Interaction of Cationic Polypeptides with Electroactive Polypyrrole/Poly(styrenesulfonate) and Poly(*N*-methylpyrrole)/Poly(styrenesulfonate) Films

Lynette A. Prezyna,[†] Yong-Jian Qiu,[‡] John R. Reynolds,*[,‡] and Gary E. Wnek*[,†]

Department of Chemistry and Polymer Science and Engineering Program, Rensselaer Polytechnic Institute, Troy, New York 12180-3590, and Center For Advanced Polymer Research, Department of Chemistry, The University of Texas at Arlington, Arlington, Texas 76019-0065

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ABSTRACT: The binding of cationic polypeptides to polypyrrole (PP)/poly(styrenesulfonate) (PSS) and poly(*N*-methylpyrrole) (PNMP)/PSS films, along with the effect of binding on film electroactivity, has been studied by using the electrochemical quartz crystal microbalance (EQCM). Two polypeptides were studied, namely, poly(L-lysine hydrochloride) and the arginine-rich histone H3. Poly(L-lysine hydrochloride) binds to either the oxidized or reduced forms of the films, although the electroactivity and ability of the films to transport small cations upon electrochemical cycling remain intact. Histone H3 shows little tendency to bind to oxidized films, but upon electrochemical reduction PSS is extracted. This unusual behavior is thought to be the result of strong electrostatic and hydrophobic interactions between the PSS and H3 at the PP/PSS surface. The films retain their electroactivity after interaction with H3. These observations lead us to propose a model for the surface structure of the electroactive composite films. We suggest that there exist in these films three types of PSS polyanions. These are well-entangled chains, most of whose sulfonates are employed as dopants; polyanions existing primarily near the surface, with only a portion of the chain acting as a dopant; and some PSS chains that are weakly adsorbed or very loosely held by physical interactions and contribute few sulfonates as dopants.

Introduction

Since polypyrrole (PP) was first electrochemically synthesized by Diaz et al.[1] nearly a decade ago, there has been substantial interest in research dealing with its electroactive properties. Of particular interest is the fact that conductive, oxidized polypyrrole is reasonably stabe in aqueous solutions, making it an attractive material for biomedical applications. Various devices can be envisioned that utilize the electroactivity, providing unique systems that exploit the combination of an electrochemical stimulus with the selectivity and specificity of biological systems. Miller et al.[2–4] nicely illustrated the use of conductive polymers in drug delivery by demonstrating that electrochemical codeposition of poly(*N*-methylpyrrole) (PNMP) and poly(styrenesulfonate) (PSS) afforded conductive polymer films that cathodically bind and anodically release small cations such as dopamine. The cations, bound to the PSS, are released upon electrochemical oxidation of the PNMP as the sulfonate moieties of the PSS become the counteranions for the conductive polymer. We note that cation binding and release can also be accomplished with "self-doped" polyheterocycles containing pendant sulfonates.[5–7] Anions of biological interest, such as ATP, can also be incorporated into polypyrrole.[8]

We are interested in polyheterocycle/polyelectrolyte composite films such as PNMP/PSS (and/or PP/PSS) as model systems to bind and possibly release polycationic polypeptides. Binding via cooperative, electrostatic interactions may be a useful means to modify the surface charge concentration and biological activity of the composite films. Strong electrostatic interactions between the PSS and polycation, however, may preclude the release of the polycation upon oxidation of the electroactive polymer. We note that PSS, a synthetic polyanion, is capable of decondensing chromatin, resulting in the activation of RNA synthesis.[9] PSS successfully competes with DNA for cationic binding sites on the DNA-packaging proteins, known as histones, which contain numerous positively charged residues. Thus, a motivation for our work is the intriguing possibility of activating chromatin decondensation electrochemically. Histone H3 is utilized in our study as one of the polycations because of its high concentration of positively charged arginine residues[10] and some limited knowledge about the interaction of H3 with PSS in solution.[11]

