Investigation of the Relationship Between Conductivity and Protein-Binding Properties of Polypyrrole

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SYNOPSIS

Polypyrrole was synthesized using two different methods of chemical synthesis to obtain a product with different electrical conductivity values. The two products were found to possess about the same ability to bind protein. After prolonged exposure to aqueous solutions of different ionic composition and at different pH values, the polymer was tested for electrical conductivity and protein-binding ability. Electrical conductivity was found to vary with the conditions under which the polymer was stored, and of these, the pH of the medium was found to be the most important. On the other hand, the ability to bind protein did not show any such variation and only chemical reduction of the polymer produced a significant reduction in the level of protein binding.

INTRODUCTION

Conducting polymers have recently attracted much attention.[1] The realization that they possess the ability to bind oppositely charged molecules in their oxidized conducting state and to release them in their neutral insulating state has led to their being investigated as ion-exchange resins.[2] Polypyrrole (Ppy) has been the most widely studied of the conducting polymers because of its relative stability to environmental factors such as oxygen, temperature, and humidity. In its oxidized conducting form, Ppy carries a positive charge and would therefore be expected to bind negatively charged molecules. The molecules would be released when the polymer is reduced to its neutral nonconducting state.

The conducting properties of Ppy have been ascribed to the resonance character of the polymer that consists of a linear chain in which pyrrole molecules are linked via α-2,5 bonds. The conductivity of Ppy is of the order of 10^2 $(\Omega\ \text{cm})^{-1}$. This compares with conductivity values of 10^4–10^6 $(\Omega\ \text{cm})^{-1}$ for metals and about 10^{-14} $(\Omega\ \text{cm})^{-1}$ for insulators.[3]

The ion-exchange behavior of Ppy toward small ions has been examined by several authors.[4,5] We recently reported the protein-binding properties of the polymer.[6] We showed that Ppy binds protein in a manner similar to ion-exchange resins and that active enzyme can be desorbed from the polymer. In this paper, we examine the relationship among the electrical conductivity of Ppy, its degree of oxidation, and its ability to bind protein for freshly prepared Ppy and after chemical reduction or prolonged exposure of Ppy to aqueous solution under a variety of conditions.

EXPERIMENTAL

Reagents

Diethylether, naphthalene, ferric chloride (anhydrous), pyrrole, sodium, and tetrahydrofuran were obtained from Aldrich. Acetate, bis(2-hydroxyethyl)imino-tris (hydroxymethyl)-methane (bis-Tris), histidine, *N*-morpholinoethane sulfonic acid (Mes), pyridine, and tris(hydroxymethyl)aminomethane (Tris) were from Sigma. All the chemicals were of analytical grade. Alkaline phosphatase (type I-S, from bovine intestinal mucosa) was also from Sigma. Pyrrole was distilled under nitrogen and

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stored at −20°C. All the other reagents were used without further purification. Buffers of the required pH were prepared by adjusting solutions of the buffers to the desired pH with HCl.

Chemical Synthesis

Chemical synthesis of Ppy in aqueous solution was carried out by the method of Armes with few modifications.[7] Anhydrous ferric chloride (36 g) was dissolved in 400 mL double-distilled water and cooled to 0°C in an ice bath. Distilled pyrrole (4 mL), precooled to 0°C, was added to the solution of ferric chloride with vigorous stirring and maintained with stirring for at least 2 h. The insoluble polymer that formed was filtered on a Whatman No. 6 filter membrane and washed extensively with water until a clear solution was obtained. It was further rinsed with ethanol and finally with diethyl ether before overnight drying at 35°C.

Synthesis of Ppy in organic solvent was carried out by the method of Myers.[8] Diethyl ether (300 mL) was added to 19.6 g of anhydrous ferric chloride contained in a 500 mL beaker. Cold distilled pyrrole (2.1 mL) was added to the stirred ferric chloride/ether solution precooled to 0°C and stirred at this temperature for at least 1 h. The insoluble product was recovered, washed, and dried as described above.

Conductivity Measurement

The conductivity of Ppy was determined on the dried polymer using the four-in-line-probe method, as described previously.[9] The polymer was ground into a fine powder and pressed into pellets of 12 mm in diameter using a model C-30 press (Research and Industrial Instruments Co., London, England) using an operating pressure of 9 tons. Conductivity values were determined on the pellets using a homemade probe constructed from four stainless steel pins and a Teflon encasement.[10] The test current (1–50 milliamps) was provided by a potentiostat/galvanostat, Model 173 (EG&G Princeton Applied Research, Princeton, NJ) and the corresponding voltage recorded on a Keithley 177 voltmeter (Keithley Instruments Inc., Cleveland, OH). A minimum of five current–voltage values were recorded for each sample of Ppy and the values computed to determine the electrical conductivity.

