The Role of Template in the Enzymatic Synthesis of Conducting Polyaniline

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Received July 22, 1999

Abstract: It was recently reported that water soluble conducting polyaniline may be prepared using a new template-guided enzymatic approach. To address the mechanistic role of the template in this reaction, various macromolecular and surfactant templates were investigated. It was found that the template provides a necessary type of "local" environment where the pH and charge density near the template molecule is different from that of the bulk solution. ^{13}C and ^{1}H NMR studies showed that this "local" environment serves as a type of nanoreactor that is critical in anchoring, aligning, and reacting the aniline monomers and ultimately controls what form of polyaniline (conducting or insulating) is obtained during reaction. Strong acid polyelectrolytes, such as sulfonated polystyrene (SPS), are the most favorable because they provide a lower, local pH environment that serves to both charge and preferentially align the aniline monomers through electrostatic and hydrophobic interactions to promote the desired head-to-tail coupling. Interestingly, it was found that micelles formed from aggregating, strong acid surfactant molecules such as sodium dodecylbenzenesulfonic acid (SDBS) also provide suitable local template environments that lead to the formation of conducting polyaniline. ^{1}H NMR spectral data showed that the aniline monomers in these micelle systems intercalate between the sulfonated styrene headgroups of the micelles. However, if the reaction media was such that micelles were not formed or if the distance between the sulfonated headgroups in the mixed micelle systems was too large, then the conducting form of polyaniline could not be obtained. The information gained from this study strongly supports the existence and importance of "local" template environments in guiding the enzymatic synthesis of polyaniline. A fundamental understanding of these types of mechanisms should lead to the design and optimization of a broad range of other interesting template-guided reactions.

Introduction

Among conducting polymers, polyaniline is remarkable for its excellent environmental stability[1] and ease with which its properties may be tuned by changes in the oxidation state [2] or in the degree of protonation.[3] Typically, polyaniline is synthesized either chemically[4] or electrochemically[5] in strong acid media. The use of enzymes as biological catalysts in the synthesis of polyanilines has also attracted great interest in recent years since enzymes can offer environmentally benign reaction conditions, a higher degree of control over the kinetics of the reaction, and a higher yield of product.[6]

Horseradish peroxidase (HRP) is able to catalyze the oxidation of a wide range of compounds including aromatic amines and phenols in the presence of hydrogen peroxide.[7] A major limitation of the enzymatic oxidation of anilines and phenols from aqueous solutions however has been that as soon as polymer begins to form, it precipitates out, and only very low molecular weight polymers (oligomers) are produced.[8] To obviate this and improve processability, a variety of modified enzymatic polymerization reactions have been investigated including solvent mixtures,[9] modified monomers in aqueous solutions,[10] micelles,[11] reverse micelles,[12] and reactions at the

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10.1021/ja9926156 CCC: $18.00 © 1999 American Chemical Society
Published on Web 11/19/1999

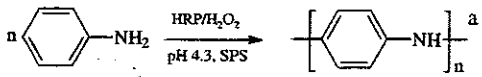

Figure 1. Schematic representation of template-directed enzymatic synthesis of conducting polyaniline. (a) The resulting polyaniline is in its doped state.

air−water interface.[13] Although these modifications have improved the molecular weight and processability, the ortho- and para-directed coupling of the enzymatic reaction typically results in a mixture of branched polymeric structures. In the case of polyanilines, the presence of these branched ortho- and para-substituted structures severely limits the degree of conjugation and hence the electrical and optical properties of the resulting polymer.

Recently a new enzymatic approach, shown schematically in Figure 1, was developed to synthesize water-soluble conducting polyaniline in the presence of sulfonated polystyrene (SPS) under mild, aqueous pH 4.3 buffered conditions.[14] This approach is based on preferential electrostatic alignment of aniline monomer onto an anionic template to minimize branching and promote a linear polyaniline chain growth. Since aniline has a pK_a of 4.63[15] it is primarily positively charged at pH 4.3. Conversely, the sulfonate groups on the SPS are negatively charged (SPS is a strong polyelectrolyte that will totally dissociate in almost the entire pH range).[16] Therefore, it is believed that the aniline monomer interacts with the SPS electrostatically and preferentially complexes with the template prior to and during the reaction. This approach inherently minimizes the parasitic branching and promotes a more para-directed, head-to-tail polymerization of aniline and produces a water-soluble conducting polyaniline and SPS complex.[14] The properties of this polyaniline/SPS complex are comparable to previously reported chemically synthesized polyaniline[1] and self-doped sulfonated polyaniline.[17,18]

It is known that the SPS in this approach serves three critical functions. One is to provide the necessary counterions for doping of the synthesized polyaniline to the conducting form. The second is to maintain water solubility of the final PANI/SPS complex for facile processing. These first two functions are well understood and are not discussed. The third function is to serve as a template that preferentially organizes the aniline monomers prior to polymerization and promotes the head to tail coupling. Although it is known that the resulting polymer complex is in fact, the linear benzenoid-quinoid form of polyaniline, the mechanistic role of how the template directs the chain growth of the conducting polyaniline has not been completely understood. To gain insights into this mechanism, a series of different macromolecular and surfactant templates were investigated. The detailed synthesis of polyaniline with each of these templates and characterization using ^{13}C, ^{1}H NMR and UV−vis−near-IR spectroscopy is presented.

Experimental Section

Materials. Horseradish peroxidase (HRP) (EC 1.11.1.7) (200 unit/mg) was purchased from Sigma Chemicals Co., St. Louis, MO, with RZ > 2.2. A stock solution of 10 mg/mL in pH 6.0, 0.1 M phosphate buffer was prepared. Aniline (99.5%) and each of the templates: poly(sodium 4-styrenesulfonate)(SPS), 70 kDa, sodium salt; poly(diallydimethylammonium chloride) (PDAC), medium molecular weight; poly(acrylic acid) (PAA), 6 kDa, sodium salt; poly (maleic acid co-olefin), (PMO), 12 kDa, sodium salt; poly(ethylene glycol) (PEG), 10 kDa; sodium dodecylbenzenesulfonic acid (99%, SDBS); polyoxyethylene(10) isooctylphenyl ether (99%, Triton X-100) and benzenesulfonic acid sodium salt (99%, SBS) were obtained form Aldrich Chemicals Co. Inc., Milwaukee, WI, and used as received. Ribonucleic acid (RNA, type VI, from torula yeast) was obtained from Sigma Chemicals Co., St. Louis, MO. Deuterium oxide (99%, D_2O) and $^{13}C_6$-aniline (99%) were purchased from Cambridge Isotope Laboratories, Inc., Cambridge, MA. All other chemicals and solvents used were commercially available, of analytical grade or better and used as received.

General Methods. The UV−vis−near-IR spectra were recorded on a Perkin-Elmer Lambda-9 UV/vis/near-IR spectrophotometer. In each measurement, the control was 0.1 M, pH 4.3 phosphate buffer. The ^{13}C and ^{1}H NMR spectra were recorded on a Bruker ARX 500-MHz NMR spectrometer. The instrumental operating parameters were as follows: temperature 300 K, pulse width 4.9 μs (30° pulse), 32 kW data points, 3.17 s acquisition time, 1 s relaxation delay, and 16 transients. The operating frequency on the ARX 500 instrument for performing ^{13}C NMR was 125 MHz. Typically 8 and 128 scans were used for collecting ^{1}H and ^{13}C NMR spectra, respectively.