It was expected that, upon reduction of the oxidized form of PP/PSS or PNMP/PSS, sulfonates would become available at the film surface, which could bind polycations. However, we recently observed[12] that during reduction of PP/PSS in the presence of H3 at pH ≈ 5.4, precipitates are formed in the electrochemical cell. Our preliminary study[10] of PSS/H3 interactions in solution indicate that precipitates readily form when H3/PSS exceeds ca. 1:1. We were thus tempted to conclude that PSS was extracted from the conductive polymer film, rather than histone being bound, contrary to expectations. We decided to study this system in more detail by coupling electrochemical methods with the quartz crystal microbalance (QCM). This combination, termed EQCM, gives us the ability to analyze both polyheterocycle electrosynthesis and mass flux into and out of the film during switching. The EQCM technique allows us to monitor electrochemical parameters (current, charge, etc.) and mass changes simultaneously with any applied waveform (scanning or stepping potential). Of particular interest to us is the electroactivity of the films after exposure to polycations. Details of the EQCM technique are well-known and documented in previous literature.[13,14]

We report here on the interaction of H3 and, for comparative purposes, the homopolycation poly(L-lysine hydrochloride) with PP/PSS and PNMP/PSS films.

[†] Rensselaer Polytechnic Institute.

[‡] University of Texas at Arlington.

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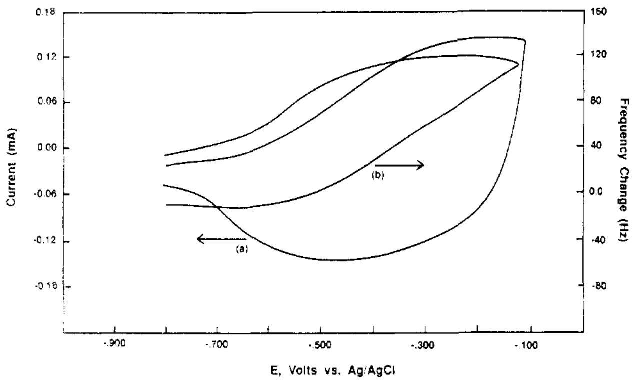

Figure 1. (a) Amperometric cyclic voltammogram of PP/PSS in 0.1 M aqueous Na^+PSS^-. Scan rate = 25 mV s^{-1}. (b) Gravimetric cyclic voltammogram of PP/PSS in 0.1 M aqueous Na^+PSS^-. Scan rate = 25 mV s^{-1}.

Experimental Section

Chemicals. Sodium poly(styrenesulfonate), 100% sulfonated, was obtained from Aldrich Chemical Co. Pyrrole (Aldrich) and *N*-methylpyrrole (Aldrich) were passed over neutral alumina until colorless and stored in the dark under argon until use. Arginine-rich histone (H3; calf thymus), tris(hydroxymethyl)aminomethane (Tris), its hydrochloride (Tris-HCl), and poly(L-lysine hydrochloride) (Pl) were obtained from Sigma Chemical Co. and used as received. The number-average molecular weight of the Pl was ca. 30 000 g/mol. Solutions of Tris-HCl used as an electrolyte (pH ≈ 5.4) and Tris/Tris-HCl buffer solutions (pH ≈ 7.2) were obtained by using only triply distilled water.

