Evidence for the Addition of Nucleophiles to the Surface of Polypyrrole Latex

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ABSTRACT: Aqueous polypyrrole latex reacted at room temperature with good nucleophiles such as thiols and amines, while alcohols showed no reaction. The reactions were followed by visible, XPS, and ESR spectroscopy, radiolabeling, and conductometric titration. This finding provides for a facile method for the modification of polypyrrole surface chemistry. A mechanism of chemical addition into the conjugated polymer chain is proposed which is consistent with these results.

Introduction

Polypyrrole has unique properties in the fields of biosensors[1,2] and immunodiagnostics[3] due to its inherent conductivity and intrinsic black color. In colloidal form the intensely black particles can serve as visual labels for the detection of immune complex formation. In this application, the particles can be surface-modified for subsequent ligand attachment or the proteinaceous ligand can be "adsorbed" to the particle surface in an appropriate buffer, without the need for an active coupling step. If the coated, unmodified surface is subjected to overnight incubation with sodium dodecyl sulfate solution, a displacement of approximately 15% of the immobilized antibody from the polypyrrole surface has been observed.[3] In contrast, subjecting antibodies bound to polystyrene by adsorption under similar conditions resulted in approximately 70% displacement.[4]

To explain these results on polypyrrole, a chemical interaction or coupling with the surface was suspected.[5] Others later observed irreversible decreases in conductivity when polypyrrole was exposed to ammonia and water vapor for extended periods of time.[6] Mechanisms were proposed involving nucleophilic attack on the electrophilic polypyrrole chain. Visible spectrophotometric and resistivity data were shown as a function of exposure to ammonia, which indicated that a chemical interaction was occurring.[7]

This work is a study of the chemical interaction of small nucleophilic species with polypyrrole latex and the resulting products. The results have implications for the interaction of proteins with polypyrrole surfaces.

Experimental Section

Materials. Polypyrrole latex was synthesized as aqueous latex using $FeCl_3$ and a poly(vinyl alcohol) stabilizer as previously described.[3] It was purified by continuous diafiltration using a 0.1 μm cutoff hollow fiber cartridge manufactured by Microgon, until the conductivity of the effluent reached a constant value of approximately 400 times that of distilled water. This resulted in 10−15 volume exchanges. Ion exchange was done using a mixed-bed prepared from equal amounts with respect to exchange capacity of Dowex 1 × 8 anion (1.5 mequiv/mL) and Dowex 50WX8 cation (2.2 mequiv/mL). Each individual resin was washed by the method of Vanderhoff et al.[8] Ethylene glycol-*1,2*-^{14}C, ethanolamine-*1,2*-^{14}C, and 2-mercaptoethanol-*1,2*-^{14}C were obtained from Sigma Chemical Co. They had specific radioactivities of 35.7, 17.2, and 12.8 mCi/mmol, respectively. Disposable PD-10 columns were obtained from Pharmacia.

Methods. *Reaction of Polypyrrole Latex with Radiolabeled Species.* Aliquots of polypyrrole latex (95 mL, 1.4% solids, which contained 19 mequiv of pyrrole, repeat units) were diluted to 1 mL in a buffer. The buffer contained 50 mM morpholinopropanesulfonic acid sodium salt (MOPS), pH 7.0, and DMF containing 0.2% Brij-35, 2.3:1 v/v. The 1 mL aliquots were mixed with 9 mequiv of labeled compound (3−9 mL) and incubated overnight in tubes rotated slowly end-over-end at room temperature. The samples were then passed through equilibrated PD-10 columns. The first two-thirds of the polypyrrole fraction was collected and tested for solids content and radioactivity.

Reaction of Polypyrrole Latex with Mercaptoacetic Acid. A 500 mL round-bottom flask was equipped with a magnetic stirrer, and 300 mL of purified polypyrrole latex (2.10% solids, 0.094 equiv with respect to pyrrole repeat units) was added. A molar equivalent of mercaptoacetic acid (7.1 mL) was added dropwise, and the reaction was allowed to stir overnight at ambient temperature. No aggregation of the particles was evident. The latex was then purified by diafiltration, followed by ion exchange.

