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Biocatalytic approaches for synthesis of conducting polyaniline nanoparticles*

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Abstract: A biocatalytic approach has been developed to synthesize conducting polyaniline (PANI) nanoparticles. The method involves the formation of nanocomposites of PANI and poly(acrylic acid) (PAA) by polymerizing aniline (AN)-camphorsulfonic acid (CSA) macromonomer (AN/CSA) using a biocatalyst in the presence of template PAA. The second step involves the separation of PANI polymers from PAA and CSA in the nanocomposites. The formation of PANI nanoparticles by this two-step approach is studied by transmission electron microscopy (TEM). High-resolution solution ^{13}C NMR and UV/visible spectroscopic techniques have been used to characterize the formation of conducting PANI chains by the enzymatic method.

INTRODUCTION

Polyaniline is one of the most promising electrically conducting polymers because of its chemical stability and relatively high conductivity [1,2]. The ability to build and control engineered polymeric materials at the nanometer (nm) scale is important for current and future development of materials for a wide range of applications from drug delivery to electronic applications, including photovoltaic cells, plastic batteries, and polymer light-emitting diode (PLED) displays. At the present time, the development and applications of these polymeric materials at the nm scale is also technological challenging for innovative applications. The key to this technological explosion of applications is how electro- and opto-active polymers such as conducting polyaniline (PANI) and other polymers could be synthesized to form nm scale polymeric materials. A simple yet elegant biotechnological approach has been developed to synthesize conducting PANI nanoparticles. It is possible to form PANI nanoparticles in the 30–50 nm size range using this novel approach.

There are two simple steps involved in the formation of PANI nanoparticles by enzymatic synthesis: (a) formation of nanocomposites by in situ polymerization of a monomer complex (AN/CSA) of aniline (AN) and camphorsulfonic acid (CSA) using a biocatalyst such as horseradish peroxidase (HRP) in the presence of polyelectrolyte template such as poly(acrylic acid) (PAA) [3,4], and (b) separation of PANI polymer chains from the template in the PAA/PANI/CSA composite. The formation of nanoparticles is studied by transmission electron microscopy (TEM) images of these samples.

Biocatalytic polymerization using a naturally occurring enzyme is advantageous in that it is a simple, one-step, and environmentally compatible synthesis with the potential for producing industrial polymers in high yield due to the high efficiency of the biocatalyst [5–9]. The enzyme, HRP, has re-

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cently been successfully employed as a catalyst for polymerization of phenols and anilines [3–7,10–12]. The free radicals of the monomer (substrate) in aqueous solution undergo oxidative coupling to produce dimers and other oligomeric products. The successive oxidation and coupling of oligomers eventually resulted in the formation of high-molecular-weight polymers [6]. This biocatalytic approach has been developed to synthesize water-soluble conducting polyanilines. The mechanistic cycle of HRP involves a two-electron oxidation step and two one-electron reduction steps [13]. Recently, we have reported the synthesis of conducting nanocomposites guided by the PAA template, suggesting that HRP influences directly the stereospecificity of the PANI in the nanocomposites, PAA/PANI/(+)CSA, and PAA/PANI/(–)CSA [3,4]. In this paper, we extend this work to the formation of PANI nanoparticles.