Polymerization using Polyelectrolyte Templates. The enzymatic polymerization of aniline in the presence of each of the templates discussed here was carried out following the procedure described in more detail in our previous work.[14] Generally, the reactions were carried out at room temperature in a 30 mL, 0.1 M sodium phosphate buffer solution at pH 4.3 which contained a 1:1 molar ratio of aniline (6 mM) to template (6 mM, based on the monomer repeat unit). Template was added first to the buffered solution, followed by the addition of the aniline under constant stirring and the adjustment of the pH to 4.3 with 1 M HCl. To the solution, 0.2 mL of HRP stock solution (10 mg/ml) was then added. The reaction was initiated by the addition of a stoichiometric amount of H_2O_2 under vigorous stirring. To avoid the inhibition of HRP due to excess H_2O_2,[9a] diluted H_2O_2 (0.02 M) was added dropwise, incrementally, over 1.5 h. After the addition of H_2O_2, the reaction was left stirring for at least 1 h, and then the final solution was dialyzed (cutoff molecular weight of 2000) against pH 4.3 deionized water overnight to remove any unreacted monomer, oligomers and phosphate salts.

Polymerization using Micellar Templates. The aqueous micelle solutions were prepared by dissolving surfactants into 30 mL of a 0.1 M, pH 4.3 phosphate buffer to a concentration over the CMC (critical micellar concentration) with continuous stirring. Typically the concentration used was 10 mM, where the known CMC of SDBS is 1.6 mM. An equivalent molar amount of aniline was then added and stirred until dissolved. The pH of the solution was adjusted to 4.3 with 1 M HCl. To this solution, 0.2 mL of HRP stock solution (10 mg/mL) was added, and the reaction was then initiated by the addition of diluted H_2O_2 (0.02 M). After dropwise addition of a stoichiometric amount of H_2O_2 under vigorous stirring for 1.5 h, the reaction was left to stir for at least one more hour.

Similar procedures were used for the enzymatic polymerization of aniline in non-micelle-forming SDBS solutions. This included reaction media with the SDBS concentration under the CMC in pure aqueous

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buffer and over the CMC but in a 50% acetone/50% aqueous buffered mixture. The enzymatic polymerization of aniline was also carried out in mixed micelle systems using Triton X-100. The mixed micelle solutions were prepared by ultrasonically mixing SDBS and Triton X-100 in a 0.1 M, pH 4.3 phosphate buffered solution. The molar ratios of SDBS to Triton X-100 were varied from 1:1 to 1:5 respectively and enzymatic polymerization was carried out using the same procedure. In each case the SDBS and aniline were kept at the same concentration (10 mM), and only the concentration of Triton X-100 was varied in each system.

^{13}C NMR pH Studies. ^{13}C-labeled aniline was used to obtain data with a fewer number of fids (ns = 128) and to minimize interference in the spectra from SPS. For these studies, solutions of 8 mg of ^{13}C-labeled aniline in 4 mL of pure D_2O and 8 mg of ^{13}C-labeled aniline with an equivalent molar amount of SPS in 4 mL of pure D_2O were titrated to the desired pH with 1.0 M HCl and 1.0 M NaOH. The pH was monitored using an Orion 520A pH meter. ^{13}C NMR spectra of the labeled aniline in these solutions were measured at pHs ranging from 7 to 1.

^{1}H NMR Studies of Aniline with Polyelectrolyte Template Systems. For these studies, 2.0 mg of aniline was dissolved in 1.0 mL of D_2O. The pH of the solution was adjusted to 4.3 using 1.0 M HCl. To the NMR tube, 0.6 mL of the solution was added, and a ^{1}H NMR spectrum of the aniline in this solution was measured. An SPS stock solution was then prepared by dissolving 44.1 mg of SPS in 1.0 mL of D_2O at pH 4.3. Incremental aliquots (10 μL) of the SPS stock solution were then added to the aniline solution in the NMR tube and ^{1}H NMR spectra were measured after each addition. NaCl (15 mg) was subsequently added to the solution when the molar ratio of aniline to SPS was 1:1 (after 6 additions). When the NaCl was totally dissolved, a final ^{1}H NMR spectrum was measured. Similar measurements were carried out using PAA as the template.

^{1}H NMR of Aniline with Micelle Template Systems. ^{1}H NMR spectra of aniline with SDBS micelle template systems were taken using procedures similar to that described previously for the polyelectrolyte templates. Here, a 2.0 mg/mL aniline stock solution in D_2O solution was prepared at pH 4.3. An aliquot of 0.6 mL was withdrawn and transferred to a NMR tube, and the ^{1}H NMR spectrum of just the aniline was first recorded. A stock SDBS micelle solution was prepared by dissolving 37.31 mg of SDBS into 1.0 mL of D_2O at pH 4.3. Incremental amounts (10 μL) of the SDBS micelle stock solution were then added, and ^{1}H NMR were recorded after each addition. Similar procedures were repeated with aniline in the presence of SBS.

Results and Discussion

Polyelectrolyte Template Systems. UV−vis−near-IR Absorption Studies on Different Template Systems. To better understand the mechanistic role of the template in these enzymatic reactions, a series of different polyelectrolyte templates were investigated. Polyelectrolytes were specifically chosen to investigate the effects of ionizability (pK_a), ionic charge (anionic, cationic, or neutral) and surface charge density of the template in the reaction. The molecular structures of each of the template polymers studied in this work are given in Figure 2. All reactions were carried out under identical conditions (0.1 M, pH 4.3 phosphate buffer with a 1:1 molar ratio of template to aniline) so that only the template was varied in each case. Figure 3 shows the UV−vis−near-IR absorption spectra of the resulting polyaniline complexes that were obtained with each of the polyelectrolyte template systems. In the following discussion, the absorption band at approximately 800−1200 nm (due to polaron transition[19]) is compared as a signature of the formation of conducting polyaniline.

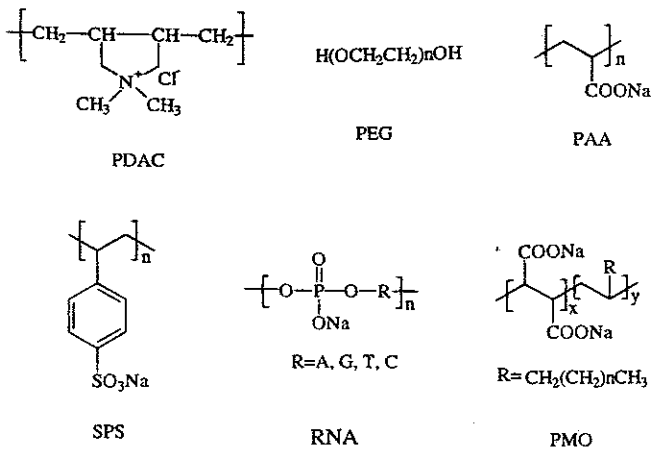

Figure 2. Molecular structures of the macromolecular templates investigated.

The polyanilines formed using both a polycationic template, PDAC, and a neutral template, PEG, are very similar to that which is obtained when there is no template present in the reaction medium. The resulting polymer solutions are purple in color, show strong absorption at approximately 400−600 nm with only a weak tail at 800−1200 nm due to the polaron transition band, and eventually precipitate. This absorption is attributed to the presence of primarily branched, low molecular weight polyaniline that is formed from the ortho- and para-directed enzymatic coupling of aniline.[10a] In addition to PDAC and PEG, other cationic and neutral template polymers such as poly(vinyl alcohol) and poly(vinylamine) showed similar behavior (data not shown here).

Enzymatic polymerization in the presence of weak acid polyelectrolyte templates, such as PAA, shows slightly stronger absorption from 800 to 1200 nm in comparison. However, the major absorption is again observed from 400 to 600 nm and is indicative of a branched, low molecular weight form of polyaniline. Similar results were obtained with other weak acid polyelectrolytes, such as poly(glutamic acid) and poly(aspartic acid) (data not shown). Therefore, at pH 4.3 polycations, neutral polymers and weak polyelectrolytes are not suitable templates to produce a highly conducting form of polyaniline by HRP catalysis.