Analysis. (a) *%* Solids: When only small volumes of latex were available, i.e., radiolabeling experiments, determinations were made spectrophotometrically by measuring the apparent absorbance at 800 nm and comparing the values to a standard curve. This curve was prepared using dilutions of a polypyrrole suspension of known solids content, previously determined gravimetrically. (b) Titration of acid groups: After reaction and purification by diafiltration and ion exchange, the latex was titrated conductometrically using standard barium hydroxide as previously described.[1] (c) X-ray photoelectron spectroscopy (XPS): The latexes were dried as thick films on 1 cm^2 silicon wafers and analyzed with a Physical Electronics XPS/SIMS system. All XPS data were obtained using a monochromatic Al (Al Kα = 1486.6 eV) X-ray source. (d) Electron spin resonance (ESR) spectroscopy: A Bruker ESP 300E spectrometer was used for the ESR measurements. Purified unreacted polypyrrole latex was solvent exchanged into *N*-methylpyrrolidone (NMP) and rendered anhydrous as previously described.[3] A polypyrrole solids content of 0.37% was used in all experiments. Dilutions of mercaptoacetic acid and ethanolamine were made in NMP, and appropriate aliquots were added to the latex prior to the ESR measurement. (e) Scintillation counting: A Beckman LS 5000 CE scintillation counter was used for the ^{14}C radioactivity determinations. Typically 0.1 mL

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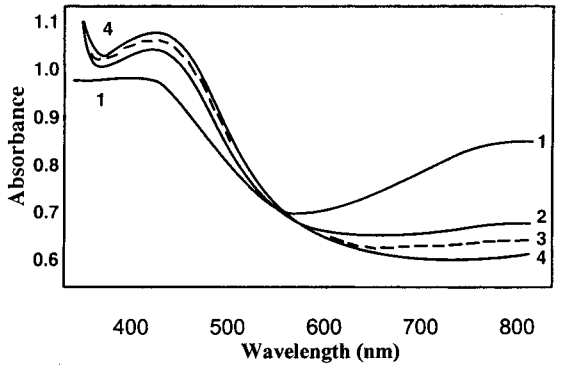

Figure 1. Absorption spectra of polypyrrole latex (0.007% solids) in 1-methyl-2-pyrrolidone: (1) control, (2) 1 h, (3) 7 h, and (4) 24 h after addition of 2-mercaptoethanol. The thiol was a 33 mol equiv excess relative to pyrrole repeat units.

Table 1. Reaction of ^{14}C-Labeled 2-Mercaptoethanol, Ethanolamine, and Ethylene Glycol with 19 μequiv of Polypyrrole Latex

nucleophile	equiv fraction added	μequiv incorporated
2-mercaptoethanol	0.46	3.0
ethanolamine	0.46	1.2
ethylene glycol	0.46	0.0

samples were mixed with 10 mL of scintillation cocktail in 20 mL scintillation vials. (f) Visible spectra: Visible spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer.

Results

Table 1 shows the results of the interaction of three radiolabeled species with polypyrrole latex. When 2-mercaptoethanol is reacted with the polypyrrole latex at a molar equivalent ratio of 0.46 with respect to pyrrole repeat units, 34% of the input species becomes immobilized. Under the same conditions, ethanolamine, a weaker nucleophile, showed 14% incorporation. Reaction of ethylene glycol, a very weak nucleophile, was undetectable.

XPS analysis of polypyrrole before and after reaction with mercaptoacetic acid indicated 3 at. % sulfur on the surface after diafiltration. This amount remained constant even after ion exchange of the latex. Ion exchange would be expected to remove any noncovalently bound ionic species from the particle's surface. The XPS result relative to sulfur implies that approximately 15% of the surface contained immobilized weak acid. The significant oxygen content is most likely due to adsorbed poly(vinyl alcohol), used as a colloidal stabilizer.

Titration of latex aliquots reacted with mercaptoacetic acid (MAA), after diafiltration and ion exchange, yielded an average (n = 2) weak acid titer of 660 mequiv/g of polymer. If this value is used to calculate a surface density of weak acid groups using the measured average particle diameter of 180 nm, a value of 1 group/5 Å^2 is found. Clearly weak acid groups are titrated several monolayers beneath the particle surface.

The visible spectra of the latex before and after exposure to nucleophiles provide further insight into the chemical interaction. Figure 1 illustrates the changes in visible absorbance of the latex with time after the addition of mercaptoethanol. The absorbance in the violet region increases, while that in the red decreases over 24 h with a tight isosbestic point at 545 nm. Similar, but less pronounced, effects are seen with

