 MHz universal counter (USA) and silver-coated resonators (Sanwa Tsusho Co., Ltd., Japan). Each resonator consisted of a thin circular quartz plate 0.15 mm thick and 8.3 mm in diameter, both sides sputter-coated with silver to form electrodes. The nominal natural frequency was 9 MHz. The resonator cleaning procedure can be found in ref 14. Change in resonant frequency of the quartz crystal Δf was assumed to be proportional to mass deposited on the basis of the Sauerbrey equation.[21] Mass increase Δm can be calculated from Δf and the resonator surface area A as $\Delta f = -\Delta m(1.83 \times 10^8)/A$. The mass sensitivity constant has units of Hz·cm^2·g^{-1} and here $A \approx 0.16 \pm 0.01$ cm^2.

Results

PLL and PLGA. Figure 1a presents spectra of polypeptide multilayers made of 14.6 kDa PLL and 13.6 kDa PLGA. The final peptide layer was PLGA. A positive Cotton effect is evident in the CD spectrum near 197 nm and a negative one near 216 nm. This implies the substantial presence of β sheet and random coil in the film, consistent with previous reports.[10,14] Deposition of a bilayer of ca. 70 kDa PAH and ca. 70 kDa PSS onto the film resulted in a decrease in intensity and broadening of the negative Cotton effect, indicating disruption of internal structure. The positive band was blue-shifted in this process by about 1 nm. UVS showed a small increase in the spectrum near 195 nm on deposition of $(PAH-PSS)_1$, owing to nonpolypeptide polymer deposition (see PAH−PSS film spectra in Supporting Information) and, possibly, the dependence of peptide bond absorbance on polypeptide conformation. The characteristic absorption peak of PAH−PSS is seen near 230 nm.

Figure 1b,c shows the effect of depositing PAH and PSS on a PLL−PLGA film when PLL was the final peptide layer. PAH (panel b) or PSS (panel c) was deposited first. The film spectrum after deposition of $(PAH-PSS)_1$ resembled that of PLL−PLGA films ending in PLGA (Figure 1a). The result of depositing $(PSS-PAH)_1$ was rather different: the positive and negative Cotton effects nearly disappeared, and in the UVS spectrum there was an obvious decrease in magnitude near 195 nm and an increase near 230 nm. The 230 nm peak proves that nonpeptide polymers were deposited, and the 195 nm peak is consistent with some mass loss. To summarize, the extent of structural change depends on whether PSS was deposited before or after PAH, and it would appear that film structure is influenced more by deposition of PSS, a strong polyelectrolyte, than PAH, a weak polyelectrolyte.

We have also studied whether polypeptide chain length has any bearing on the extent of perturbation of polypeptide film structure by PAH and PSS (Figure 2). CD spectra

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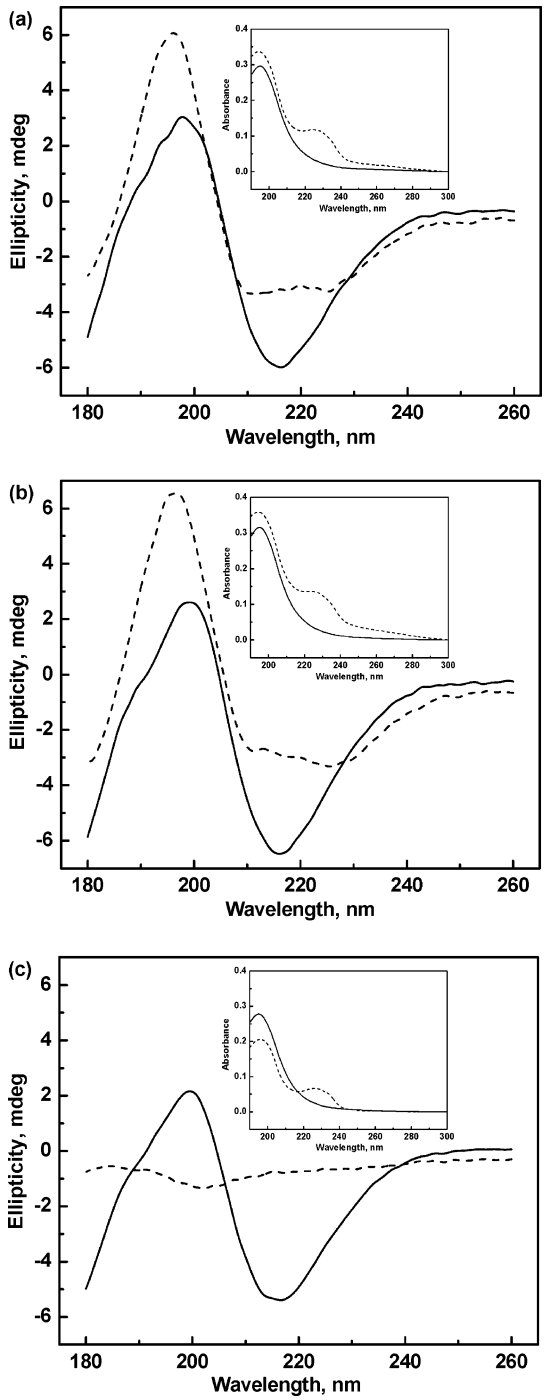