When the polymerization is carried out in the presence of strong acid polyelectrolytes such as SPS and RNA, however, dark green solutions are formed which show strong polaron absorption bands from 800 to 1200 nm and minimal absorption from 400 to 600 nm. These spectra confirm that the linear, conducting polyaniline is primarily formed when enzymatic polymerization is carried out in the presence of such strong acid polyelectrolyte templates. Similar results are also observed in other strong acid polyelectrolyte template systems such as poly(vinyl phosphate) and DNA.[20]

This favoritism to strong acid polyelectrolyte templates may be explained when one considers both the mechanism of polymerization of aniline and general polyelectrolyte behavior. The mechanism of the oxidative polymerization of aniline (both chemical and electrochemical) has been extensively studied.[21] Although the detailed mechanism of polymerization remains

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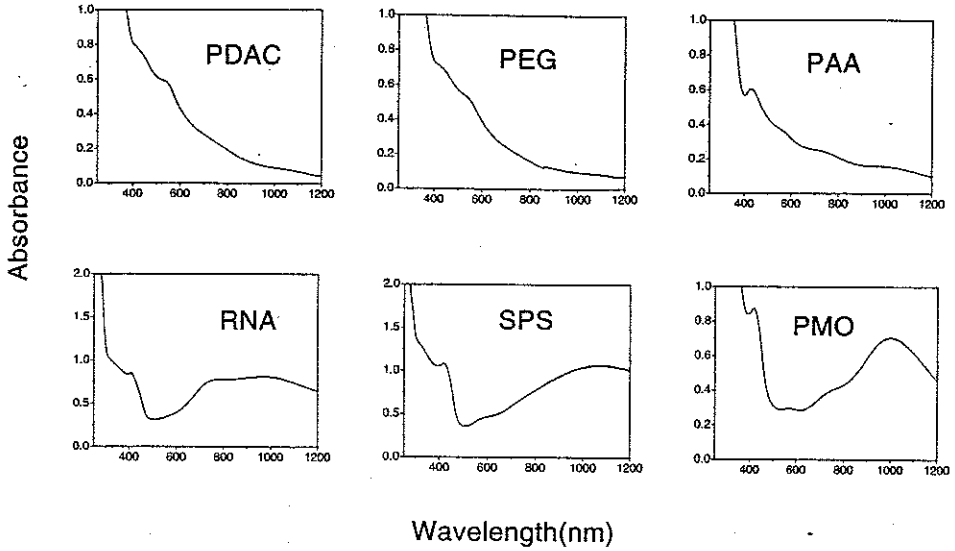

Figure 3. UV−vis−near-IR spectra of the polyaniline produced with various template systems. The enzymatic polymerization was carried out at pH 4.3 with 6 mM aniline. In each case, the molar ratio of aniline to template in the reaction medium is 1:1.

controversial due to the experimental difficulties in identifying the reaction intermediates, it is generally accepted that highly acidic medium is necessary for the synthesis of conducting polyaniline. This is strongly supported by the recent findings of Gospodinova et al.,[22] who demonstrated that the initial stage of formation of *N*-phenyl-1,4-benzoquinonediimine occurred in the whole pH range, whereas the linear chain growth of polyaniline occurred solely in a strongly acidic medium. Other studies have also confirmed that the resulting properties of the polyaniline prepared either chemically or electrochemically are strongly dependent on the pH of the reaction media.[23] To date, both chemical and electrochemical polymerization of aniline must be carried out in strong acid media to obtain the conducting form of polyaniline.

The enzymatic template polymerization of aniline is also pH-dependent. It was previously determined that conducting polyaniline is not formed when the pH is over 6.0 in the case of the SPS template system.[14b] It is known that in the enzymatic polymerization of aniline, only the initiation step of generating aniline radicals is enzyme dependent.[7] The following radical−radical coupling and radical transfer steps are controlled exclusively by radical and solvent chemistry.[24] Therefore, one would expect that a low pH medium is also necessary for the synthesis of conducting polyaniline by enzymatic catalysis once the aniline radicals have been generated. Zemel et al.[25] have shown that the pH of the reaction media for enzymatic polymerization of aniline must be sufficiently acidic to cause protonation of the aniline monomer and yet high enough to preserve as much of the bioactivity of the enzyme as possible. They found that conducting polyaniline is most effectively synthesized, enzymatically, at a pH of around 3.0 with acidifying agents that have a pK_a lower than that of the aniline monomer. However, these pH conditions are still low enough to quickly denature the enzyme and cause loss of activity. To circumvent these problems, the aniline and a stoichiometric amount of H_2O_2 was added to the reaction media first, followed by incremental addition of the HRP to minimize exposure of the enzyme to the low acid conditions. Despite these modifications, however, large amounts of HRP are still consumed, and poor solubility of the resulting polyaniline has severely limited this approach. Thus, a medium is needed that can provide both a high enough pH environment for efficient enzymatic free radical generation and yet low enough to protonate the aniline monomer and promote head-to-tail, radical−radical coupling, and radical-transfer steps for a conducting form of polyaniline. The presence of strong acid polyelectrolyte templates in the reaction media can provide these conditions.

Several theories have been postulated to account for the distribution of ions in polyelectrolyte solutions.[16] The counterion condensation theory, developed by Manning,[26] assumes that the effective polyelectrolyte charge density has a definite maximum. Higher charge densities are reduced to the maximum by counterions or "condensation" on the polyelectrolyte. Therefore, acidic polyelectrolytes will electrostatically attract hydrogen ions (while basic polyelectrolytes will repel them), and the pH at acidic polyelectrolyte surfaces will be lower than that of the bulk aqueous medium. The opposite situation prevails for basic polyelectrolytes where their surface pH will be higher than that of the surrounding bulk solution.[27] Theoretical calculations by Lochhead et al.[28] have shown that, although the pH of the bulk solution remains constant, the localized pH near the negatively charged surface is lowered and will vary for different bulk solutions. The extent to which the pH is lowered depends on the magnitude of the surface potential, which in turn is a function of both monolayer (chemistry and geometry) and bulk solution properties. Since it is already established that a low pH environment is necessary for the linear chain growth of polyaniline, it is understandable that polycations and neutral polymers will not provide a sufficient acidic environment to facilitate the formation of conducting polyaniline. On the other hand, Manning's theory suggests that strong acid polyelectro-

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lytes may provide a sufficiently low, local pH environment for protonation and head-to-tail coupling of the aniline. At the same time, the pH of the bulk solution will remain at a higher pH to prolong the activity of the enzyme.

The difference in template behavior between the strong acid polyelectrolyte and the weaker acid polyelectrolytes in the synthesis of conducting polyaniline may be further accounted for by different dissociation behavior. SPS is a strong acid polyelectrolyte that completely dissociates in aqueous solution into polyions and counterions over the entire pH range. Weak acid polyelectrolytes such as poly(acrylic acid), however, form a polyion-counterion system only in a limited pH range and remain as undissociated polyacid in the acidic range. This behavior is typical for weak polyelectrolytes and quite analogous to weak small molecular electrolytes. The dissociation behavior of polyacids is defined by the well-known Henderson–Hasselbalch equation similar to that of small molecules.[16]

$$pK_{app} = pH + \log(1 - \alpha)/\alpha$$

But the resulting pK_{app} in the case of polyacids, depends on the degree of dissociation (α) and is only an apparent one. By potentiometric titration the pK_{app} and α can be determined. For example, at pH 4.3, very few carboxylic groups ($\alpha < 0.1$) are dissociated,[29] and the charge density of the PAA is much lower in solution than that obtained with SPS. Thus, the lower charge density of the PAA attracts fewer hydrogen ions and does not provide a sufficiently low pH, local environment to facilitate growth of conducting polyaniline.