Quartz Crystal Microbalance Studies of H3 and Poly(lysine) Binding. PP/PSS and PNMP/PSS films were prepared by potentiostatic polymerization at 0.8 V versus Ag/AgCl, using 0.1 M pyrrole and 0.1 M Na^+PSS^- in triple distilled and filtered water. The working electrode was a gold-coated (0.71 cm^2) quartz plate described previously[13] and the counter electrode was a platinum plate. Films were rinsed with water and placed in an aqueous solution of Na^+PSS^- free from monomer. Cyclic voltammetric, chronogravimetric, and chronoamperometric studies were carried out to demonstrate the initial electroactivity of the composite films in Na^+PSS^-(aq). The frequency responses, corresponding to mass changes, were monitored upon exposure to 0.01 M Tris-HCl electrolyte (pH ≈ 5.4) and to 50 mM arginine-rich histone (H3) in 0.01 M Tris-HCl (pH ≈ 5.4) and Tris/Tris-HCl buffer (pH ≈ 7.4). The reduction of the PP/PSS and PNMP/PSS films was carried out at -1.0 V and oxidation at 0.0 V, versus Ag/AgCl. Similar experiments were carried out using Pl (0.01 M) in water (pH ≈ 5) and in 0.01 M Tris-HCl.

Results and Discussion

We first demonstrate that, as expected, mass is *gained* when the composite films are electrochemically reduced in the presence of aqueous solutions of alkali metal and organic cations. We then use the EQCM technique to investigate the interaction of H3 and Pl by the films and the electroactivity of the films after exposure to the polypeptides.

Alkali Metal Cation Binding and Release. PP/PSS and PNMP/PSS composites were grown with a 3000-Hz frequency change on the bare electrode. Using the value of 18 ng Hz^{-1} cm^{-2} and a density of ca. 1.27 g/cm^3, we calculate that these films have a thickness of ca. 400 nm. A typical amperometric cyclic voltammogram (A-CV) and a gravimetric cyclic voltammogram (G-CV) for PP/PSS in Na^+PSS^- are shown in Figure 1. The A-CV shows a broad oxidation peaking at ca. -0.2 V versus Ag/AgCl and a reduction at ca. -0.45 V. The breadth of the A-CV can be attributed to the use of a large (0.7 cm^2) working electrode in the EQCM experiment. This A-CV sharpens considerably when carried out on a PAR Model 273 potentiostat/galvanostat and Pt button (0.018 cm^2) working electrode. Scanning anodically, the concurrent G-CV exhibits a frequency increase (mass decrease) commencing at ca. -0.55 V. As the oxidation of the film proceeds, the expulsion of Na^+ from the PP/PSS leads to ca. a 120-Hz change. Reversing the scan, a mass increase is observed during reduction as Na^+ ions are taken back into the membrane to compensate the now free sulfonate anions. The magnitude of this frequency change is a function of the mass of the mobile species. For example, replacement of the Na^+ with Cs^+ leads to 300-Hz changes during switching. The 40-Hz difference between the initial and final states at -0.8 V may be attributed to either kinetic effects in the ion transport or a variable amount of solvent moving in and out of the film during switching.[15]

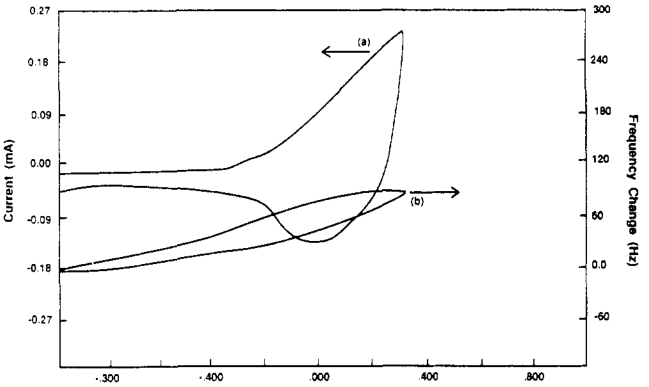

Figure 2. Amperometric (a) and gravimetric (b) cyclic voltammograms of PNMP/PSS in 0.1 M aqueous Na^+PSS^-. Scan rate = 25 mV s^{-1}.