Figure 1. Effect of deposition of PAH and PSS on CD and UVS spectra of multilayer films of PLL (14.6 kDa) and PLGA (13.6 kDa). (a) Final layer of polypeptide PLGA: (−) $(PLL-PLGA)_{10}$ or (---) $(PLL-PLGA)_{10}-(PAH-PSS)_1$. (b) Final layer of polypeptide PLL: (−) $(PLL-PLGA)_{10}$ or (---) $(PLL-PLGA)_{10}-PLL-(PAH-PSS)_1$. (c) Final layer of polypeptide PLL: (−) $(PLL-PLGA)_{10}$ or (---) $(PLL-PLGA)_{10}-PLL-(PSS-PAH)_1$. The order of deposition of PAH and PSS is reversed with respect to panel b. The solid spectrum should be approximately the same in all three cases. The data, however, are from three separate experiments, and the film ends in PLGA in panel a and in PLL in panels b and c. After slight adjustment of the horizontal and vertical axes, the three solid spectra are nearly identical.

of films of ca. 14 kDa (a) peptides and of ca. 90 kDa peptides (c) show similar changes on deposition of $(PAH-PSS)_1$. The positive Cotton effect is shifted slightly to the UV, and the negative one is diminished in magnitude and flattened out. As to spectral differences, the lower mass peptides show an increase in the positive Cotton effect, and the higher mass ones show an obvious increase in absorbance near 195 nm in the UV absorption spectrum. Other combinations of chain length of PLL and PLGA—viz., 48.1 kDa PLL and 50.3 kDa PLGA (b), and 222 kDa PLL and 97.8 kDa PLGA (d)—behaved somewhat differently. The negative Cotton effect nearly vanished on perturbation, the positive Cotton effect remained about the same, and the UVS absorption peak near 195 nm declined in magnitude.

QCM also has been used to study PLL−PLGA film assembly and perturbation by PAH−PSS (Figure 3). Mass buildup as a function of adsorption step was nonlinear, and the overall frequency shift was substantially smaller for the shorter peptides (a) than the longer ones (b), consistent with previous reports.[10,19] The data would suggest that material was lost from the short peptide film on deposition of $(PAH-PSS)_1$. The assembly behavior of other chain lengths of PLL and PLGA (48.1 kDa PLL and 50.3 kDa PLGA, and 222 kDa PLL and 97.8 kDa PLGA) was not substantially different from the given examples, nor was the effect of depositing PAH and PSS (data not shown).

Designed Polypeptides. PLL−PLGA film properties have been compared with those of films made of designed polypeptides. Figure 4 shows the spectrum of a designed polypeptide multilayer film before and after deposition of $(PAH-PSS)_1$. Cotton effects are seen near 197 and 216 nm prior to adsorption of PAH and PSS, similar to PLL−PLGA films. Evidently, a large quantity of β sheet and random coil was present.[13] In marked contrast to $(PLL-PLGA)_n$, however, the designed peptide films showed little structural change on deposition of a PAH−PSS bilayer. The positive and negative Cotton effects decreased in magnitude by only a few percent, and the peak maxima did not shift. The UVS absorption peak near 195 nm increased only slightly on exposure to PAH, though substantially more when PSS was deposited, owing presumably to deposition of nonpolypeptide polymer on the surface and, perhaps, some structural change in the peptides. There was, however, almost no difference between depositing $(PAH-PSS)_1$ (Figure 4a) and $(PSS-PAH)_1$ (Figure 4b) on the film when the outermost layer was negative, again rather different from $(PLL-PLGA)_n$.