To further illustrate the effects of dissociation constants of the templates in these enzymatic reactions, a comparison of PAA to poly(maleic acid co-olefin)(PMO) is carried out. In contrast to the PAA, the PMO serves as a suitable template for enzymatic polymerization of aniline to a conducting form. In this case a dark green solution is formed which exhibits strong polaron bands at 800–1000 nm (Figure 3). Here, although both polymer templates are functionalized with carboxylic groups, their dissociation behaviors are quite different. It has previously been shown that the maleic acid moieties in the polymer exhibit a two-step dissociation behavior similar to that observed with molecular maleic acid.[29] The dissociation constants of these two carboxylic acids are $pK_{a1} = 2.0$ and $pK_{a2} = 6.26$.[15] The magnitude of this difference is sufficient to expect the weaker carboxylic acid to remain essentially undissociated until the stronger acid is completely dissociated. Therefore, at pH 4.3, approximately half of the carboxylic groups are dissociated in the PMO, unlike the PAA, and the requisite lower, local pH environment is provided.

^{13}C NMR Titration. The local template environment was investigated using NMR titration of ^{13}C-labeled (all carbon positions were labeled) aniline in the reaction media with and without SPS template. The chemical shifts of the aniline resonance peaks are plotted as a function of the pH of the bulk solution and are shown in Figure 4. The ^{13}C NMR spectrum of aniline at pH 7 shows four triplets at 145, 130, 123, and 117 ppm which are assigned as ipso, meta, para, and ortho carbons, respectively.[30] The splittings in the ^{13}C NMR spectra are due to ^{13}C–^{13}C J-couplings in the ^{13}C-enriched aniline molecules. The observed changes of chemical shift with the decrease of pH shown in Figure 4 reflect the protonation process of aniline.

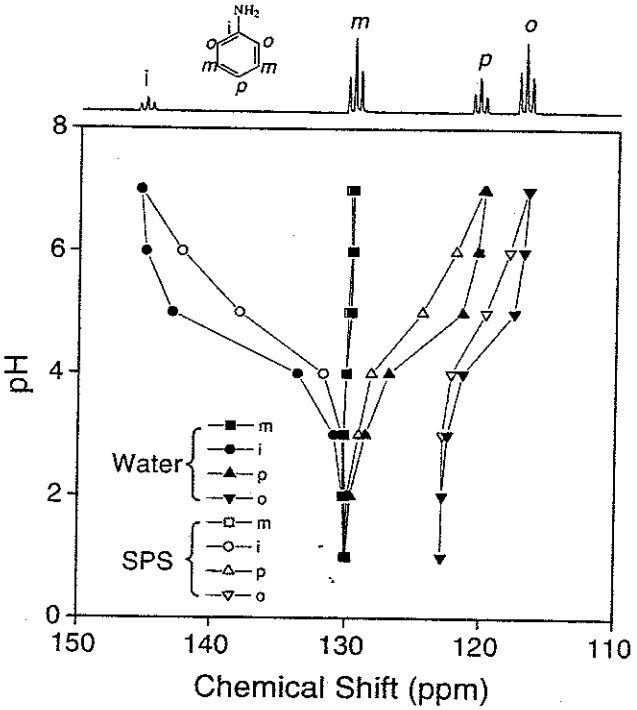

Figure 4. The pH dependence of ^{13}C NMR chemical shift of $^{13}C_6$-aniline(2 mg/mL) in pure D_2O, and SPS/D_2O solution. The molar ratio of aniline to SPS is 1:1 in the SPS/D_2O solution. The solutions are titrated by 1 M HCl and NaOH.

The chemical shift changes of the ortho, para, and ipso carbons for both the pure aniline and aniline/SPS systems are most dramatic when the pH is close to the pK_a of aniline (4.63). As the bulk pH decreases, the ipso carbon peak shifts upfield, while the para and ortho carbons shift downfield. These results are similar to that previously observed in pyridine[31] and pyridoxal phosphate systems.[32] These changes of chemical shifts are explained by the alteration of the electron density on the aromatic ring due to the protonation of the amine group.[33] The lower the pH, the larger is the fraction of protonated aniline. In this case, the observed chemical shifts represent an average of the charged and uncharged anilines which are not distinguishable in ^{13}C NMR due to the rapid exchange on the NMR time scale.

It is worth noting that the chemical shifts of ipso, para, and ortho carbons of aniline show significant difference in the presence of SPS template compared to that in a pure water system at the same bulk pH around its pK_a. In some cases the chemical shift difference is as much as 6 ppm. These differences in fact reflect different protonation levels of aniline in these two systems. For example, at pH 5, the ipso carbon shows a peak at 143 ppm in the pure aniline system, while in the SPS system the peak shifts upfield to 137 ppm. This indicates that more charged aniline species are formed when SPS is present in the solution. Since the bulk solution pH is the same in both systems, these differences show that the template is providing a lower, local pH environment that in turn promotes additional protonation of the aniline monomer.

When the pH of the bulk solution, however, varies too much from the pK_a of the aniline, then the lower, local pH environment of the template does not effect the level of protonation. This is because the local pH environment is still strongly dependent

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(31) Breitmaier, E.; Bauer, G. *Pharm. Unserer Zeit* **1976**, *5*, 97.

(32) Witherup, T. H.; Abbott, E. H. *J. Org. Chem.* **1975**, *40*, 2229.

(33) Breitmaier, E.; Bauer, G. *^{13}C NMR Spectroscopy: A Working Manual with Exericises*, translated from German by Cassels, B. K.; Harwood Academic Publishers: New York, 1984; Chapter 7.

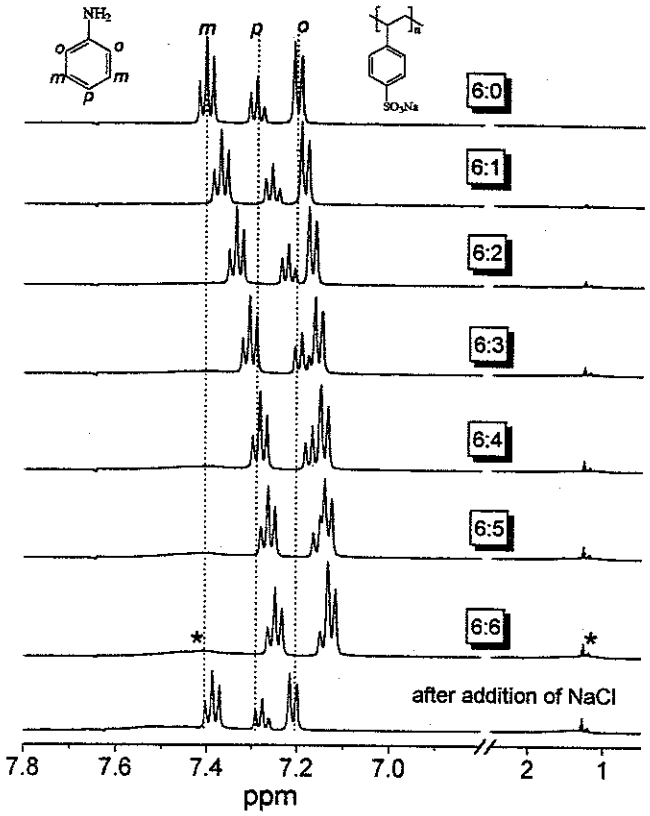

Figure 5. ^{1}H NMR of aniline (2 mg/mL) in D_2O solution with various molar ratios of aniline to SPS at pH 4.3. All the curves are plotted with an axis break from 6.8 to 2 ppm. (*) Peaks are assigned to SPS.

on the bulk pH,[27] and at pH 7.0 the local template environment, although lower, remains considerably higher than the pK_a of the aniline. The majority of the aniline remains neutral, and the NMR is identical to what is observed when SPS is not present. Conversely, as the bulk pH is lower than 3.0, most of the aniline in the system becomes protonated, with or without the template, and the chemical shifts of the ipso, para, and ortho carbons show little difference in the presence of SPS. In addition, at very low pH the ipso and para carbon peaks are found to merge together with the meta carbon at 130 ppm.