Similar results are obtained when PNMP/PSS composites are employed, with one important difference. This is illustrated in the amperometric and gravimetric cyclic voltammograms carried out in Na^+PSS^- (Figure 2). The lack of a distinct anodic peak in the amperometric cyclic voltammogram, and the lower frequency shift in the gravimetric CV, is due to the fact that the PNMP/PSS composite has a relatively low electroactivity, which is not surprising considering its low conductivity (ca. 10^{-3} S/cm versus ca. 10^{+1} S/cm for PP).[4]

Electrochemical Cycling in Tris-HCl. Exposure of reduced PP/PSS or PNMP/PSS to solutions of Tris-HCl (pH ≈ 5.4) reveals a small initial mass gain and then a slow mass loss. It should be pointed out that, in the electrochemically grown films, it is unlikely that all of the sulfonate anions are charge compensated by PP cations, the remainder being compensated by Na^+ or protons. Immersion in Tris-HCl is expected to lead to ion exchange,[16] and we believe the initial gain in mass is the result of this phenomenon. We observe a ca. 75-Hz change after 30 min, which is rather small compared with the frequency changes to be discussed later. It is not clear if this slow mass loss represents instrumental drift or slow removal of PSS from the composite. The chronogravimetric response of PS/PSS in 0.01 M Tris-HCl, during repeated oxidation and reduction, is shown in Figure 3. It can be seen that electrochemical switching causes a mobile cation (most likely Tris-H^+) to be released when the polymer is oxidized and bound upon reduction. The robust electroactivity of the composite films in the aqueous buffer is evident from the reproducible frequency changes observed when the polymer film is electrochemically switched. The small net loss of mass (frequency increase) as cycling proceeds is, again, likely the result of instrumental drift.

Experiments with Pl. Figure 4 shows the QCM

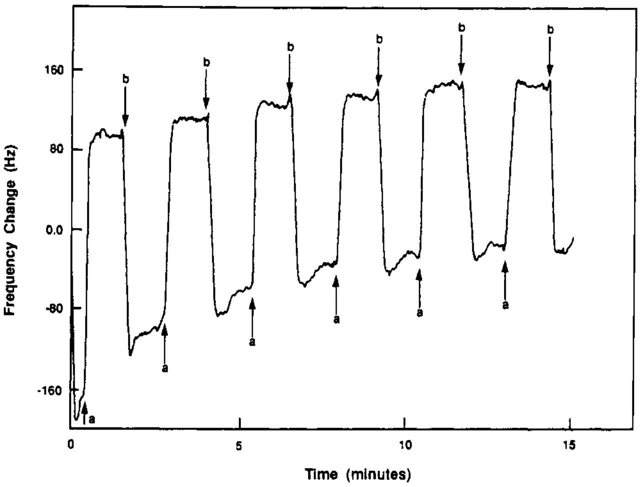

Figure 3. Chronogravimetric response of PP/PSS in 0.01 M aqueous Tris-HCl during repeated oxidative (a, 0.0 V versus Ag/AgCl) and reductive (b, −1.0 V versus Ag/AgCl) steps.

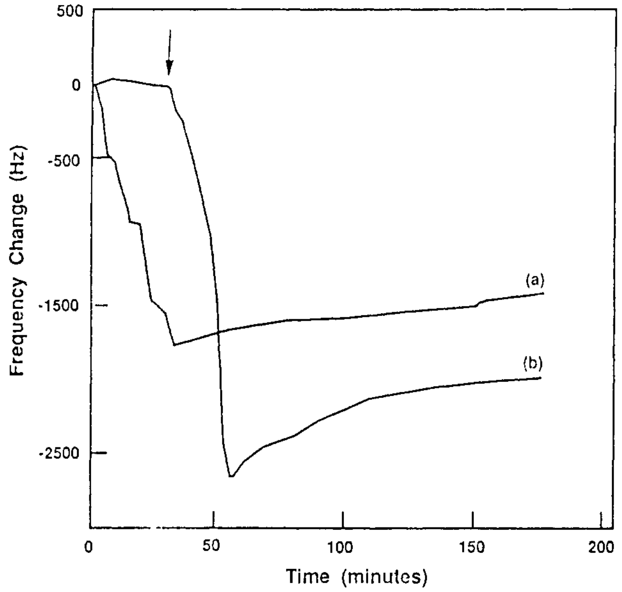

Figure 4. EQCM frequency response with time of (a) reduced and (b) oxidized PP/PSS exposed to poly(L-lysine hydrochloride) at pH = 5.