Previous work has shown that disulfide cross-linking can have a large impact on film stability under harsh conditions, for example, acidic pH.[6,13] Here, *stability* means susceptibility of film structure to environmental perturbation of any kind. We have used CD and UVS to determine whether disulfide cross-linking of the designed polypeptides in a multilayer film would influence susceptibility to perturbation of structure by PSS and PAH. Both of the designed peptides contain the amino acid cysteine, which can form a disulfide bond under oxidizing conditions. The data show that even smaller changes in the CD spectrum occurred on deposition of PAH−PSS than in the absence of cross-linking, and there was no dependence on the sequence of deposition of the non-peptide polyelectrolyte (see Supporting Information). This implies that the designed peptide films were relatively dense.

Discussion

Interpretation of CD Spectra. Two methods have been used in studies of polypeptide multilayer films to assess secondary structure content: FTIR[10,11,23,24] and

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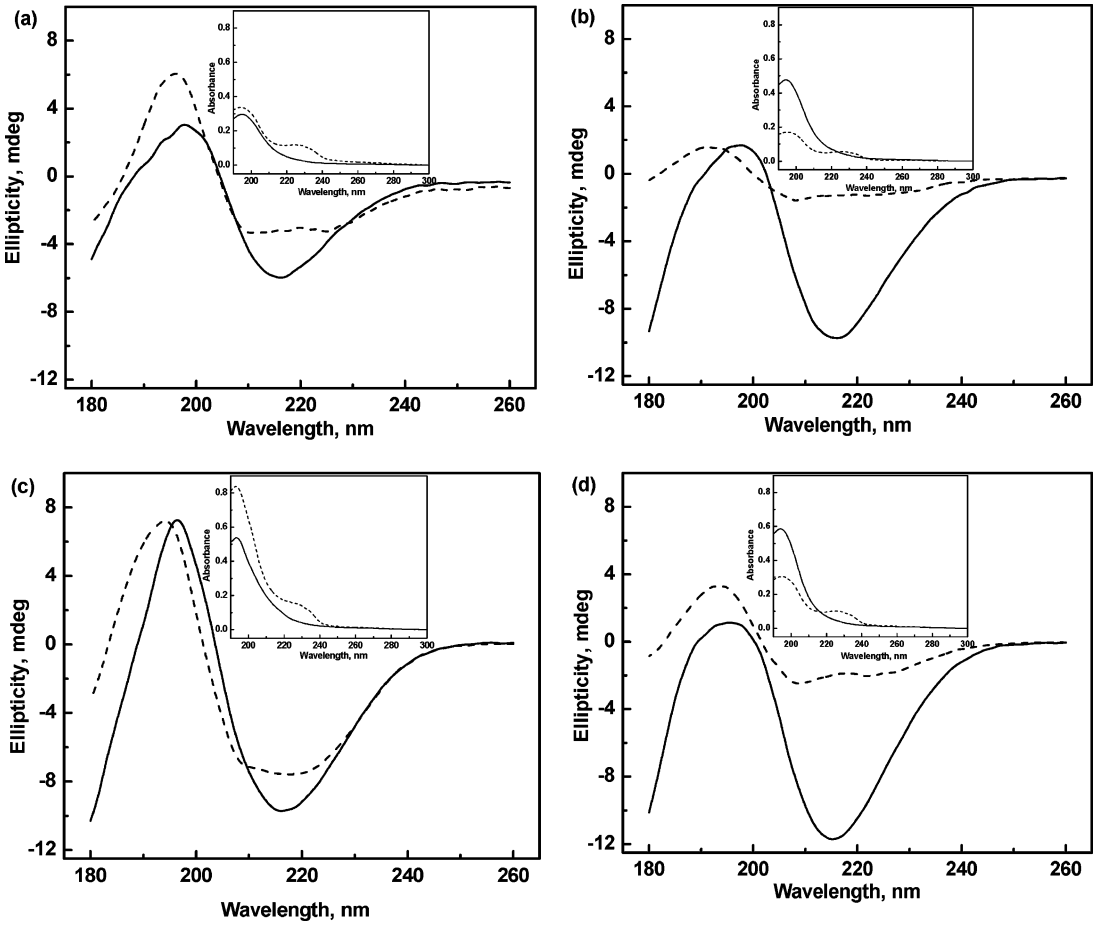