EXPERIMENTAL

In a typical procedure, 0.093 g (1 mmole) of aniline was added to 10 ml of 0.01 M sodium phosphate buffer. Then, 0.072 g (1 mmole, per monomer unit, MW 250 000) of poly(acrylic acid) was added. The reaction solution was stirred for 6 h to ensure complete formation of adduct. To this adduct solution, 0.6 ml of 2 M (–)10-camphorsulfonic acid was added. The reaction was carried out at pH 4.3. A solution of HRP, 3 mg in 0.5 ml of deionized H_2O, was then added and the contents continuously stirred. The reaction was then initiated with incremental addition of hydrogen peroxide (H_2O_2). To avoid the inhibition of HRP due to excess H_2O_2, a 0.5 % solution of H_2O_2 (8 aliquots of 10 µl each) was added drop-wise within a time interval of 5 min between each addition. A dark green solution was formed, indicating the formation of doped polyaniline. The unreacted monomer was removed by using Spectra/Pro dialysis membrane with molecular weight cut off at 1000 daltons. The same procedure was used to prepare polyaniline nanocomposites using (+)CSA and (±)CSA. Circular dichroism (CD) spectra show the maximum ellipticity for the sample molar ratio of PAA to aniline (1:1). It appears that the molar ratio of 1:1 for PAA:aniline is an optimum condition for synthesis of chiral polyaniline nanocomposites. For the purpose of further characterization and analysis, a molar ratio of 1:1 (PAA:aniline) was used in the reaction medium. A Bruker DRX 500 MHz NMR Spectrometer operating at 125 MHz for ^{13}C nuclei was used to collect and process ^{13}C NMR spectral data.

RESULTS AND DISCUSSION

HRP appears to be a suitable enzyme for the synthesis of these types of polymers that we are interested in [3,7,10–12], although other peroxidases such as that derived from soybeans are viable alternatives. HRP is able to catalyze the oxidation of a wide range of compounds, including aromatic amines and phenols, in the presence of hydrogen peroxide to generate corresponding free radicals [13].

$$\text{HRP} + \text{H}_2\text{O}_2 \rightarrow \text{HRP I}$$

$$\text{HRP I} + \text{RH} \rightarrow \text{R*} + \text{HRP II}$$

$$\text{HRP II} + \text{RH} \rightarrow \text{R*} + \text{HRP}$$

Here, the native enzyme (HRP) receives two oxidizing equivalents from hydrogen peroxide to form an intermediate HRP I, which in turn oxidizes the substrate (RH) by carrying out two sequential one-electron reduction steps through a partially oxidized intermediate HRP II to return back to its original native form and repeats the process again. The substrate in this case (RH) is aniline. R* is the radical species formed from aromatic amine. These free radicals then undergo coupling to produce dimer, and successive oxidation and coupling reactions eventually result in the formation of polymer [14,15]. The enzymatic approach is *environmentally benign*, offers a higher degree of control over the kinetics of the reaction, and has the potential of producing products in high yields.

Template-assisted enzymatic polymerization of monomers, in the presence of a polycationic or polyanionic template, appears to inherently minimize the parasitic branching and promotes a more para-directed head-to-tail polymerization [10]. Here the template serves a number of critical roles. First, the template serves to preferentially align the monomers prior to polymerization and minimizes the degree of branching. Lastly, the template, which is largely ionic, serves to provide water solubility and subsequently excellent processability of the final complex material.

The conducting nanocomposites are formed as a result of enzymatic synthesis of aniline-CSA macromonomer in the presence of polymer template PAA (steps a–d in Fig. 1). The conductivity of the system was measured using a Cascade Microtech four-point probe which showed the conductivity 1.8×10^{-2} S/cm for the nanocomposite, PAA/PANI/(–)CSA. Polymerization reaction is initiated by adding H_2O_2 initiator to the reaction medium. A series of UV/visible spectra were recorded during the PAA/PANI/(–)CSA nanocomposite formation by incrementally adding a known amount of H_2O_2 to the reaction medium. To avoid precipitation of the material, during enzymatic polymerization, H_2O_2 was added incrementally to attain the levels shown in Fig. 2. The band at 325 nm is due to a π-π^* transition of the benzenoid ring structure, and the two absorption peaks at 414 and a band in the 650–900 nm region are due to polaron transitions. These characteristic peaks suggest that the polyaniline formed in the enzymatic polymerization of nanocomposites is spectroscopically similar to the conducting form of the polyaniline synthesized through traditional chemical or electrochemical methods.