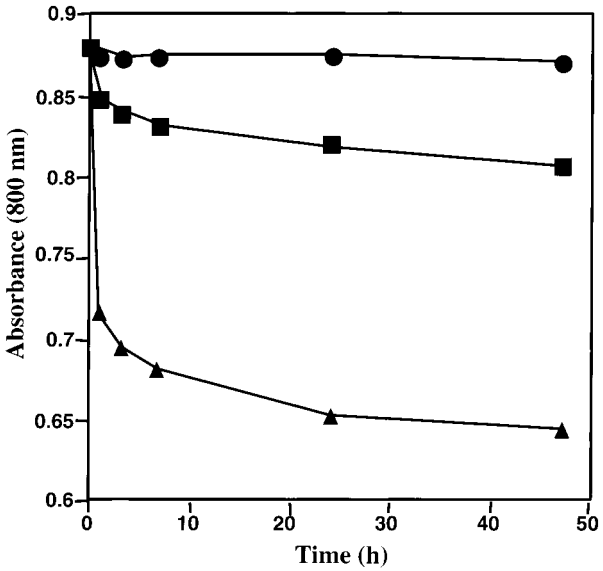

Figure 2. Absorbance of polypyrrole latex in 1-methyl-2-pyrrolidone (0.007% solids) at 800 nm after reaction with ethylene glycol (●), ethanolamine (■), and 2-mercaptoethanol (▲). Reactants were added to the latex in molar equivalent excess of 50-, 50-, and 33-fold, respectively.

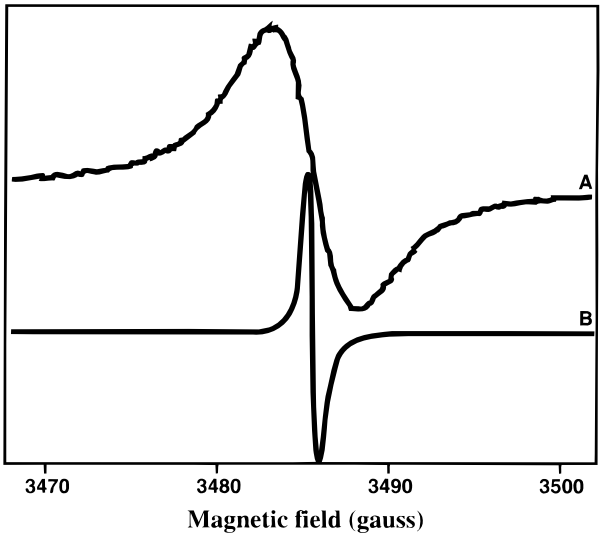

Figure 3. ESR spectra of polypyrrole latex at 0.37% solids in 1-methyl-2-pyrrolidone: (A) control and (B) 5.25 h after addition of 0.1 equv of MAA.

amines; ethylene glycol has no effect on the spectrum. Figure 2 compares the spectral change at 800 nm absorbance with time for a model mercaptan, amine, and alcohol. The same spectral trends are observed in both water and *N*-methylpyrrolidone (NMP), but the spectral changes occur much more rapidly in water.

Spectral changes are also evident in the ESR spectra of polypyrrole latex in NMP when exposed to both mercaptans and amines. Pure polypyrrole latex shows a broad line at 3485 G, which narrows significantly after exposure to mercaptoacetic acid (Figure 3). Figure 4 plots a time course of the line width change for three equivalent ratios of polypyrrole to mercaptan. During the course of the measurements the integrated intensity of the signal remains constant (±10%). A similar time course is seen with amines, and the rate of line width narrowing is approximately the same. This is in contrast to the significant difference between mercaptoethanol

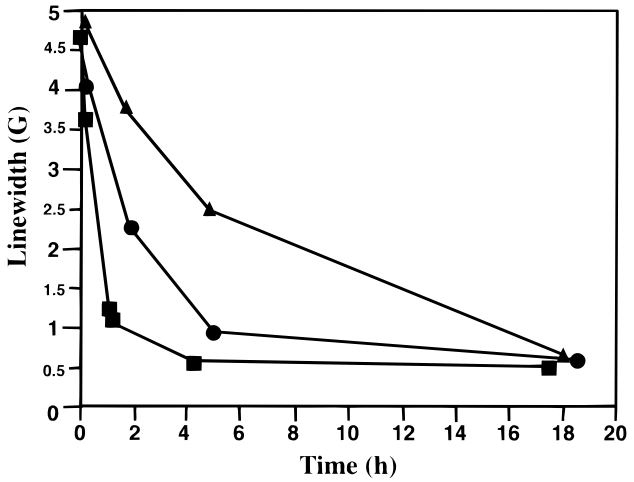

Figure 4. ESR line width (G) of polypyrrole latex in 1-methyl-2-pyrrolidone (0.37% solids) as a function of time after addition of mercaptoacetic acid (MAA) at the equivalent ratios of polypyrrole/mercaptoacetic acid of 1:1 (■), 10:1 (●), and 100:1 (▲).