Figure 2. Optical spectra of multilayer films of PLL and PLGA of different average molecular weight, before and after deposition of PAH and PSS. (−) $(PLL\text{-}PLGA)_{10}$; (---) $(PLL{-}PLGA)_{10}{-}(PAH{-}PSS)_1$. Horizontal and vertical scales are the same in each case for the sake of comparison. (a) PLL (14.6 kDa) and PLGA (13.6 kDa). (b) PLL (48.1 kDa) and PLGA (50.3 kDa). (c) PLL (84.0 kDa) and PLGA (84.6 kDa). (d) PLL (222 kDa) and PLGA (97.8 kDa).

CD.[6,13,14,17,18] In analysis of film structure by FTIR, the amide I band is decomposed into contributions corresponding to the various types of secondary structure. Assignment of a unique absorption band to a certain structural group can be difficult, owing to extensive band overlapping. CD makes use of chirality to provide moderate-resolution structural information on proteins and peptides. The peptide bond absorbs strongly at wavelengths below 240 nm, and the character of absorbance depends substantially on conformation.[16] CD thus can provide information on secondary structure content of a polypeptide film and can be used to monitor changes in molecular structure on perturbation of environment.

Percentage contribution of the various types of secondary structure to a CD film spectrum can be estimated by deconvolution. The basic principle of the procedure is to regard a spectrum as a linear combination of spectra corresponding to the various types of secondary structure. Each type of secondary structure has a distinctive spectrum. Several assumptions and a set of reference spectra, then, underlie the usual deconvolution process.[20,25] Here we have made the additional assumption that PAH and PSS make no direct contribution to the CD signal; neither polymer is chiral, and the CD spectrum of a PAH−PSS multilayer film is not substantially different from that of unmodified quartz plate (data not shown). We have also assumed that the absorption of side chains is insignificant in the far-UV region of the spectrum. This assumption is not very important in the present context, however, given the low percentage content of aromatic groups in the peptides, the low probability that they will be in a substantially asymmetrical environment in the film, as required to contribute to the CD signal, and the focus on the far-UV region of the spectrum. Film spectra thus were deemed to consist of polypeptide backbone contributions alone. Of course, this perspective hardly entails that deposition of PAH−PSS will not alter the structure of a polypeptide film.

PLL-PLGA Films. $(PLL{-}PLGA)_n$ films formed at neutral pH (Figures 1 and 2) contain a large amount of β sheet, β turn, and random coil but only a small amount of α helix (Figure 5), in agreement with previous studies.[10,14,19] PLL and PLGA are weak polyelectrolytes, so charge density can vary substantially with pH. At neutral pH both polypeptides are relatively highly charged, enabling strong attractive electrostatic interactions between adjacent layers and strong repulsive electrostatic interactions within a layer. This combination of interactions can induce secondary structure and stabilize it. The more globally organized β sheet appears to be favored over the more locally organized α helix under the conditions studied here. It may be that the huge variety of possible fully hydrogen-bonded β strands will provide greater entropic stability to β structure than α helices in the film.

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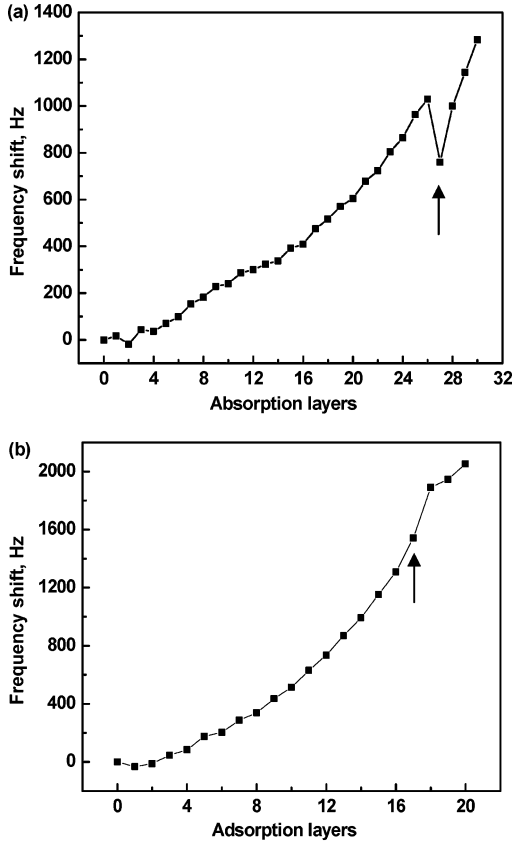

