Macromolecules

Volume 39, Number 25 December 12, 2006

Communications to the Editor

Ionic Liquid Immobilized Enzyme for Biocatalytic Synthesis of Conducting Polyaniline

Valérie Rumbau, Rebeca Marcilla, Estibalitz Ochoteco, Jose A. Pomposo, and David Mecerreyes*

New Materials Department, CIDETEC—Centre for Electrochemical Technologies, Parque Tecnológico de San Sebastián, Paseo Miramón 196, E-20009 Donostia-San Sebastian, Spain

Received May 29, 2006

Revised Manuscript Received October 9, 2006

Polyaniline (PANI) is one of the most popular conducting polymers due to its stability and interesting electrical and optical properties with technological applications in lightweight batteries, microelectronics, electrochromic displays, electromagnetic shielding, organic light emitting diodes, and sensors among others.[1] The use of enzymes as biological catalysts in the synthesis of functional polymers in general[2] and PANI in particular has attracted great interest in recent years. Enzymes can offer environmentally benign reaction conditions,[3] high conformational specificity,[4] a higher degree of control over the kinetics of the reaction, and a high yield of PANI. In the biocatalytic approach, the aniline monomer is coupled together by horseradish peroxidase (HRP) or another peroxidase[5] catalyzed oxidation in the presence of an anionic polyelectrolyte[6] or a micellar template[7] leading to a PANI aqueous dispersion. For practical applications the expensive enzymes must be purified, recovered, and reused after the reaction. This is the main reason behind the well-established strategy of immobilization of enzymes into solid supports which was applied to the HRP enzyme.[8] The goal of this communication is to report a new method which allows to recycle and to reuse the HRP enzyme in the biocatalytic synthesis of PANI. This method is based on a biphasic catalytic system where the enzyme is immobilized into an ionic liquid (IL).

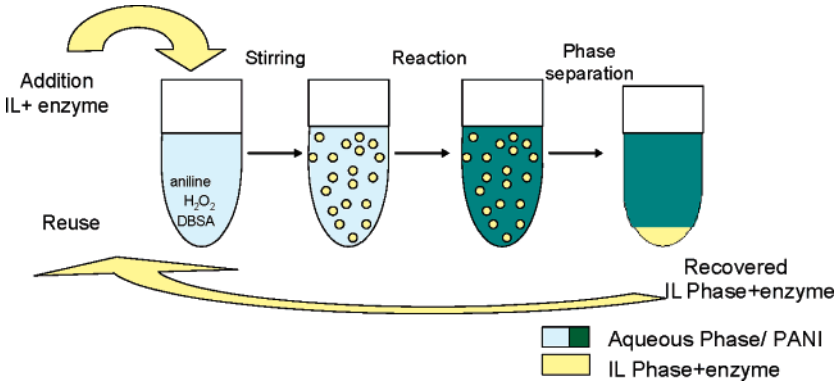
* Corresponding author. E-mail: dmecerreyes@cidetec.es. Telephone: 34943309022. Fax: 34943309136.

Ionic liquids (IL) are organic salts with a low melting point (<100 °C) that are being the focus of many investigations because of their chemical stability, low flammability, negligible vapor pressure, and high ionic conductivity.[9] One of the most interesting properties of ILs is their tunable and unique immiscibility in water and/or organic solvents. Furthermore, IL are known as good solvents for enzymes, showing high activity and stability in these solvents which makes them good candidates as an alternative media for biocatalysis[10] due to the possibilities of dissolving polar compounds. In the particular case of biocatalytic polymerizations, ILs have recently been used as alternative solvents in the enzymatic synthesis of polyesters.[11] As another original example in biocatalysis, Lee et al. reported the use of IL to immobilize a lipase enzyme in enantioselective transesterification reactions.[12] Due to the immiscibility between the IL and the organic solvent, the enzyme could be recovered and reused. Consequently, we thought that room-temperature ILs immiscible with water would be ideal to immobilize HRP enzyme and to be subsequently recovered by liquid/liquid phase separation after the biocatalytic reaction. Our simple idea is depicted in Scheme 1 where the HRP enzyme is immobilized in the IL phase and the other components of the reaction such as aniline monomer, H_2O_2 oxidant, and dodecylbenzene sulfonic acid (DBSA) template in the aqueous phase. By this method, the biocatalytic process should took place at the aqueous/IL interface.