^{1}H NMR. At pH 4.3, in the presence of a strong acid polyelectrolyte, a majority of the aniline monomers are protonated. It has been postulated that these positively charged monomers act as counterions that complex with the anionic polyelectrolyte template through electrostatic forces.[14b] ^{1}H NMR spectroscopy was used to monitor this complexation process of aniline with the SPS template prior to polymerization. Figure 5 shows a series of ^{1}H NMR spectra for aqueous solutions of pH 4.3 that contain varying molar ratios of aniline to SPS. The ^{1}H NMR spectrum of aniline in a pure water system shows three peaks which are assigned as two triplets at 7.41 and 7.30 ppm for the meta and para protons, respectively, and one doublet at 7.21 ppm for the ortho proton. The resonance peak for the protons of the amine group is not observed due to proton exchange with water in the system.[34] It was found that each of these proton peaks shift upfield as the molar ratio of SPS in the system increases. Eventually, the resonance peak of the para proton completely merges with the ortho proton when the molar ratio of aniline to SPS reaches 1:1. Also, two broad peaks appear at about 7.4 and 1.3 ppm (labeled with asterisks), that are due to the protons from the sulfonated styrene ring (another peak

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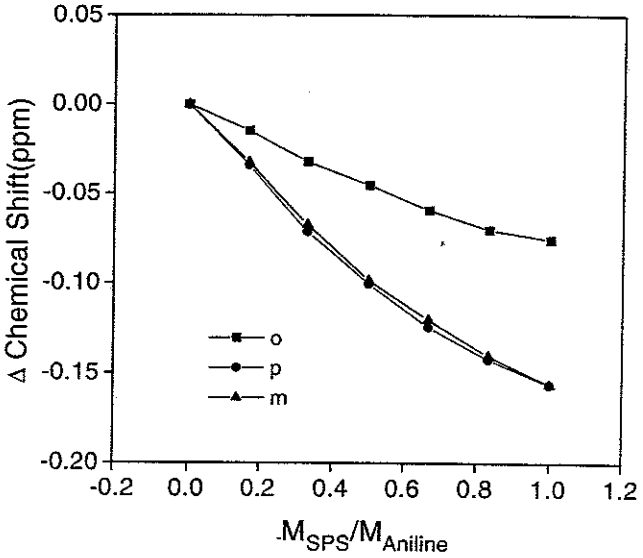

Figure 6. ^{1}H NMR chemical shift changes of aniline as a function of the molar ratio of SPS to aniline . The changes of chemical shift are relative to that in pure water.

at around 6 ppm is not shown), and the methyl protons from the SPS backbone, respectively. The signals of the SPS protons are very weak in comparison to those of the aniline monomer and thus do not interfere with the characterization of the aniline protons. A plot to summarize the change of chemical shift for each of the aniline protons with molar ratio of SPS to aniline is given in Figure 6. Each of the three proton peaks is observed to shift upfield with increasing amounts of SPS. However, the para and meta protons shift more dramatically than the ortho protons.

These observed changes of the ^{1}H NMR spectra strongly support intimate interaction of the aniline monomer with the SPS template. Although electrostatic forces most likely dominate this interaction because of the nature of the charged species, other intermolecular forces, such as van der Waals, hydrogen bonding, and dipole-charge transfer are also likely to contribute. According to Anton and co-workers[35] some polyelectrolyte solutions form hydrophobic "pocket" regions that can act as emulsifiers to solubilize low molar mass hydrophobic molecules. Even though most of the aniline in this case is charged at pH 4.3, the aromatic ring remains hydrophobic and may anchor into the hydrophobic regions in the SPS template. The larger chemical shift of the para and meta protons over the ortho protons (Figure 6) suggests that these protons prefer to orient into the more shielded hydrophobic regions of the SPS template. Similar shielding of the para and meta protons of aromatic species due to location in the hydrophobic regions of micelle systems was reported previously[36] and was also observed with micelle systems in this work (following section). The chemical shift change of the ortho proton is explained by the presence of the sulfonate groups on the SPS, as proposed by Macdonald et al.[37] The proximity of the electron density of the sulfonate group shields the protons of the quaternary nitrogen as well as the nearby ortho protons, leading to an upfield shift in the observed resonance.

Upon addition of NaCl to the 1:1 molar ratio of SPS to aniline solution, the aniline protons become deshielded. Here the aniline

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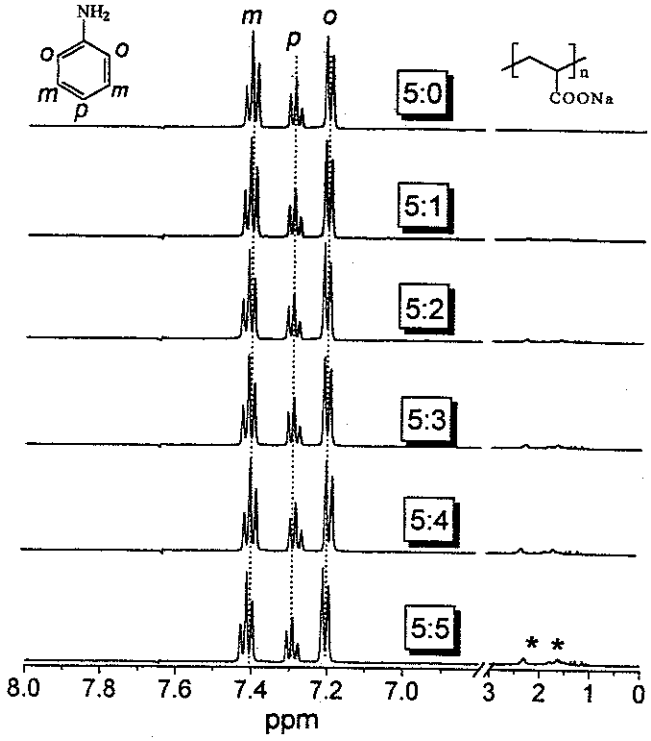

Figure 7. ^{1}H NMR of aniline (2 mg/mL) in D_2O solution at pH 4.3 with various molar ratios of aniline to PAA. (*) Peaks are assigned to the backbone methyl protons of PAA.

proton peaks shift dramatically downfield, and the para hydrogen peak reemerges. At high concentrations of NaCl in the reaction media (0.4 M), the sodium ions compete with the charged aniline in the SPS local environment and the aniline is "squeezed" out from the shielding hydrophobic environment. This behavior is strong evidence that the complexation of aniline with the SPS template molecules is driven by electrostatic interaction. Careful inspection of the aniline ^{1}H NMR spectra after the addition of NaCl, however, reveals that the chemical shifts of the para and meta peaks remain slightly upfield shifted when compared to those in the pure water system, and the ortho hydrogens show a slight downfield shift. These results show that even in high salt concentration, the aniline monomers remain loosely complexed with the SPS molecules. This is further confirmed by ^{13}C NMR titration experiments of aniline/SPS with (0.4 M NaCl) and without salt. In this case (data not shown) there was no observable difference in the chemical shifts for the aniline carbons when salt was added to the reaction medium. For example, at pH 4.3, the chemical shifts of the ipso carbons in each case were observed at 132.6 ppm. This shows that even in the presence of salt, the aniline carbons remain in a lower, local pH environment. Thus, even though the addition of salt alters the hydrophobic environment, the local pH environment remains preserved to promote the head-to-tail coupling of the aniline monomers.