Figure 3. Frequency shift data of polypeptide film fabrication and perturbation by deposition of PAH and PSS. The absolute value of the frequency shift is shown. (a) $(PLL-PLGA)_{13}-(PAH-PSS)_2$: PLL (14.6 kDa) and PLGA (13.6 kDa). (b) $(PLL-PLGA)_8-(PAH-PSS)_2$: PLL (84.0 kDa) and PLGA (84 6 kDa). Note that two bilayers of PAH and PSS were deposited in these experiments. Arrows indicate where the first nonpolypeptide polyion was deposited.

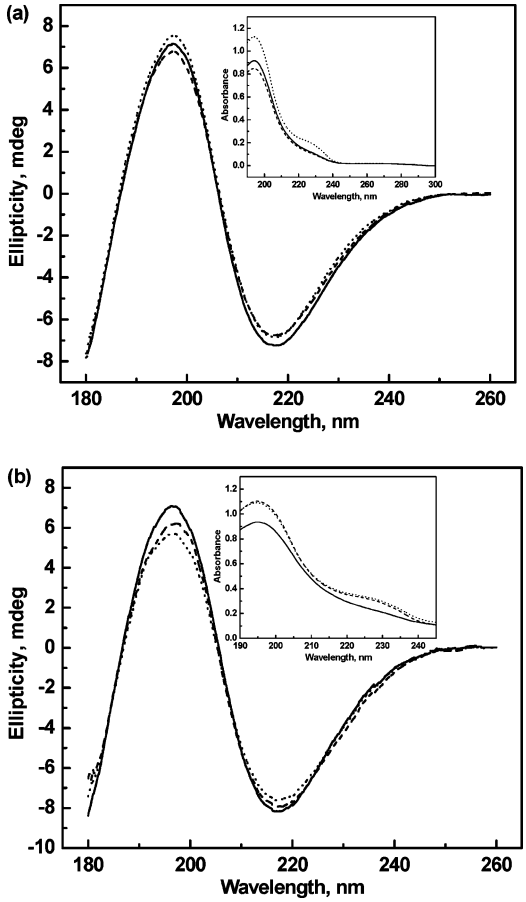

Figure 4. Optical spectra of multilayer films of designed polypeptides, before and after deposition of PAH and PSS. P = positive designed peptide, N = negative designed peptide. (a) (−) $(P-N)_{10}$; (---) $(P-N)_{10}-PAH$; (····) $(P-N)_{10}-(PAH-PSS)_1$. (b) (−) $(P-N)_{10}$; (---) $(P-N)_{10}-PSS$; (····) $(P-N)_{10}-(PSS-PAH)_1$. The order of deposition of PAH and PSS was reversed with respect to panel a. Cross-linked polypeptides showed relatively little difference in behavior (see Supporting Information).

Deconvolution of CD spectra suggests that deposition of $(PAH-PSS)_1$ on $(PLL-PLGA)_n$ results in an increase in the percentage of α helix in the film at the expense of random coil (Figure 5). The change in relative content of β structure is small. This conflicts to some degree with the work of Boulmedais et al.,[10] who have reported that, during film preparation, the percentage of α helix switches between a low value when the film is in contact with a PAH solution and a high value when it was in contact with a PLGA solution and that deposition of $(PSS-PAH)_2$ on $(PLGA-PLL)_n$ destroys β sheet structure throughout the film. Structure was analyzed by FTIR. By contrast, the present analysis by CD would suggest that only a small amount of α helix was present at any step of the film assembly process and that only a small fraction of β structure was lost on perturbation by PSS−PAH, but that the distribution of secondary structure changed on perturbation. Boulmedais et al.[10] have reported an increase in film thickness and mass also on deposition of $(PSS-PAH)_2$ on $(PLGA-PLL)_8$. Our experiments, however, indicate a loss of mass in some cases but not in others. When mass loss did occur, presumably it was due to resolubilization of PLGA on deposition of PSS or formation of a soluble complex involving material from the film and from solution.