frequency responses of oxidized and reduced PP/PSS films to Pl at pH = 5 (unbuffered) as a function of time. Mass uptake is significant in both cases (Δf = 1500–2500 Hz), indicating that both forms adsorb the polycation.[17] Importantly, the PP/PSS films remain electroactive as indicated in Figure 5, which shows the A-CV and G-CV behavior of PP/PSS in aqueous NaCl after it had been soaked in a Pl solution for 3 h in the reduced form. The data reveal a definite, reproducible initial mass gain (beginning between −1.0 and −0.8 V versus Ag/AgCl) upon scanning anodically. We believe that some of the PSS anion (dopant) sites are deactivated because of binding to the Pl. Hence, anions from the electrolyte (e.g., Cl^-) are being used to balance positive charges on the PP created by oxidation. Subsequently, the films exhibit cation transport behavior as suggested by reversible mass changes upon cycling. The initial mass gain is not observed when an oxidized PP/PSS film is exposed to Pl for a similar period and then cycled, suggesting that interactions between oxidized PP/PSS and the polycation are relatively weak. As a result, fewer PSS anionic sites have been deactivated due to binding with Pl. In no case do we see evidence for release of Pl upon oxidation, which is expected since there is no driving force to dissociate the PSS/Pl polyelectrolyte complex. Also, negligible mass gain is observed when a PP/tosylate film (oxidized or reduced) is allowed to contact aqueous Pl, indicating that the polyanionic dopant is necessary for Pl adsorption.

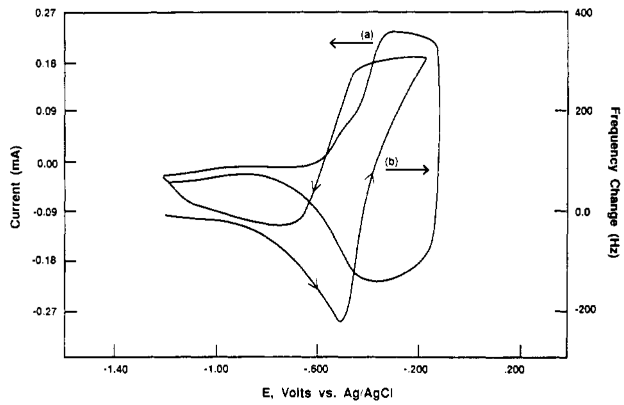

Figure 5. Amperometric (a) and gravimetric (b) cyclic voltammogram of PP/PSS in 0.1 M aqueous NaCl after a 3-h exposure to poly(L-lysine hydrochloride) in the reduced form.

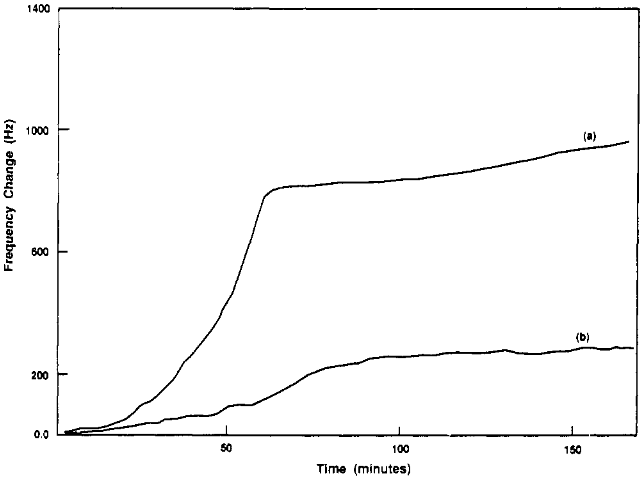

Figure 6. EQCM frequency response with time for the reduced form of (a) PP/PSS and (b) PNMP/PSS exposed to histone H3 in 0.01 M Tris-HCl.