In a first attempt, HRP was immobilized by simple solution in the hydrophobic IL butyl-3-methylimidazolium bistrifluoromethanesulfonimide. Next, the HRP/IL mixture was added to the aqueous solution of aniline, DBSA and H_2O_2 at pH 4.3. Right after the addition of the IL/enzyme, a dark green PANI precipitate appeared blocking the IL/aqueous interface and preventing the polymerization process to proceed quantitatively. This precipitation can be associated with the strong interactions between PANI and the hydrophobic 3-methylimidazolium bistrifluoromethanesulfonimide IL. Next, we repeated the same process dissolving the HRP enzyme in a less hydrophobic IL such as 1-butyl-3-methylimidazolium hexafluorophosphate. In this second case, right after the addition of the IL containing

10.1021/ma061196b CCC: $33.50
Published on Web 11/08/2006

Scheme 1



the immobilized enzyme, the aqueous phase became green which is the typical color of conducting PANI dispersions. After 0.5 h of reaction time, two liquid phases were observed in the reaction. Then, the IL phase was easily extracted from the aqueous phase by liquid/liquid (IL/water) phase separation. The UV−vis spectrum of the obtained aqueous phase was compared to the PANI obtained by classical biocatalytic synthesis dissolving the HRP enzyme in the aqueous reaction media (Figure 1). Both spectra are very similar and clearly showed an absorption peak maximum at 420 nm. Furthermore, a well-known absorption tail extends toward the near-IR region between 800 and 1100 nm due to the polaron transition which is a sign of the formation of conducting PANI.

The question now is whether the enzyme is really immobilized into the IL phase or migrates to the aqueous phase. To answer this question, 6 mg of HRP enzyme were dissolved in the IL leading to a brown solution showing a band in the UV with a maximum at 400 nm. We took 1 mL of this solution mixed with 10 mL of distilled water and stirred for 6 h. After this time, 30% of the enzyme migrated to the aqueous phase as calculated by UV spectroscopy. However, interestingly once the aniline reactant is added, this migration is limited to less than 5% in 6 h which gives an indication that under the reaction conditions the enzyme stays almost quantitatively into the aqueous phase. As a further probe, we carried out the same biocatalytic reaction described before but having an increased aniline/DBSA ratio of 4. It is well-known that using this ratio a solid PANI is obtained. Consequently, after the reaction the solid PANI could be filtered off. Then, the remaining aqueous phase was analyzed by UV spectroscopy. Interestingly, we could not detect any measurable amount of HRP. These two results indicated that under the reaction conditions, in the presence of aniline and for 0.5 h, the enzyme was immobilized inside the IL.

The recovered HRP/IL mixture was added to a new aqueous reaction media, similar to the initial one, including fresh aniline, DBSA and H_2O_2. Again, the aqueous phase became green indicating the synthesis of conducting PANI. After reaction, the HRP/IL phase could be easily recovered again by liquid−liquid phase separation. UV-spectrum of the recovered aqueous PANI in the second run showed a similar spectrum than the first run. The same process was successfully repeated up to 5 times using the same HRP/IL phase demonstrating the validity of our approach and the possibility of recycling and reusing the enzyme inside the IL phase.

To compare the quality of the obtained conducting polymer, Figure 2 shows the electrical conductivity of the PANI films prepared by solvent casting from the aqueous solutions. Interestingly, the electrical conductivity compares favorably with the PANI obtained without immobilization of the enzyme (run 0) and showed a similar high value even after the fifth run. In other words, the quality of the PANI obtained by this new method is similar than the PANI obtained without immobilization of the enzyme or by immobilization of the enzyme in solid supports.[8a] Overall, these results indicate that the enzyme inside the IL phase is easy to recycle and can be reused leading to good quality PANI even after several runs.