Analysis by QCM has shown that growth of $(PLL-PLGA)_n$ films was nonlinear (Figure 3), consistent with previous findings.[3−5,19] Each PLL adsorption step involved the interaction of newly adsorbed chains with PLGA on the film surface and, possibly, the diffusion of PLL into the film to overcompensate the negative charge of the surface layer. CD suggests that deposition of $(PAH-PSS)_1$ on $(PLL-PLGA)_n$ involved the diffusion of PAH and/or PSS chains into the film. In any case, the result was disruption of secondary structure. Assuming inward diffusion of the nonpeptide polymers, it was driven in part by an increase in entropy, as the mixing of PAH and PSS with PLL and PLGA will decrease film order.

$(PLL-PLGA)_n$, though rich in secondary structure, is apparently not so dense or rigidly hydrogen-bonded that PAH and PSS cannot disrupt its structure. Individual electrostatic interactions between PLL residues and PLGA residues and any existing secondary structure will be weakened by thermal fluctuations, dielectric properties of water, the presence of highly mobile counterions, and the inward diffusion of PSS and PAH. The rate and overall extent of polyion exchange, assuming it occurs, will be influenced by polymer length, thermostability of secondary structures, and the density of the polypeptide film. Even in cases where polyion exchange goes to equilibrium very slowly, the process will provide a mechanism for rearrangement of secondary structure in the film and, probably, loss of deposited polypeptides. Deposition of PAH−PSS thus can serve as a useful probe of the strength of a polypeptide multilayer film and its susceptibility to environmental perturbation.

PAH is more similar to PLL than PSS is to PLGA with regard to dependence of linear charge density on pH.

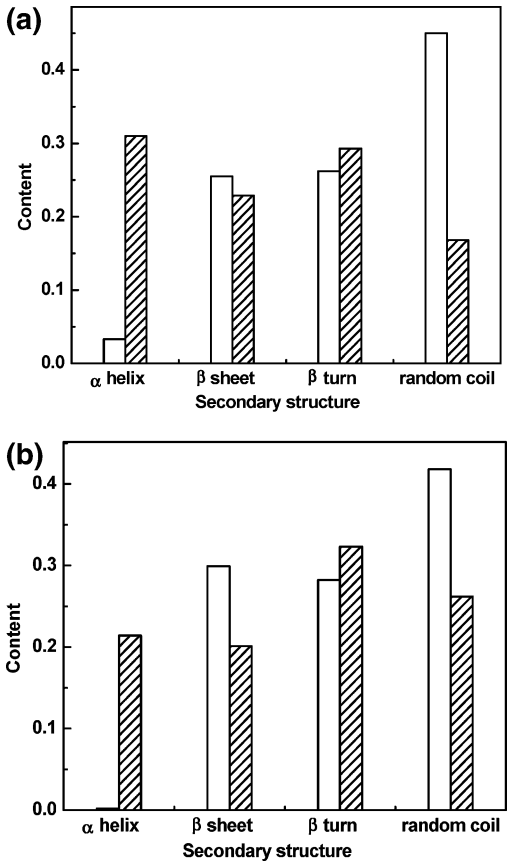

Figure 5. Secondary structure content of PLL−PLGA multilayers, before and after deposition of PAH and PSS. The data were obtained by deconvolution of the CD spectra in Figure 2a,c with the program CONTINLL. (Open bars) $(PLL-PLGA)_{10}$; (hatched bars) $(PLL-PLGA)_{10}-(PAH-PSS)_1$. (a) PLL (14.6 kDa) and PLGA (13.6 kDa); (b) PLL (84.0 kDa) and PLGA (84.6 kDa). The residuals of fitting are substantially smaller in magnitude than the fitted values. See ref 13 and Supporting Information.

Exchange between PAH and PLL therefore is expected to lead to smaller changes in film structure than exchange between PSS and PLGA. Figure 1b,c is consistent with this conclusion. PSS chains will also influence the extent of proton dissociation in a polypeptide film, changing the local network of electrostatic interactions and shifting pK_as even further from solution values.

Polymer length and degree of similarity of length between oppositely charged polypeptides would appear to have some bearing on the response of a polypeptide film to environmental perturbation by PAH−PSS (Figure 2). When the average molecular weight of PLL and PLGA was small (Figure 2a) or about the same (Figure 2c), $(PLL-PLGA)_n$ structure changed little on deposition of $(PAH-PSS)_1$. By contrast, when the molecular weight of the polypeptides was rather different, there was a relatively large change in secondary structure content on perturbation (Figure 2b,d).