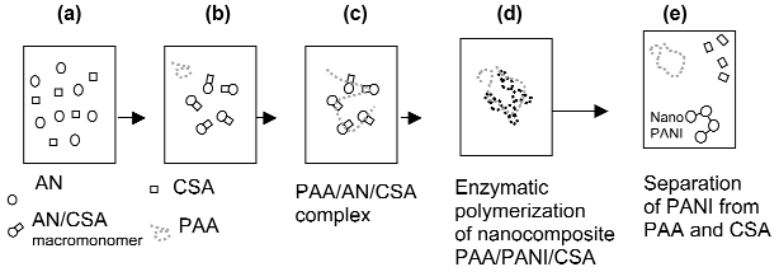

Fig. 1 A scheme showing the steps (a–e) that are involved in the enzymatic polymerization of nanocomposite PAA/PANI/CSA possessing a specific helical conformation [3] and PANI nanoparticles.

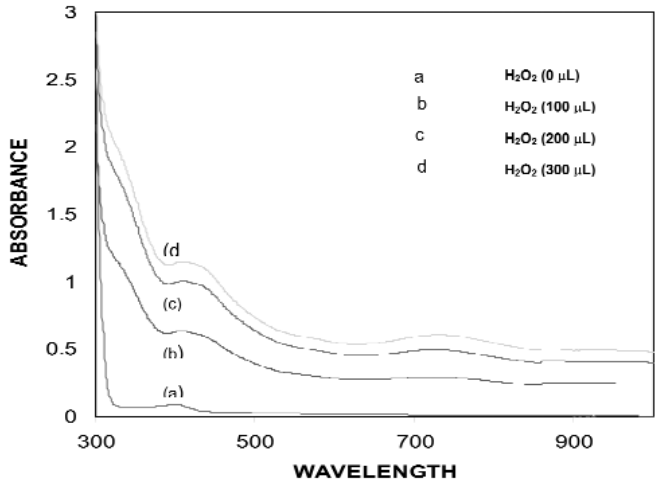

Fig. 2 UV/vis spectra recorded during the enzymatic polymerization of PAA/PANI/(–)CSA nanocomposites at different levels of H_2O_2. The formation of nanocomposites is confirmed by TEM images. H_2O_2 was added incrementally to attain the levels (a) 0, (b) 100 µl, (c) 200 µl, and (d) 300 µl.

The transmission electron micrograph (TEM) (Fig. 3a) of enzymatically synthesized PAA/PANI/(–)CSA material suggests that nanocomposites are evenly dispersed and have a fairly narrow dispersed size distribution of 25–50 nm. It is also of interest to note here that CD spectrum of these nanomaterials suggests that PANI chains in PAA/PANI/CSA have specific helical conformation regardless of whether induced chirality in the macromonomer (Fig. 1, step b) AN/CSA is +ve or –ve [3].

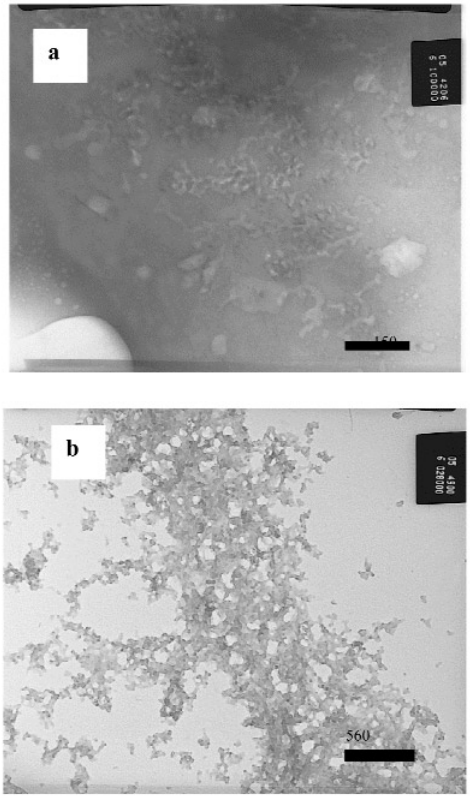

Fig. 3 TEM images of (a) conducting nanocomposite PAA/PANI/(–)CSA, (b) after treating with NH_4OH. The bar scale unit is in nm.