As previously discussed, PAA is not a suitable template in this enzymatic reaction for the synthesis of the conducting form of polyaniline. To compare the interaction of monomer with this template prior to reaction, similar proton NMR studies were carried out and are shown in Figure 7. In sharp contrast to the SPS at pH 4.3, PAA as a weak acid polyelectrolyte does not exhibit any significant effects on the chemical shifts of the aniline protons under similar conditions. This behavior is consistent with the previous experimental results that show the need for a template that has a high dissociation constant. In this case the low dissociation of the PAA at pH 4.3 results in a smaller percentage of negatively charged groups[29] and this level is not sufficient to electrostatically complex and protonate the aniline monomers for optimal head-to-tail coupling

Aqueous Micellar Systems

Polymerization. Aqueous micellar systems offer several interesting variations to further probe the template mechanism in these enzymatic reactions. The aggregation behavior of surfactant molecules in aqueous medium to form micelles when the concentration is over the critical micellar concentration (CMC) is well understood.[27] The local environment created by this micellar aggregation of strong acid surfactant molecules is similar to that of strong acid polyanions. Each of these systems can form electrical double layers in which counterions such as H^+ may be condensed and can form hydrophobic pockets with which the monomers may associate.[38] The ability to specifically control the formation and surface charge density of micelles and their use as templates in the enzymatic polymerization of anilines, can offer additional insight toward elucidating the role of the template in these reactions.

In these studies the ionic surfactant, SDBS was compared to a small, non-aggregating molecule, SBS. These molecules were specifically chosen because they have identical acidic groups as that of the SPS and differ only in their ability to form aqueous micelles. The molecular structures of SDBS, SBS, and Triton X-100 (used for mixed micellar studies) are given in Figure 8. The synthetic procedure used for the enzymatic polymerization of aniline in the presence of these ionic templates is similar to that used for the macromolecular polyelectrolyte systems. Figure 9 shows the visible absorption spectra for the polyaniline formed in the presence of SPS (macromolecule), SDBS (above the CMC) and SBS (non-aggregating molecule). A comparison of these spectra shows that the polymer formed in the SDBS micelle system strongly absorbs from 800 to 1200 nm and is similar to that observed in the SPS system. The polyaniline formed with the micellar system is also water-soluble and in its doped state due to the presence of SDBS molecules.[39] These results show that the micelles formed by the SDBS are also suitable templates for the enzymatic synthesis of conducting polyaniline. However, the spectrum of the polyaniline is significantly different when the reaction is carried out in the presence of the non-aggregating molecule, SBS. In this case, the absorption peak is observed at much shorter wavelengths, near 500 nm, and is again indicative of a more branched, insulating form of polyaniline.[10a] Since the concentration of SO_3^- groups is the same for the SDBS and SBS, the primary difference in these systems is the formation of micelles with the SDBS.

The necessity of this local "micelle" environment was further investigated by carrying out the reaction in SDBS systems under conditions where micelle formation would be limited. Figures 10 a and b show the visible absorption spectra for the polyaniline formed with SDBS below the CMC (60 μM) and over the CMC but in the presence of 50% acetone, respectively. These conditions were chosen for the following reasons. First it is known that when the concentration of surfactant is lower than its CMC, few or no micelles will be formed in the solution.[26] Since the addition of aniline monomer may lower the CMC of

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(39) Cao, Y.; Smith, P.; Heeger, A. J. *Synth. Met.* **1992**, *48*, 91.

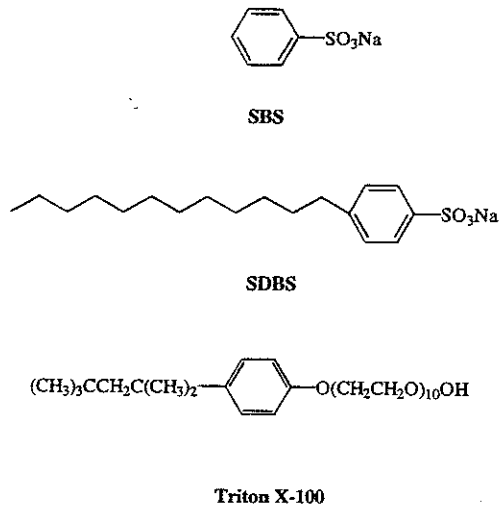


Figure 8. Molecular structures of SDBS, Triton X-100, and SBS.

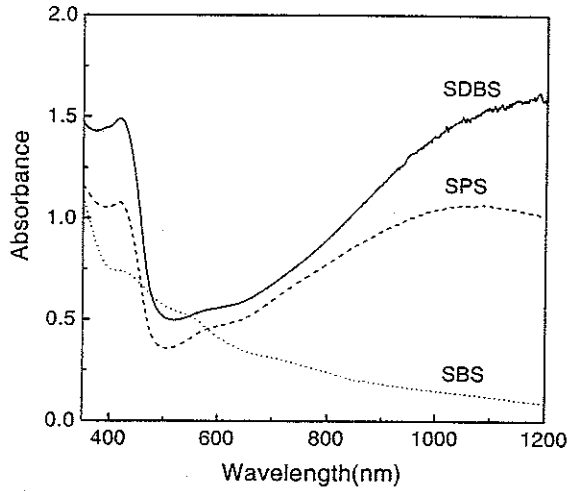
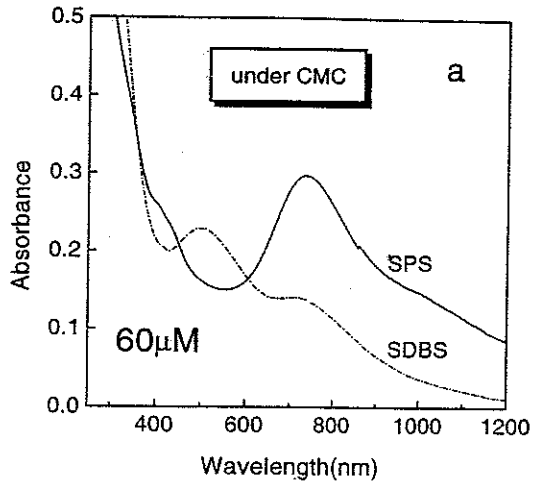


Figure 9. UV−vis−near-IR spectra of polyaniline produced in 10 mM SDBS, SPS, and SBS solution. In each case, the aniline concentration is 10 mM.

the SDBS,[40] a very low concentration of SDBS (60 μM) was added to ensure minimal micelle formation. Similarly, it is known that micelles may be collapsed by changing the polarity of the solution, such as by the addition of acetone.[41] Therefore, it was expected that under these conditions the SDBS molecules would be dispersed in the reaction media with minimal aggregation. These poor micelle-forming conditions were confirmed by light-scattering measurements.

The resulting polyaniline spectra show that under these poor micelle-forming conditions, the emeraldine salt form of polyaniline is not as readily obtained with the SDBS. Under these conditions, the SDBS molecules behave similarly to the non-aggregating SBS molecules. However, it is interesting to note that if these same conditions are used for the SPS polyelectrolyte (low concentration based on repeat unit and 50% acetone in the solution), the emeraldine salt form of polyaniline is still obtained. This behavior is clearly explained by SPS being a macromolecular anionic template, where the sulfonate groups are covalently bonded along the polymer chain. Therefore, even a small concentration SPS molecules in the reaction media is adequate to provide the requisite local charged environment and



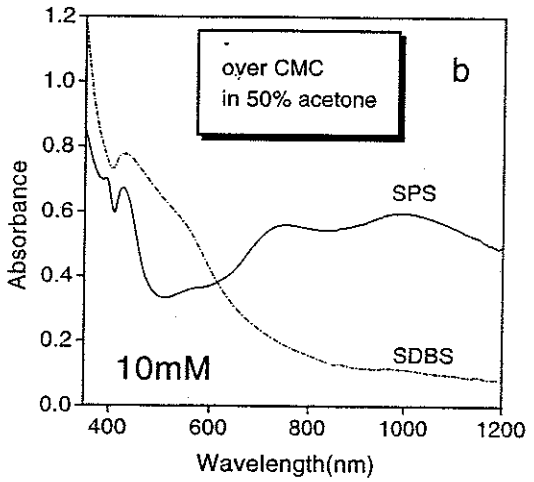


Figure 10. UV−vis−near-IR spectra of polyaniline produced in (a) 0.1 M, pH 4.3 phosphate buffer with a SDBS concentration of 60 μM (under its CMC) (b) in 50% acetone/0.1 M, pH 4.3 buffer mixture with a SDBS concentration of 10 mM (over its CMC). In each case, the UV−vis−near-IR spectrum of polyaniline produced under the same SPS concentration (calculated by unit) is also shown for comparison.

hydrophobic regions to form the template nano-reactor and promote the polyaniline linear chain growth in the doped form. It is expected that these conditions would not be disrupted by dilution or by the addition of acetone to the reaction medium. These results all strongly support the importance of a local environment that can provide a higher charge density, lower pH and hydrophobic regions to promote the formation of conducting polyaniline.