Procedure for Protein Adsorption

A sample of Ppy powder was first soaked in distilled water for 15 h at 4°C, after which it was washed with 0.5M buffer at the required pH. It was then equilibrated to the desired buffer concentration by several washes with dilute buffer of the same composition and pH. Protein adsorption experiments were routinely carried out in 50 mL of 20 mM bis-Tris buffer at pH 6 using alkaline phosphatase at a concentration of 0.5 mg/mL. Under these conditions, alkaline phosphatase, whose isoelectric point is 4.4, carries a net negative charge. The amount of Ppy used was 0.5 g (dry weight) except where otherwise stated. The preequilibrated polymer was filtered to damp dryness and added to the solution of alkaline phosphatase made up in the same buffer as the one used to equilibrate the Ppy sample. The stirred mixture was maintained at 0°C to avoid denaturation of the protein until equilibrium was obtained. This was normally achieved after 1 h. The alkaline phosphatase/polypyrrole mixture was then filtered on a 0.45 μm-pore-size Durapore membrane (Millipore), and the amount of protein remaining in solution was determined by the absorbance at 280 nm.

Measurement of Polypyrrole Stability

The stability of Ppy to various factors was determined by soaking a sample of the polymer powder in aqueous solution of the desired pH and ionic composition for 5 days at 4°C. The polymer was then adjusted to give a buffer concentration of 20 mM at pH 6 by washing with 0.5 M buffer and then with several volumes of 20 mM buffer at pH 6. A fraction of the polymer was used to determine conductivity after drying. The remaining fraction, equivalent to 0.5 g dry weight of polymer, was filtered to damp dryness and tested for protein binding by adding to 50 mL of the same buffer containing 25 mg alkaline phosphatase. The protein/Ppy mixture was allowed to equilibrate for 1 h at 0°C, and the amount of protein remaining in solution was determined by the absorbance at 280 nm.

Chemical Reduction of Polypyrrole

Chemical reduction of Ppy was carried out in 1 M solution of sodium naphthalene placed in a two-necked flask fitted with a nitrogen source and a magnetic stirrer. Sodium naphthalene was prepared as described by Closson et al.[11] Naphthalene (25.6 g) was dissolved in 200 mL of dry tetrahydrofuran previously purged with nitrogen. Sodium (4.6 g) was cleaned in petroleum ether (boiling point 100–120°C), weighed in a fresh batch of the same solvent, and cut into small pieces before adding to the so-

lution of naphthalene. The mixture was maintained at room temperature under a nitrogen atmosphere and stirred until all the sodium was dissolved. It was then filtered through a coarse sintered glass disc. Ppy was added to the resultant solution of sodium naphthalene and the mixture stirred for several hours at room temperature. The mixture was filtered and the polymer washed with tetrahydrofuran, after which excess solvent was removed by a stream of nitrogen. It was then washed and equilibrated to a concentration of 20 mM bis-Tris buffer, pH 6, and its conducting and protein-binding properties evaluated as described earlier. The control sample was exposed to tetrahydrofuran alone and treated in the same manner as the reduced polymer.

RESULTS AND DISCUSSION

Effect of Chemical Oxidation and Reduction

The protein-binding ability of Ppy prepared by two different methods of chemical synthesis is presented in Figure 1. The polymerization was carried out in either diethyl ether or water as described in the Experimental section and produced Ppy with conductivity values of 70 and 15 $(\Omega\ \text{cm})^{-1}$, respectively. Without undergoing further treatment, the two Ppy samples were evaluated for their protein-binding abilities. We observed that although the binding was slightly higher in the case of the polymer prepared in ether the increase was not commensurate with the nearly fivefold difference in their levels of conductivity. This result agrees with an earlier report that suggested that the degree of oxidation of Ppy is an intrinsic property of the polymer and that there

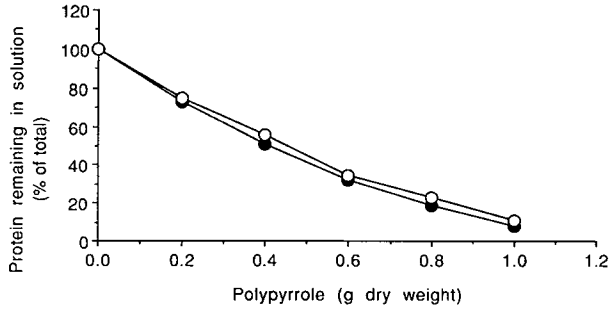

Figure 1 Measurement of alkaline phosphatase remaining in solution after adding successive batches of polypyrrole prepared chemically in either water (○) or diethyl ether (●). The polypyrrole sample was preequilibrated in bis-Tris buffer (20 mM, pH 6), and adsorption was carried out in 50 ml of the same buffer containing 0.5 mg protein/mL.