A simple procedure is used to form PANI particles from the nanocomposite material. In this method, the conducting nanocomposite sample was dedoped by simply treating with aqueous NH_4OH. During this process, PANI was detached from the PAA and CSA. The TEM image after the treatment with NH_4OH is shown in Fig. 3b. The detachment of PANI from its template PAA and the dopant CSA was investigated by a molecular spectroscopic method.

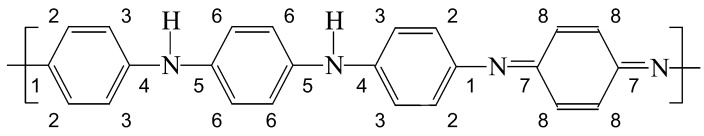

Fig. 4 Emeraldine base of PANI in the nonconducting form (dedoped) showing benzenoid (reduced) and quinoid (oxidized) structures.

To confirm the absence of template and CSA as a result of dedoping process, solution ^{13}C NMR spectrum was recorded of PANI dissolved in NMP solvent using $CDCl_3$ (Fig. 5a). The benzenoid protonated carbon resonances of PANI appear in the region of 110–130 ppm (see the inset, Fig. 5a). The peaks at 119.0 (C-6), 123.0 ppm (C-3) are assigned to benzenoid protonated carbon resonances of PANI (inset, Fig. 5a). The peak at 128.0 ppm (C-8) is assigned to quinonoid carbon resonances, and the weak resonances due to amine and imine nonprotonated quaternary carbon resonances of PANI also appear at ca. 140–160 ppm. The peaks at 178.8 ppm (Fig. 5b) and 217.8 ppm (Fig. 5c) were assigned to the carbonyl carbon resonances of CSA and PAA, respectively. The peaks at 16.9, 30.0, and 48.6 ppm are assigned to methylene carbon resonances of the NMP solvent. It is significant to note that the aliphatic carbon resonances of the template PAA and the dopant CSA are not observed in the spectrum of dedoped PANI nanocomposite (spectrum a in Fig. 5). This result suggests the absence of template and CSA after subjecting the conducting PANI nanocomposite sample to the dedoping process. The dedoped nanomaterial is in the nonconducting state and could easily be redoped by subjecting the nanomaterial to HCl to bring the material into the conducting state.

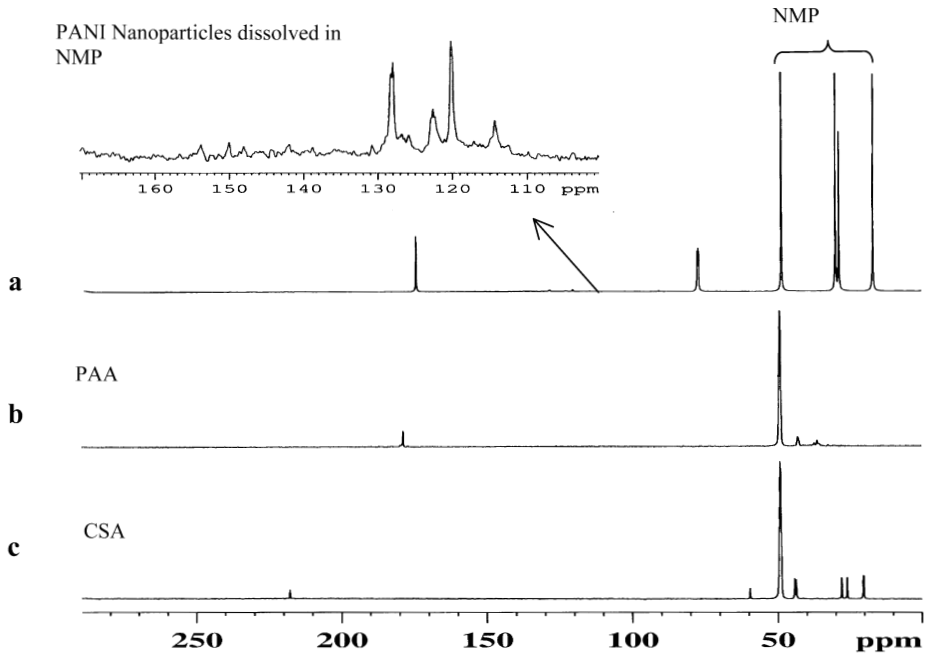

Fig. 5 125 MHz ^{13}C NMR spectrum (NMP solvent used) of PANI nanoparticles obtained after treating nanocomposite with NH_4OH (a); signatures of PAA and CSA are not observed in the Spectrum a [4]. The inset Fig. 5a shows the vertically expanded region of the top spectrum for PANI resonances.