Experiments with Histone H3. In contrast to the observations with Pl, exposure of either reduced PP/PSS or reduced PNMP/PSS films to Tris-HCl solutions of H3 results in a dramatic mass loss, as illustrated in Figure 6. For the case of PP/PSS (Figure 6a), the QCM frequency change promptly levels off at approximately 800 Hz after 70 min.[18,19] The changes between 70 min and the end of the experiment at 200 min are most likely due to instrumental drift. As the mass loss is occurring, the solution was observed to cloud significantly. We believe this mass loss and clouding, also noted earlier in our UV study,[9] reflects the loss of PSS from the polymer film and subsequent complexing with H3. (It is likely that complexation to some degree *precedes* extraction of PSS.) Experiments employing PNMP/PSS revealed less mass loss (Figure 6b), a result consistent with our UV study, which suggests that less PSS is extracted from PNMP/PSS upon exposure to H3 than from PP/PSS. Figure 7 shows that even after long (3.3 h) exposure to H3 in Tris-HCl, the film retains its electroactivity along with its cation binding and release ability. Note that no significant mass change is observed when the oxidized form of PP/PSS or PNMP/PSS is exposed to H3 solutions (Figure 8), in contrast to what is observed with Pl.

The observations in this work prompt us to suggest a crude picture of the structure of these electroactive

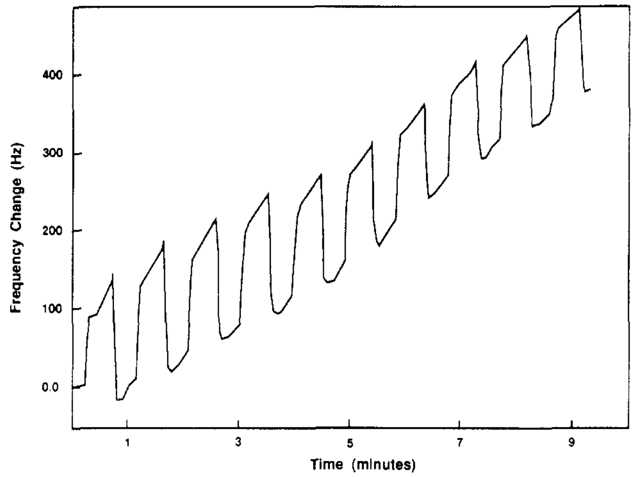

Figure 7. Chronogravimetric response of PP/PSS with repeated oxidative (0.0 V versus Ag/AgCl) and reductive (–1.0 V versus Ag/AgCl) steps after a 3.3-h exposure to histone H3 in 0.01 M Tris-HCl.

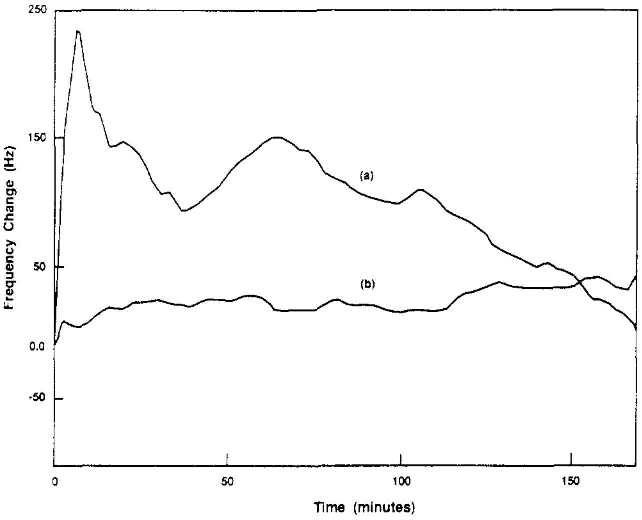

Figure 8. EQCM frequency response with time for the oxidized form of (a) PP/PSS and (b) PNMP/PSS exposed to histone H3 in 0.01 M Tris-HCl.