^{1}H NMR. Solubilization of organic molecules in micellar systems is known to be a dynamic process that involves both hydrophobic and electrostatic interactions.[27] Depending on the structure and degree of hydrophobicity of the organic solutes, they can be solubilized inside the hydrocarbon core of the micelle, or in the so-called palisade layer (the Stern layer).[42] The site of incorporation of monomer into micellar systems has been previously investigated using NMR spectroscopy.[43] Here, ^{1}H NMR spectroscopy was used to study the interaction of the aniline monomer with the micelles. Figure 11 shows the ^{1}H NMR spectra of aniline as a function of the molar ratio of aniline to SDBS (above the CMC) in the reaction media. The major

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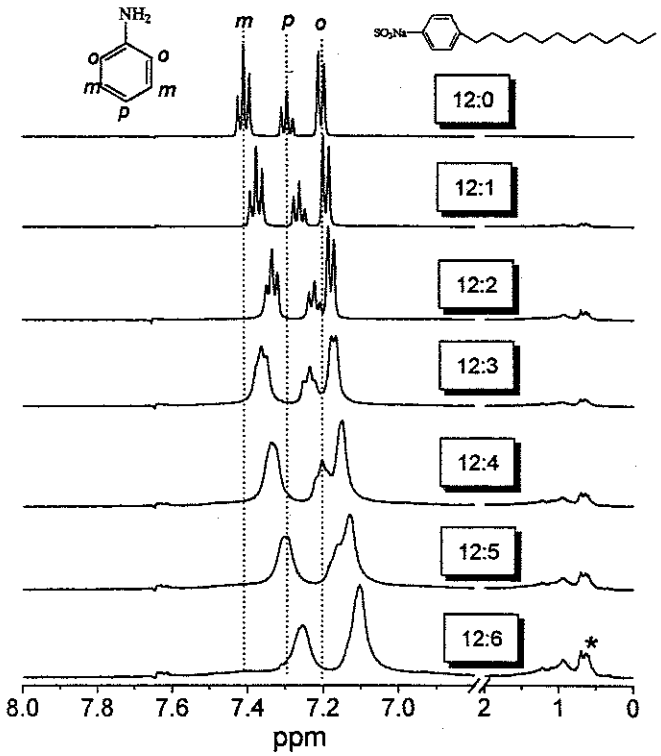

Figure 11. ^{1}H NMR spectra of aniline (2 mg/mL) in D_2O at pH 4.3 with various molar ratios of aniline to SDBS. (*) Peaks are assigned to SDBS.

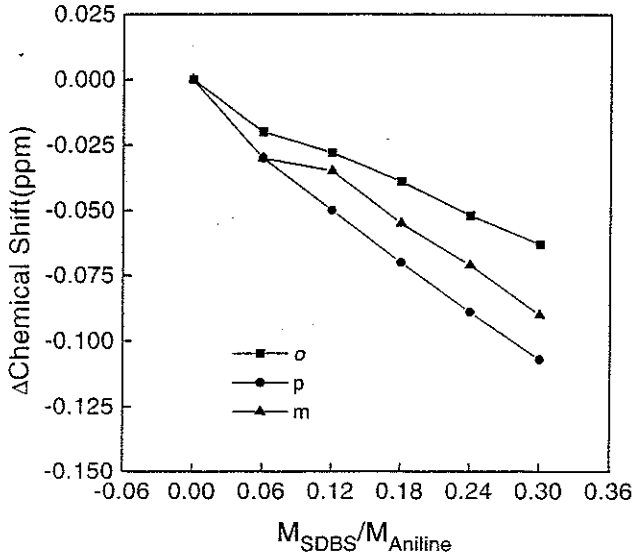

Figure 12. ^{1}H NMR chemical shift changes of aniline as a function of the molar ratio of SDBS to aniline. The changes of chemical shift are relative to that in pure water.

features of the ^{1}H NMR of aniline in the SDBS micellar systems are quite similar to that previously observed with the SPS template. Upon addition of SDBS into the reaction media the resonance peaks of the aniline protons shift upfield gradually, and the para proton peak eventually merges with the ortho proton peak. The line widths of all the aniline proton resonances are found to increase due to the inhomogeneous nature of the medium caused by the presence of micelles. A plot of the change of chemical shift as a function of the molar ratio of SDBS to aniline is given in Figure 12. Similar to what was previously observed with the SPS template, the upfield shifts of the meta and para protons are more pronounced than that observed for the ortho protons with increasing SDBS. This shielding of

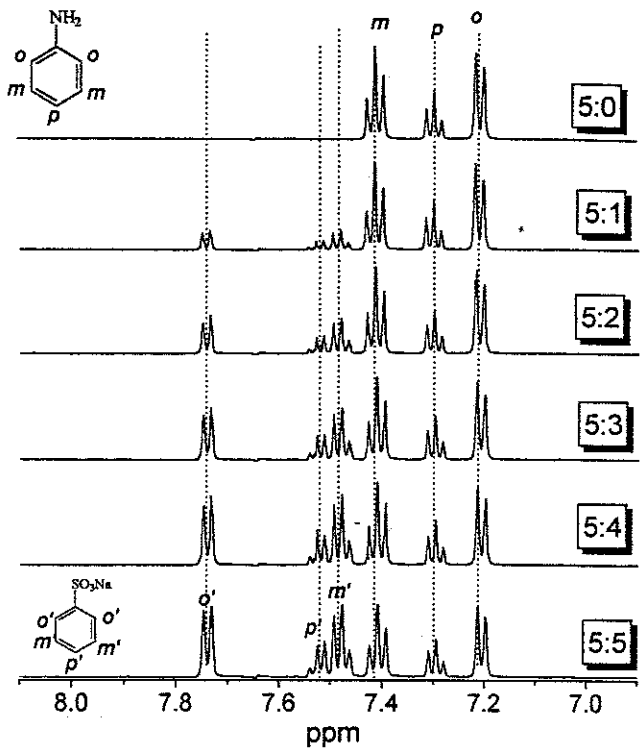

Figure 13. ^{1}H NMR of aniline (2 mg/mL) in D_2O with varied ratios of aniline to SBS at pH 4.3.

aromatic protons in micelles was also observed by Jacobs et al.[43b] with phenol and sodium dodecyl sulfate and by Das et al.[44] with oleate and sodium alkylbenzenesulfonate. It is believed that this upfield shift of the aromatic protons with increasing surfactant concentration is due to an increase in the hydrophobic micellar environment. The greater upfield shift of the meta and para protons than that of the ortho protons indicates that these protons are oriented such that they are more exposed to the hydrophobic hydrocarbon core of the micelle, while the ortho protons are more exposed to the micellar pseudo-phase. On the basis of a similar pronounced difference in the shielding of meta and ortho protons in the process of micellization of alkylbenzenesulfonate, Goon et al.[45] suggest that the water boundary in the alkylbenzenesulfonate micelle lies just between the ortho and meta protons of the phenyl ring. Therefore, we believe that the positively charged anilines in our systems are intercalated between the benzenesulfonate headgroups of the micelles with the NH_3^+ group directed toward the bulk solution. Thus the para and meta protons see a more hydrophobic environment than the ortho protons and are more shielded to the magnetic field. A similar intercalation of aromatic counterions between the headgroups of micelles (with more or less penetration) was also observed by Bijma et al.[46a] in the micelles formed by alkylpyridinium and by Kreke et al.[46b] in the mixed micelles formed by cetyltrimethylammonium derivatives.