In conclusion, a new process of enzymatic route to PANI is presented which allows purifying the obtained conducting polymer aqueous solution from the remaining enzyme. The IL 1-butyl-3-methylimidazolium hexafluorophosphate was used as immobilization media by dissolving the HRP enzyme. The IL/HRP phase acts as an efficient biocatalyst and can be easily recycled and reused several times. This new method is faster

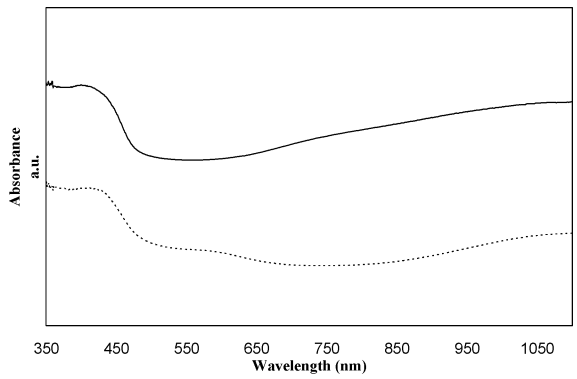


Figure 1. UV−vis spectra of PANI/DBSA aqueous solution obtained without immobilization of the HRP enzyme (upper spectrum) and with immobilization of the HRP enzyme in 1-butyl-3-methylimidazolium hexafluorophosphate (lower spectrum).

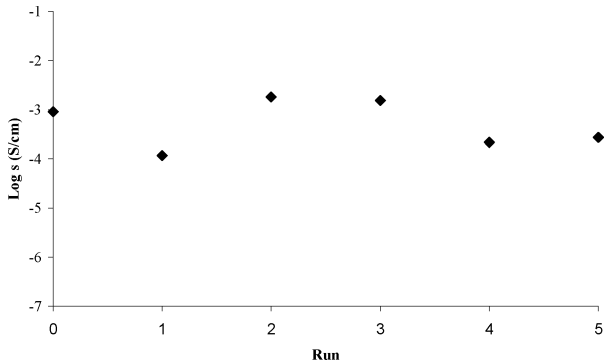


Figure 2. Electrical conductivity films obtained from the aqueous solutions after several runs. Run 0 indicates the control value obtained without encapsulation of the enzyme.

and easier than the classical immobilization of HRP into solid supports.

Experimental Section. Materials. Horseradish peroxidase (HRP, type II, 150−250 units/mg solid), dodecylbenzenesulfonic acid sodium salt (97%), and aniline monomer (purity more 99%) were purchased from Sigma-Aldrich. Hydrogen peroxide (30 wt %) and hydrochloric acid (1 N) were obtained from Quimibacter S.L. 1-Butyl-3-methylimidazolium hexafluorophosphate and 1-butyl-3-methylimidazolium bistrifluoromethanesulfonimide ILs were purchased from Solvionic S.A.

Characterization Methods. The electronic spectra were recorded on a UV−visible spectrometer (UV-1603 SHIMADZU). PANI films were obtained by casting and drying at 60 °C in a vacuum for 24 h. The electric conductivity of PANI was measured with a four-probe instrument.

Biphasic Enzymatic Polymerization of Aniline. At first, we dissolved the HRP enzyme into the IL (6 mg into 1 mL). This IL was added to 10 mL pH 4.3 aqueous solution of aniline, the template dodecylbenzenesulfonic acid (DBSA) and hydrogen peroxidase. In a typical polymerization experiment, equimolar quantities (typically 0.055 M) of the template, aniline, and hydrogen peroxide were dissolved. Reaction was carried out at 20 °C for 0.5 h. After reaction, the IL phase was separated from the aqueous phase by liquid/liquid pour off in order to purify the PANI aqueous solution and to recuperate the HRP/IL phase.

Acknowledgment. This work was carried out under a Marie Curie Research Training Network (BIOMADE, Project 505147).