Deposition of $(PAH-PSS)_1$ resulted in mass loss in some instances (Figure 3a). For instance, the overall film frequency shift decreased by about 25% after deposition of a PAH layer on $(PLL-PLGA)_{13}$ when the average molecular mass of the polypeptides was about 14 kDa. This may be attributable to the leaching of polypeptide into solution in the presence of the nonpeptide polyions. In other cases an increase in film mass was found, similar to the results of Boulmedais et al.[10] Figure 3b shows that frequency shift increased by nearly 20% on deposition of PAH on $(PLL-PLGA)_8$ when the average molecular mass was ca. 84 kDa. In the corresponding UVS experiment, however, only a small increase in photon absorption was evident. The apparent discrepancy can possibly be attributed to the well-known dependence of extinction coefficient on polypeptide backbone conformation.[16,26] It could also be that differences in surface roughness between the quartz resonator and the quartz slide have some bearing on the comparison.

Designed Polypeptide Films. Mass deposition of the designed peptides was remarkably linear (not shown), as found previously.[13] Weak polyelectrolytes as well as strong ones can exhibit linear growth. Moreover, the data show that linear versus nonlinear growth is not merely a matter of ionic strength of solution; PLL−PLGA and the designed peptides were assembled under identical conditions. Polymer size therefore could play a role in the design and engineering of polypeptide multilayer films. Figure 4 shows that secondary structure content of the designed peptide films was perturbed little on deposition of PAH and PSS. The obvious difference in behavior with respect to the PLL−PLGA films must ultimately be due to differences in chemical structure of the peptides.

Physical Basis of Polypeptide Film Stability. The data suggest that polymer size and structure influences the response of polypeptide film structure and stability to environmental perturbation. Table 1 in Supporting Information compares physical properties of the designed polypeptides, PLL, and PLGA. The average degree of polymerization of PLL and PLGA ranged from 105 to 1740 in these experiments, while the designed polypeptides were 32-mers. Polydispersity and charge per unit length also could be relevant to film assembly stability. PLL and PLGA, homopolymers prepared by solution-phase synthesis, were polydisperse, and the charge density was about 1 per monomer at neutral pH. By contrast, the designed polypeptides were heteropolymers of the same degree of polymerization, and the charge density was about 0.5 at neutral pH. Amino acid coupling in solid-phase synthesis was lower than 100% at some steps of the process, but the final products had a narrow range of dispersity (data not shown). Matching of the size of oppositely charged polyelectrolytes could influence the order and stability of a multilayer film, determining to some extent the role of counterions in the film. The degree of match in size will not precisely be reflected by average degree of polymerization of polypeptides prepared by solution-phase synthesis, as the degree of polydispersity could be quite different for the same average contour length.

The present work, Li et al.,[13] and unpublished results from our laboratory appear to indicate that small, designed polypeptides of uniform chain length and intermediate charge per unit length can self-assemble at low ionic strength into organized, stable, and apparently dense multilayer films (compare ref 18). Such films are both similar to and different from PLL−PLGA films. A schematic representation of the response of polypeptide multilayers to deposition of PAH and PSS is given in Figure 6. The designed peptide film data are consistent with a model in which the molecules self-assemble into a firmly fixed network of interactions, possibly similar in some respects to the core of a folded globular protein, providing considerable resistance to environmental perturbation. This interpretation is consistent with our

(26) Cantor, C. R.; Schimmel, P. R. In *Biophysical Chemistry. Part II: Techniques for the Study of Biological Structure and Function*; W. H. Freeman: New York, 2001; pp 385−405.