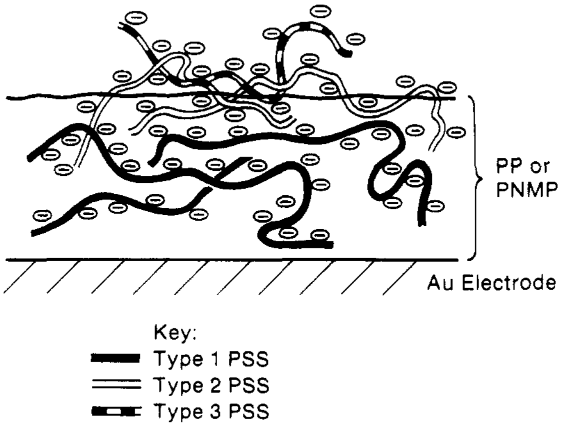

Figure 9. Proposed structure of PP/PSS or PNMP/PSS composite film showing three types of PSS chains. Pl binds to type 2 and type 3; H3 extracts type 3 and some type 2 chains that are not buried too deeply in the composite.

composites (Figure 9). The films are thought to be entangled chains of PSS and PP (or PNMP), with some of the sulfonates serving as dopant anions for the latter. We suggest that there exist in these films three types of PSS polyanions. The first ("type 1" polyanions) are well-entangled chains, and a relatively large portion of its sulfonates are employed as dopants in the PP oxidized form. Exposure of the films to either H3 or Pl leads to essentially no diminution of the cation transporting capacity of the films, suggesting that the type 1 sites are unaffected by polycation binding. "Type 2" polyanions exist primarily near the surface, with only a portion of the chain acting as a dopant, while the remainder of the chain is neutralized by cations in the electrolyte (Na^+, Tris-H^+). It is also conceivable that some PSS chains are very loosely held by physical interactions and contribute few (or no) sulfonates as dopants ("type 3" polyanions).[20] The presence of type 2 and type 3 sulfonates accounts for the binding of Pl by the oxidized form of the electroactive composite film. Some type 2 sulfonates that initially served as dopant anions are complexed when the reduced form is contacted with Pl, as suggested by the mass gain in Figure 4, which is presumably due to uptake of small anions as dopants to replace some of the type 2 sites. Most type 1 (and possibly type 2) sites remain, as evidenced by the continued cation binding ability of the films. In the case of histone H3, we propose that this polymer again interacts mainly with the type 2 and type 3 polyanions. The number and/or accessibility of these sulfonates in the oxidized form is apparently insufficient for complex formation with H3. However, upon reduction, the number and/or accessibility increases and some PSS chains are removed from the film, owing to the high mutual affinity of the polycation and polyanion. The film is still electroactive and able to exchange cations upon electrochemical switching.

In conclusion, we find that Pl binds irreversibly to either oxidized or reduced PP/PSS composite films. The Pl-derivatized films remain electroactive and continue to be able to transport cations. In contrast, histone H3 does not bind to PP/PSS or PNMP/PSS upon reduction. Rather, PSS is extracted from the film, most likely because of the strong affinity of the polycation H3 for the polyanion PSS. The loss of PSS is only partial as is demonstrated by the continued small ion binding and release properties of the composites. Our results suggest that binding of selected polyelectrolyte may be a useful approach to modify the surfaces of these electroactive composite films. The strong interaction between H3 and the reduced films, leading to extrusion of some PSS, prompts us to consider exploring the use of these electroactive films to modulate chromatin structure.