The structural changes as a result of doping–dedoping–redoping of enzymatically synthesized PANI using different biocatalysts have been investigated in depth by solid-state ^{13}C and ^{15}N NMR to correlate structure–conductivity relationship [16]. In addition, the contribution of polarons and bipolarons to the PANI's conductivity is also probed by variable-temperature spin-lattice relaxation T_1 study in the solid state. Heterogeneous and homogeneous line broadenings in the solid-state NMR spectra are differentiated using solid-state spin-echo experiments to understand electron-nuclear interactions by solid-state NMR [16].

In summary, a simple two-step enzymatic approach provides nanomaterials with electro-active properties for their potential use in a wide range of electronic applications.

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REFERENCES

1. A. G. MacDiarmid, L. S. Yang, W. S. Huang, B. D. Humphrey. *Synth. Met.* **18**, 393 (1987).
2. A. G. MacDiarmid and J. A. Epstein. *Synth. Met.* **65**, 103 (1994).
3. M. Thiyagrajan, L. Samuelson, J. Kumar, A. L. Cholli. *J. Am. Chem. Soc.* **125**, 11502 (2003).
4. M. Thiyagarajan, J. Kumar, L. Samuelson, A. L. Cholli. *J. Macromol. Sci., Pure Appl. Chem*. **A40**, 1347 (2003).
5. K. Ryu, J. P. Mc Eldoon, A. R. Pokora, W. Cyrus, J. S. Dordick. *Biotechnol. Bioeng.* **42**, 807 (1993).
6. J. A. Akkara, K. J. Senecal, D. L. Kaplan. *J. Polym. Sci., Polym. Chem*. **29A**, 1561 (1991).
7. S. Kobayashi, H. Uyama, A. Kimura. *Chem. Rev*. **101**, 3793 (2001).
8. H. Uyama and S. Kobayashi. *Chem. Tech.* **22**, 29 (1999).
9. T. Fukuoka, H. Tonami, N. Maruichi, H. Uyama, S. Kobayashi. *Macromolecules* **33**, 9152 (2000).
10. L. A. Samuelson, A. Anagnostopoulos, K. S. Alva, J. Kumar, S. K. Tripathy. *Macromolecules* **31**, 4376 (1999).
11. W. Liu, J. Kumar, S. Tripathy, K. J. Senecal, L. Samuelson. *J. Am. Chem. Soc*. **121**, 71 (1999).
12. W. Liu, A. L. Cholli, R. Nagarajan, J. Kumar, S. Tripathy, F. F. Bruno, L. Samuelson. *J. Am. Chem. Soc.* **121**, 11345 (1999); S. K. Sahoo, W. Liu, A. Samuelson, J. Kumar, A. L. Cholli. *Macromolecules* **35**, 9990 (2002).
13. H. B. Dunford. In *Peroxidases in Chemistry and Biology*, J. Everse, K. E. Everse, M. B. Grisham (Eds.), CRC Press, Boca Raton, FL, **2**, 24 (1991).
14. P. Xu, J. Kumar, L. Samuelson, A. L. Cholli. *Biomacromolecules* **87**, 191 (2002).
15. S. K. Sahoo, W. Liu, L. Samuelson, J. Kumar, A. L. Cholli. In *NMR Spectroscopy of Polymers in Solution and Solid State*, H. N. Cheng and A. D. English (Eds.), pp. 258–269, American Chemical Society, Washington, DC (2002).
16. S. K. Sahoo, R. Nagarajan, S. Roy, L. A. Samuelson, J. Kumar, A. L. Cholli. *Macromolecules* **37**, 4130 (2004).