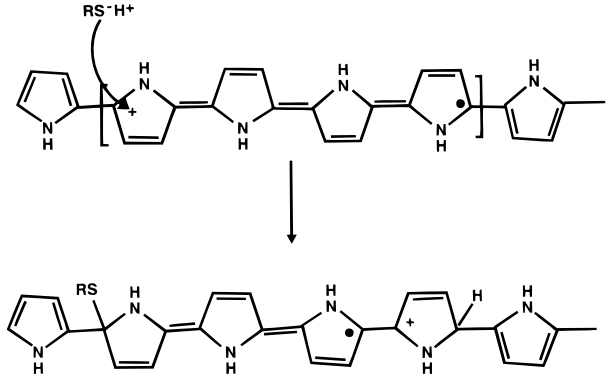

Figure 5. Nucleophilic addition of thiol to the polaron unit of polypyrrole.

and ethanolamine in their effect on the temporal changes of the visible spectrum, as seen in Figure 2.

Discussion

The results of this study are consistent with a covalent functionalization of the polypyrrole by thiols and amines. Although we did not carry out a detailed mechanistic study, the mechanism may be rationalized in terms of nucleophilic addition of thiolate to electrophilic sites of polypyrrole as represented in Figure 5. Radiolabeling experiments summarized in Table 1 show that the incorporation of ^{14}C label in polypyrrole was 40% higher for 2-mercaptoethanol than for ethanolamine, with complete absence of radiolabel incorporation for ethylene glycol. This follows the order of reactivity of these three species toward polypyrrole, as clearly demonstrated in Figure 2.

We have also demonstrated preparatively the incorporation of mercaptoacetic acid into polypyrrole. XPS confirmed the incorporation of sulfur onto the surface from the reaction between mercaptoacetic acid and polypyrrole and absence of this element in the unreacted latex (Table 2).

Time-dependent changes of the electronic spectra of polypyrrole incubated with mercaptoacetic acid, shown in Figure 1, are consistent with a covalent functionalization of the polypyrrole by the thiol. Time-dependent disappearance of the broad band at 800 nm with the concomitant increase of the higher energy band at 400 nm and a tight isosbestic point at 542 nm are strongly indicative of a covalent interaction between the chromogenic polypyrrole and the nucleophile, without accumulation of intermediates. Such spectral changes are consistent with interruption of extended conjugation of polypyrrole by reaction with thiols and resultant rehybridization of carbon atoms from sp^2 to sp^3. As the number of conjugated centers decrease, the energy of the electronic transition at the longest wavelength increases.[9] The electronic changes can also be discussed employing band structure formalism.[10] Polypyrrole in the ground state at low doping has a quinoid structure with the polaron spread over four units, as shown in Figure 5. Upon reaction with the thiol or amine, the band at 800 nm (~1.4 eV), corresponding to transition between the polaron levels, decreases as the band gap p → p* transition at 400 nm (~3.2 eV) increases.[11]

Table 2. XPS Atomic Concentrations (%) for Polypyrrole Latex

sample	O	N	C	Cl	S
polypyrrole latex control	18	9	72	1	0
reacted with MAA and diafiltered	20	9	68	0.3	3
ion-exchanged	19	8	69	0.1	3

As shown by Figure 3, reaction between nucleophiles and polypyrrole is accompanied by a time-dependent narrowing of the ESR signal without changes in its integrated intensity. This is consistent with conservation of the total number of polarons in polypyrrole before and after the reaction. Although the total number of polarons may not change after reaction, the introduction of sp^3 carbons as the consequence of nucleophile addition may create radical-cation polarons separated in the product by less than four pyrroles, resulting in faster spin cation exchange. Synergistically, the product of the reaction should be intrinsically more solublilized, leading to narrowing of the ESR signal.

ESR line narrowing has been reported for lithium phthalocyanine[12,13] and for the conducting polymers polypyrrole[14] and polyaniline[15] as a function of decreasing oxygen concentration. The ESR experiments reported here were done in closed tubes with an air headspace. We cannot rule out that the oxygen concentration decreased during the ESR experiment by a catalytic process, contributing to the line narrowing. One of us had reported previously on a catalytic property of polypyrrole latex in the presence of oxygen for the oxidative cleavage of vicinal diols.[16] The catalytic properties of such latexes are under further investigation.

In summary, our data indicate a covalent interaction between polypyrrolic electrophilic centers and nucleophiles. Amines and thiols are ubiquitous functionalities displayed by many proteins. Since polypyrrole is important as a solid phase in biosensors and other biomedical applications, our work suggests that one-step covalent derivatization of polypyrrole by simple exposure of this material to protein solutions may be possible. Past observations have indicated immunoglobulins become irreversibly immobilized on the polypyrrole latex surface. The present work increases the understanding of such interactions.

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