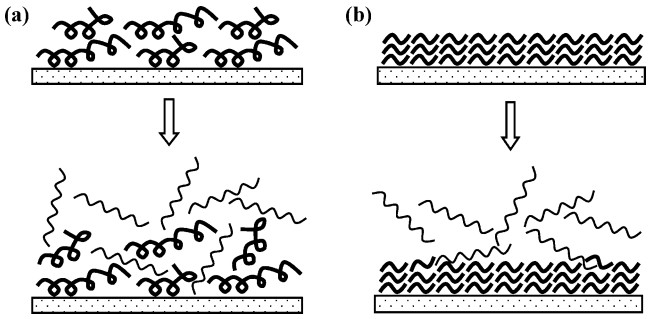

Figure 6. Schematic representation of the response of polypeptide multilayer films to deposition of PAH and PSS. (a) PLL and PLGA; (b) designed polypeptides. Symbols are as follows: boldface wavy lines indicate designed polypeptide chains, corkscrew shapes are PLL/PLGA chains, and lightface wavy lines are PAH/PSS chains. Destruction of PLL−PLGA multilayers by deposition of PAH and PSS suggests a relatively loose film structure, enabling PAH/PSS to diffuse into the film and perturb film structure. By contrast, designed polypeptide multilayers resisted perturbation by PAH and PSS, suggesting that the film is dense and crystallike and that PAH and PSS do not diffuse in.

finding that such films are stable under a variety of extreme conditions, for example, dehydration, extreme heating or cooling when dehydrated, heating when hydrated, and immersion in aqueous solution for an extended period of time or in an organic solvent (B.L., J. Rozas, and D.T.H., manuscript submitted for publication). It would appear, then, that the structure and stability of the designed polypeptide films inhibits the inward migration of PAH and PSS chains, limiting polyion exchange. Of course some such diffusion and exchange will necessarily occur because of the requirement for entropy to increase, but apparently to a much smaller extent than in PLL−PLGA films because of favorable enthalpic interactions between designed peptides.

Amino acid composition differs significantly between PLL, PLGA, and the designed polypeptides. The relative proportions of hydrophobic and hydrophilic surface areas are different. Table 2 in Supporting Information shows the accessible surface areas and hydropathy values of the amino acid residues in the peptides studied here. Hydrophobicity and hydrophilicity can be quantified as the accessible nonpolar and polar surface areas of the extended polypeptide chain. The positively charged polypeptides, when fully extended, have a substantially larger fraction of nonpolar accessible surface area than the negatively charged polypeptides. PLL and PLGA have much lower hydropathy values than the designed polypeptides due to differences in charge density; the designed polypeptides are more hydrophobic than PLL and PLGA. This suggests that if electrostatic interactions play the most significant role in polypeptide multilayer film formation and stability, hydrophobic interactions will still play a role, and charge per unit length and chain length will affect film density.

Conclusions

Multilayer thin films have been formed from various types of polypeptide, and CD, UVS, and QCM have been used to study film assembly and changes in film properties on deposition of PAH and PSS. CD would appear to be better suited than FTIR to determination of the secondary structure content of polypeptide multilayer films. Weak polyelectrolytes as well as strong ones can exhibit linear growth at relatively low ionic strength, depending on polymer structure. Gain or loss of deposited mass occurs on deposition of a PAH−PSS bilayer, the response varying with the polypeptides used to construct the multilayer. PAH and PSS chains would appear to diffuse into $(PLL-PLGA)_n$, resulting in rearrangement of polymer chains and/or replacement of PLGA by PSS. There is some induction of α-helical structure in PLL−PLGA films on deposition of PAH and PSS. By contrast, the relative content of β structure, which is energetically favorable than α structure and generally more abundant in polypeptide multilayer films, would appear to change little. The degree of structural change on perturbation depends on polymer molecular weight, polymer primary structure, and polydispersity. Films built of designed polypeptides resist perturbation by deposition of PAH and PSS. The amount of adsorbed material and the secondary structure content of these films remained almost unchanged on deposition of the nonpeptide polymers, regardless of chemical cross-linking. The apparent reason is that the designed polypeptides are much smaller and more hydrophobic than PLL and PLGA and therefore assemble into more dense and more stable multilayers. Control of polypeptide structure enables control over polypeptide film properties. Peptide design therefore could play a key role in the engineering of thin films, particularly in biomedicine, biotechnology, food technology, environmental technology, and other areas where biocompatibility, biofunctionality, edibility, and environmental benignity are design requirements.

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Supporting Information Available: Figures showing CD and UV spectra of cross-linked polypeptide multilayers, goodness of fit in deconvolution of CD spectra, and assembly of PAH−PSS film, and tables of degrees of polymerization of polypeptides and the hydrophobicity/hydrophilicity of polypeptides. This information is available free of charge via the Internet at http://pubs.acs.org.

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