Table I Effect of Chemical Reduction on the Conductivity and the Protein-Binding Property of Polypyrrole

	Conductivity $(\Omega\ \text{cm})^{-1}$	% Protein Remaining in Solution
Nonreduced Ppy	3	45 ± 3 (4)[a]
Reduced Ppy	$5 \cdot 10^{-4}$	61 ± 5 (6)

[a] Mean ± standard deviation (number of experiments with separate samples).

is little variation in oxidation with either variation of the conditions used in synthesis or composition of the polymer.[12] On the other hand, conductivity has been reported to depend on factors such as polymer chain length or length of the regions of conjugation within the polymer, which are themselves determined by the conditions used in either synthesis of the polymer or prevail during prolonged storage of it.[13,14] It emerges from these results that the oxidation state of Ppy is likely to be the primary factor that determines its protein-binding ability.

We have also examined the protein-binding property of Ppy after reducing it using sodium naphthalene, which is a very strong reducing agent.[15] Such a reduction should lower the level of oxidation of the polymer as well as its level of conductivity. Although only a partial reduction of the polymer could be obtained, possibly because of the susceptibility of reduced Ppy to reoxidation by oxygen,[16] when the partially reduced polymer was evaluated for its ability to bind proteins, we observed a significant decrease in the level of protein binding to the partially reduced polymer in comparison with the nonreduced polymer (Table I). This observation provides support for the view that it is the oxidation state of Ppy that is the primary factor which determines its protein-binding ability.

Buffer, Ionic Strength, and pH Effects

We have examined the stability of Ppy in aqueous solution to a number of factors and the effects of these on the electric conducting and protein binding properties of the polymer. The results presented in Table II show that prolonged suspension in water has a destabilizing effect on the conductivity of Ppy. However, when the conductivity values of the polymer that had been exposed to various buffers were compared with that of the polymer exposed to water alone, it was observed that the conductivity of Ppy was relatively stable to the buffers examined except

Table II Effect of Prolonged Exposure of Polypyrrole to Water and Buffer Ions (0.5M, pH 6) on Its Conductivity and Protein-Binding Ability

Type of Buffer	Conductivity (Tested After 5 Days) $(\Omega\ cm)^{-1}$	% Protein Remaining in Solution (Tested After 5 Days)
Initial values	(9.0)	(34)
Water	2.3	35
Acetate	0.2	29
Bis-Tris	0.9	34
Histidine	0.5	35
Mes	0.1	—[a]
Pyridine	1.0	34
Tris	1.1	35

[a] Not tested; the buffer caused precipitation of the protein.

toward acetate, Mes, and to some extent histidine. Interestingly, these buffers produce negatively charged species in solution. Previous studies have established the susceptibility of Ppy to similarly charged ions[17,18] and to water,[19] due to nucleophilic attack on the polymer chain. The limited extent of the loss of conductivity suggests that the mechanism by which it occurs is a slow process. The other buffers examined in our study, bis-Tris, pyridine, and Tris, exist in solution as cationic species that do not, as a result, interact directly with the positively charged polymer. Any effect on conductivity in the presence of these buffers can therefore be assumed to be due to the conjugate base of the acid used to adjust the pH of the buffer. However, this was shown not to be important by exposing Ppy to increasing concentrations of bis-Tris buffer (pH 6) up to a concentration of 1 M. This produced no effect on either the electrical conductivity or the subsequent protein-binding ability of the polymer. The relatively high conductivity value obtained when the polymer was exposed to water in comparison with the other solutions (Table II) could be due to the difference in pH of the solution. In effect, Ppy is a highly acidic polymer, and the pH for the nonbuffered medium was about 3 compared with pH 6 for the buffered solutions. As shown in Table II, the protein-binding property of Ppy is unaffected by prior exposure to water or the buffer ions described above.

Figure 2 shows the effect of exposing Ppy, for 5 days, to 0.5 M bis-Tris buffer prepared at varying pH values between 3.0 and 9.5, on its electrical conductivity and ability to bind proteins. This was cross-checked by also carrying out the experiment in 0.5 M pyridine buffer for pH values lower than pH 6 and in 0.5 M Tris buffer for values higher than pH 7. The results show that a dramatic loss of conductivity occurs at alkaline pH values. However, the polymer was relatively stable to exposure to acidic pH values. The pH-induced effect on the conductivity of Ppy was far greater than was the effect caused by any of the other factors examined in this study. These results are in accord with data published elsewhere, although previous experiments have been conducted in nonbuffered medium and often involving the use of strong alkali.[20,21] The loss of conductivity observed at alkaline pH has been attributed by some authors to hydrolysis of the polymer leading to a break in either the chain length or the length of the regions of conjugation within the polymer chain.[21] In strong contrast to the effect on conductivity, the protein-binding ability of Ppy was little affected by prior exposure to alkaline pH. The level of protein binding was maintained at nearly the same level even after a drop in conductivity of four orders of magnitude (Fig. 2).