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References and Notes

(1) Diaz, A. F.; Gardini, G. P.; Gill, W. F.; Grant, D. M.; Kanazawa, K. K.; Kwak, J. F.; Street, G. B. *Synth. Met.* **1980**, *1*, 329.
(2) Miller, L. L. In *Functional Polymers*; Bergbreiter, D. E., Martin, C. R., Eds.; Plenum: New York, 1989; pp 61–68 and references therein.
(3) Miller, L. L.; Zhou, Q. X. *Macromolecules* **1987**, *20*, 1594.
(4) Zhou, Q.-X.; Miller, L. L.; Valentine, J. R. *J. Electroanal. Chem.* **1989**, *261*, 147.
(5) Sundaresan, N. S.; Basak, S.; Pomerantz, M.; Reynolds, J. R. *J. Chem. Soc., Chem. Commun.* **1987**, 621.
(6) Reynolds, J. R.; Sundaresan, N. S.; Pomerantz, M.; Basak, S.; Baker, C. K. *J. Electroanal. Chem.* **1988**, *250*, 355.
(7) Basak, S.; Rajeshwar, K.; Kaneko, M. *Anal. Chem.* **1990**, *62*, 1407.
(8) Boyle, A.; Genies, E.; Fouletier, M. *J. Electroanal. Chem.* **1990**, *279*, 179.

(9) Berlowitz, L. *Genetics* **1974**, *78*, 311. Miller, G. I.; Berlowitz, L.; Regelson, W. *Exp. Cell Res.* **1972**, *71*, 409.

(10) The molecular weight of H3 is about 15.3 kD, and its primary structure is known. Also, it is susceptible to aggregation in aqueous solutions. See: van Holde, K. E. *Chromatin*; Springer-Verlag: New York, 1989; Chapter 4.

(11) Lee, J. J.; Wnek, G. E. *ACS Div. Polym. Chem. Prepr.* **1989**, *30* (2), 175.

(12) Wnek, G. E.; Prezyna, L. A.; Lee, J. J.; Qiu, Y.-J.; Reynolds, J. R. *ACS Div. Polym. Chem. Prepr.* **1989**, *30* (2), 178.

(13) Baker, C. K.; Reynolds, J. R. *J. Electroanal. Chem.* **1988**, *251*, 307.

(14) Deakin, M. R.; Buttry, D. A. *Anal. Chem.* **1989**, *61*, 1147A.

(15) Baker, C. K.; Qiu, Y. J.; Reynolds, J. R. *J. Phys. Chem.*, in press.

(16) Tsai, E. W.; Pajkossy, T.; Rajeshwar, K.; Reynolds, J. R. *J. Phys. Chem.* **1988**, *92*, 3560.

(17) It should be noted that the mass gains are almost completely suppressed when the experiments with either reduced or oxidized PP/PSS films are carried out using a Tris-HCl (0.01 M, pH $\approx$ 5.2) solution of the Pl. We believe that the Tris electrolyte helps to shield charges on the PP/PSS and Pl, reducing their ability to bind electrostatically to the PSS. However, addition of Tris-HCl after Pl binding does not appear to lead to any desorption of the Pl.

(18) The frequency change varies from 300 to 800 Hz, depending on the batch of commercial PSS employed.

(19) The mass loss is only modestly suppressed in experiments with the reduced form of PP/PSS in Tris buffer (pH $\approx$ 7.2) containing H3. For example, an experiment at pH $\approx$ 5 showed a frequency increase of about 320 Hz, while a parallel experiment in pH $\approx$ 7 Tris buffer showed a frequency increase of about 280 Hz. In both cases clouding of the solution was observed, consistent with the proposal that some PSS leaves the films and complexes with H3.

(20) That the composite film surfaces are rich in sulfonates has been recently demonstrated by using XPS: Reynolds, J. R.; Gieselman, M. B.; Qiu, Y.-J.; Pyo, M. H. *Polym. Mat. Sci. Eng. Prepr.* **1991**, *64*, 202.

Registry No. Pl (homopolymer), 26124-78-7; Pl (SRU), 28575-12-4; PSS, 50851-57-5; PP, 30604-81-0; PNMP, 72945-66-5.