References and Notes

(1) Shimano, J. Y.; MacDiarmid, A. G. *Synth. Met.* **2001**, *123*, 251.
(2) (a) Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, *101*, 3793. (b) Gross, R.; Cheng, H. N. *Biocatalysis in Polymer Science*; ACS Symposium Series 340; American Chemical Society: Washington, DC, 2003.
(3) (a) Liu, W.; Kumar, J.; Tripathy, S.; Senecal, K. J.; Samuelson, L. *J. Am. Chem. Soc.* **1999**, *121*, 71−78. (b) Cruz-Silva, R.; Romero-García, J.; Angulo-Sanchez, J.; Ledezma-Perez, J.; Arias-Martín, E.; Moggio, I.; Flores-Loyola, E. *Eur. Polym. J.* **2005**, *41*, 1129.
(4) (a) Lim, C. H.; Yoo, Y. J. *Proc. Biochem.* **2000**, *36*, 233. (b) Thiyagarajan, M.; Samuelson, L. A.; Kumar, J.; Cholli, A. *J. Am. Chem. Soc.* **2003**, *125*, 11502.
(5) (a) Yu, I.; Sakharov, A.; Vorobiev, Ch.; Castillo Leon, J. J. *Enzymol. Micr. Tech.* **2003**, *33*, 661. (b) Karamyshev, A. V.; Shleev, S. V.; Koroleva, O. V.; Yaropolov, A.; Sakharov, A. *Enzymol. Micr. Technol.* **2003**, *33*, 556.
(6) Nagarajan, R.; Tripathy, S.; Kumar, J.; Bruno, F. F.; Samuelson, L.; *Macromolecules* **2000**, *33*, 9542.
(7) (a) Liu, W.; Cholli, A. L.; Nagarajan, R.; Kumar, J.; Tripathy, S.; Bruno, F. F.; Samuelson, L. *J. Am. Chem. Soc.* **1999**, *121*, 11345. (b) Liu, W.; Kumar, J.; Tripathy, S. *Langmuir* **2002**, *18*, 9696. (c) Sahoo, S. K.; Nagarajan, R.; Roy, S.; Samuelson, L. A.; Kumar, J.; Cholli, A. L.; *Macromolecules* **2004**, *37*, 4130.
(8) (a) Jim, Z.; Su, Y.; Duan, Y. *Synth. Met.* **2001**, *122*, 237. (b) Fernandes, K. F.; Lima, C. S.; Pinho, H.; Collins, C. H. *Proc. Biochem.* **2003**, *38*, 1739. (c) Fernandes, K. F.; Lima, C. S.; Lopes, F. M.; Collins, C. H. *Proc. Biochem.* **2004**, *39*, 957. (d) Azevedo, A. M.; Vojinovic, V.; Cabral, J. M. S.; Gibson, T. D.; Fonseca, L. P. *J. Mol. Catal. B: Enzymol.* **2004**, *28*, 121. (e) Sun, D.; Cai, C.; Li, X.; Xing, W.; Lu, T. *J. Electroanal. Chem.* **2004**, *566*, 415. (f) Moeder, M.; Martin, C.; Koeller, G. *J. Membr. Sci.* **2004**, *245*, 183.
(9) Rogers, R. D.; Seddon, K. R.; Volkov, S. In *Green Industrial Applications of Ionic Liquids*; Nato Science Series; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2002.
(10) Park, S.; Kazlauskas, R. J. *Curr. Opin. Biotechnol.* **2003**, *37*, 8793.
(11) (a) Uyama, S. J.; Takamoto, Kobayashi, S. *Polym. J.* **2004**, *40*, 2065. (b) Nara, S. J.; Harjani, J. R.; Salunkhe, M. M.; Mane, A. T.; Wadgaonkar, P. P. *Tetrahedron Lett.* **2003**, *44*, 1371. (c) Marcilla, R.; De Jeus, M.; Mecerreyes, D.; Duxbury, C. J.; Koning, C. E.; Heise, A. *Eur. Polym. J.* **2006**, *6*, 1215.
(12) Lee, J. K.; Kim, M. J. *J. Org. Chem.* **2002**, *67*, 6845.

MA061196B