From these results and those presented in Table II, there is no direct relationship between the level of conductivity of Ppy and its ability to bind proteins. This implies that a significant change in conductivity can occur without a corresponding effect on the oxidation state of the polymer, on which the protein-binding ability depends. These results can be explained in terms of anion or pH-induced hydrolysis of Ppy producing a shorter chain but without affecting the overall oxidation state of the polymer.

CONCLUSION

The results presented in this report show that although the level of conductivity of Ppy is known to

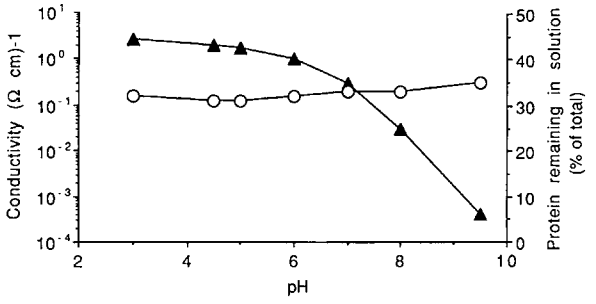

Figure 2 Effect of prolonged exposure of polypyrrole to 0.5 M bis-Tris buffer at various pH values on its conductivity (▲) and protein-binding ability (○).

be determined by intrinsic factors such as degree of oxidation of the polymer, length of polymer chain, and length of the regions of conjugation within the polymer, its protein-binding property appears to depend on the degree of oxidation alone. Moreover, conductivity is also greatly dependent on extrinsic factors such as the composition of the medium during synthesis of Ppy or during storage of the prepared polymer, whereas the protein-binding ability shows only small variations with similar factors. On the other hand, chemical reduction of the polymer leading to a partial lowering of its level of oxidation resulted in a significant decrease in the amount of protein that could be adsorbed by the polymer. Overall, these results suggest that the oxidation state of Ppy, not its conductivity *per se,* is the primary factor that governs its protein-binding property.

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REFERENCES

1. R. B. Kaner and A. G. Macdiarmid, *Sci. Am.,* **258,** 60 (1988).
2. G. Hailin and G. G. Wallace, *Anal. Chem.,* **61,** 198 (1989).
3. F. Garnier, *Recherche,* **18,** 1306 (1987).
4. L. S. Curtin, G. C. Komplin, and W. J. Pietro, *J. Phys. Chem.,* **92,** 12 (1988).
5. B. Zinger and L. L. Miller, *J. Am. Chem. Soc.,* **106,** 6861 (1984).
6. A. B. Smith and C. J. Knowles, *Biotechnol. Appl. Biochem.,* to appear.
7. S. P. Armes, *Synth. Met.,* **20,** 365 (1987).
8. R. E. Myers, *J. Electron. Mater.,* **15,** 61 (1986).
9. L. J. Van der Pauw, *Philips Res. Rep.,* **12,** 364 (1957).
10. L. D. Couves, PhD Thesis, University of Kent, Canterbury, England, 1988.
11. W. D. Closson, P. Wriede, and S. Bank, *J. Am. Chem. Soc.,* **88,** 1581 (1966).
12. M. Salmon, A. F. Diaz, A. J. Logan, M. Krounbi, and J. Bargon, *Mol. Cryst. Liq. Cryst.,* **83,** 1297 (1982).
13. K. Hyodo and A. G. Macdiarmid, *Synth. Met.,* **11,** 167 (1985).
14. K. Yakushi, L. J. Lanchlan, T. C. Clarke, and G. B. Street, *J. Chem. Phys.,* **79,** 4774 (1983).
15. A. Pron, Z. Kucharski, C. Burowski, M. Zagorska, S. Krichene, J. Suwalski, G. Dehe, and S. Lefrant, *J. Chem. Phys.,* **83,** 5923 (1985).
16. J. Bargon, S. Mohmand, and R. J. Waltman, *IBM J. Res. Dev.,* **27,** 330 (1983).
17. S. Asvapiriyanont, G. K. Chandler, G. A. Guanawardena, and D. Pletcher, *J. Electroanal. Chem.,* **177,** 229 (1984).
18. E. W. Tsai, T. Pajkossy, K. Rajeshwar, and J. R. Reynolds, *J. Phys. Chem.,* **92,** 3560 (1988).
19. T. F. Otero, R. Tejada, and A. S. Eloka, *Polymer,* **28,** 651 (1987).
20. H. Muenstedt, *Polymer,* **27,** 899 (1986).
21. O. Inganaes, R. Erlandsson, C. Nylander, and I. Lundstrom, *J. Phys. Chem. Solids,* **45,** 427 (1984).

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