In addition, the total merging of the para proton and ortho proton peaks of aniline requires much less SDBS present in the media (2:1, aniline to SDBS) than that was previously observed for the SPS system (1:1, aniline to SPS). This suggests that the micelles formed by the SDBS are able to provide a significantly stronger hydrophobic environment for the aniline than the SPS template.

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(45) Goon, P.; Das, S.; Clemett, C. J.; Tiddy, G. J. T.; Kumar, V. V. *Langmuir* **1997**, *13*, 5577.
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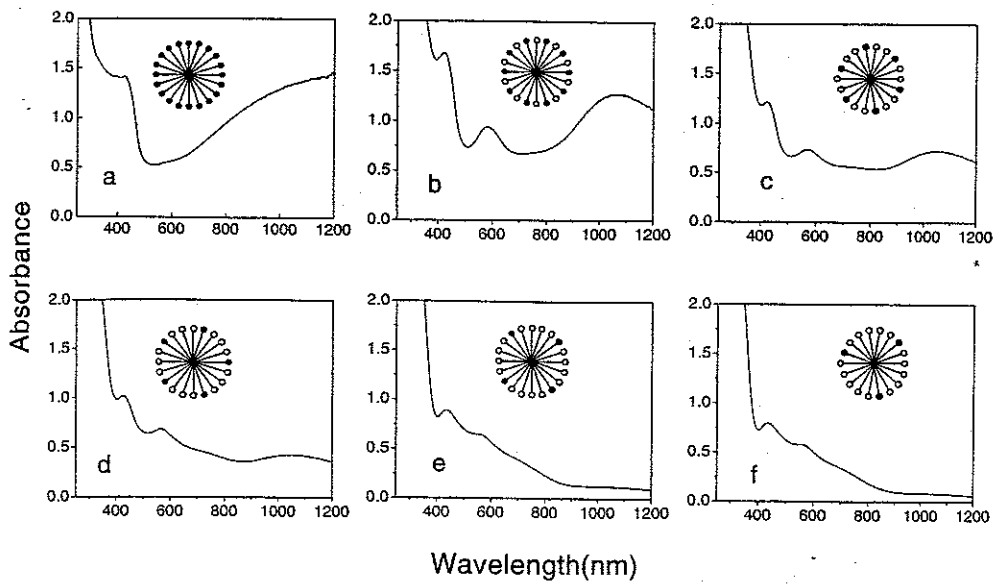

Figure 14. UV−vis−near-IR spectra of polyaniline produced in the mixed micelle solutions formed by SDBS and Triton X-100. The concentrations of both SDBS and aniline in each solution are 10 mM. The molar ratios of SDBS to Triton X-100 are: (a) 1:0, (b) 1:1, (c) 1:2, (d) 1:3, (e) 1:4, and (f) 1:5, and each mixed micelle system is schematically represented as insets with each spectrum.

^{1}H NMR spectra of aniline in the system of the non-aggregating SBS is given in Figure 13. Three new peaks appear at 7.74, 7.53, and 7.48 ppm with the addition of SBS and are assigned to the meta, ortho, and para protons of the SBS molecules, respectively. The ^{1}H NMR spectra of aniline in the presence of SBS are quite different from that observed with the SDBS system in that there is little observable shift in any of the protons with increasing amounts of SBS in the media. This is strong evidence that there is little interaction between the aniline and SBS molecules, even when the ratio is as high as 1:1. Again, this is believed to be due to the lack of a local environment that can provide both hydrophobic regions for monomer emulsification and a lower pH environment for the preferred head-to-tail coupling.

Mixed Micellar Systems. The effect of charge density of the local environment (the distance between neighboring charge groups) on the enzymatic polymerization of aniline was investigated using mixed micellar systems. Micellar systems provide a unique way to control the distance between negatively charged groups by forming mixed micelles of varying ratios with a nonionic surfactant system.[47] In the present work, Triton X-100 is used to form mixed micelles with the anionic surfactant SDBS. Triton X-100 was chosen because it is a nonionic surfactant that can serve as a spacer to control the surface charge density and because it was previously established by us that conducting polyaniline could not be formed from pure Triton X-100 micelles.

The enzymatic polymerization of aniline was carried out under the same conditions but with various molar ratios of SDBS to Triton X-100 (1:1 to 1:5). Figure 14 gives a schematic illustrating the approximate distribution of charge density in each mixed micelle system and the corresponding UV−vis absorption spectrum after polymerization. In each case, the concentration of SDBS in the system is the same, and the amount of Triton X-100 is varied. The UV−vis absorption shows that as the amount of Triton X-100 in the system is increased, the characteristic polyaniline absorption peak observed at 800−1200 nm for the pure SDBS system becomes weaker and gradually shifts to shorter wavelengths. A new peak is also observed at 580 nm that is due to the exciton transition of quinoid ring.[19] This new peak indicates that, as the charge density decreases in the mixed micelles, the formation of undoped polyaniline increases in the reaction media.

When the molar ratio of SDBS to Triton X-100 in the reaction media reaches 1:4, the absorption peak that was observed at 800−1200 nm disappears, indicating that little or no formation of conducting polyaniline occurs with these higher amounts of nonionic surfactant mixed in the system. Although a great deal of theoretical and experimental studies have been carried out, regarding the molecular level organization of mixed surfactant systems many questions remain.[48] Other studies have shown that micellization behavior of mixed surfactants may vary unpredictably under different conditions.[49] Therefore, the exact spacing between the sulfonate groups in these mixed micelles cannot be assessed. These results, however, are important in that they show a direct dependence on the type of polyaniline that is formed with the amount of nonionic surfactant. This strong dependence on charge density again supports the necessity of the template to provide a minimal charge distribution or local pH environment for the formation of conducting polyaniline.

The macromolecular ionic templates are a more straightforward case in that their charge distribution does not change significantly, if one assumes minimal effects from the conformational changes, because the ionic groups are covalently bound to the backbone. A series of SPS molecules with different molecular weights and different spacing between the neighboring sulfonated styrenes will be studied to determine what the minimal chain length and charge density is to provide this requisite local environment. These studies are underway.

Conclusions

This paper discusses the mechanistic role of the template in the enzymatic synthesis of polyaniline. UV−vis absorption, ^{13}C NMR, and ^{1}H NMR spectroscopies were used to characterize

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and compare the reaction conditions using various polyelectrolyte and micellar template systems. These studies have clearly shown that there is a direct dependence on the template structure and the type of polyaniline that is formed. The effective template provides several key "local environmental" conditions to facilitate the reaction. The template provides a lower local pH environment that increases the level of protonation of aniline in the reaction medium. This increase in protonation promotes both electrostatic interaction of the aniline monomer to the template and head-to-tail coupling of the monomers during reaction. In addition, this allows one to carry out the reaction at a higher bulk pH to prolong the bioactivity of the enzyme. The template also provides hydrophobic regions that serve to solubilize and orient the monomer molecules prior to reaction. These studies should provide new insight toward the selection of appropriate template systems for the synthesis of conducting polyaniline and important fundamental information regarding the design and optimization of a broad range of other interesting template-guided reactions.

Acknowledgment. We thank Dr. H. B. Liu for helpful discussions.

JA9926156