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Synthesis of water-soluble poly(*o*-methoxyaniline) by enzyme HRP

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Abstract: The poly(*o*-methoxyaniline) was synthesized by enzymatically oxidative polymerization of *o*-methoxyaniline using poly(sodium 4-styrene sulphonate) (SPS) as a template. The enzyme horseradish peroxidase(HRP) was used as a catalyst with a stoichiometric amount of monomer and hydrogen peroxide as an oxidant at pH 4.3 in 0.01 M sodium phosphate buffer solution medium. The formation of water-soluble poly(*o*-methoxyaniline) was characterized by UV–vis. The conducting structure of synthesized poly(*o*-methoxyaniline) complex with SPS was confirmed by FT-IR spectroscopy.This biomimetic approach offered an unsurpassed easiness in the synthesis, processability, stability and environmental compatibility.

Keywords: *O*-methoxyaniline; oxidative polymerization; enzyme HRP; water-soluble polymer.

INTRODUCTION

Polyaniline (PANI) is an intrinsically conducting polymer (ICP) that has become an appealing target of research because of its facile synthesis, having a low cost monomer and good environmental stability [1]. The main disadvantage that restricts the industrial scale application of PANI is its lack of processability, which is associated with its insolubility and infusibility, especially in its conducting form [2]. For this reason, homopolymers of substituted anilines, or aniline derivatives, such as methoxyaniline [3], ethoxyaniline [4], and methylaniline [5] are explored. Among the substituted polyaniline derivatives, Poly(o-methoxyaniline) can be easily synthesized either chemically or electro-chemically, with higher processability and solubility compared to PANI [6]. In the recent years, electrochemical polymerization of poly(o-methoxyaniline) on various substrates such as stainless steel [7], copper [8], α-iron oxide [9] and brass [10], have been investigated, mostly with the target application of a corrosion resistor. However electrochemical polymerization has some restrictions such that poly(o-methoxyaniline) layers cannot be obtained on insulating surfaces

and is less industrially favorable compared with chemical polymerization [11].

However, enzymatic polymerization provides an alternative method of a "green process", which is usually carried out at room temperature, in aqueous organic solvents around neutral pH [12].

The enzyme horseradish peroxidase (HRP), as shown in Figure 1, in the presence of hydrogen peroxide catalyzes the polymerization of phenol and aromatic amines[13]. Peroxidase-catalyzed synthesis of polyphenol and polyaromatics involves a reaction mechanism that results in a direct ring-to-ring coupling of phenol and aniline monomers [14].Dordick and coworkers reported the enzymatic synthesis of polyphenols using horseradish peroxidase (HRP) for the first time [15].The aniline monomers have been enzymatically polymerized to yield a wide range of soluble polyanilines [16].

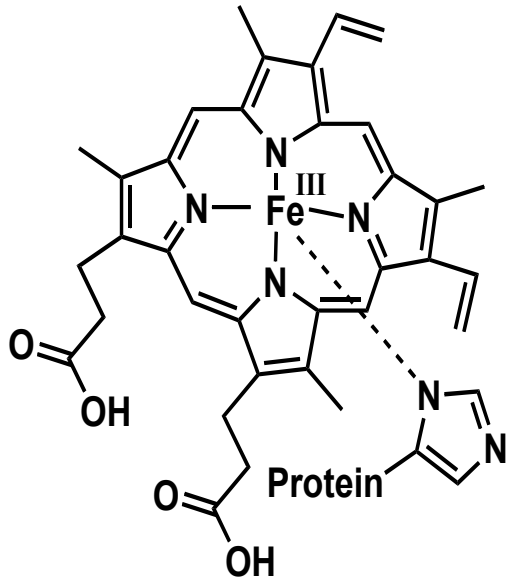

Figure1. The structure of active site of HRP

Since then, a variety of modified enzymatic reactions have been investigated to optimize these reactions for polyphenol and polyaromatic synthesis [17-19].

The conductivity of polyaniline is related with the backbone structure of polyaniline [20]. The branched structure lowered the conductivity [21], while a linear structure with the help of a template like SPS led to the conducting materials [22].

This paper describes an enzymatic polymerization of *o*-methoxyaniline in presence of an anionic polyelectrolyte, SPS as a template. The reaction was carried out in mild condition pH 4.3 buffer aqueous solution. The final product was a water-soluble poly(*o*-methoxyaniline)/ SPS complex. The details of the synthesis and characterization of this simple, cost-effective and environmentally compatible method are presented.

MATERIALS AND METHODS

Materials

Poly(sodium 4-styrene sulphonate) (MW of 70000), used in this study was purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without any further purification. HRP (EC 1.11.1.7) (about 170 units/mg), hydrogen peroxide (H_2O_2) (30 wt%), *o*-methoxyanilinewas obtained from Merck.

Polymer Synthesis

A procedure for the preparation of the poly(*o*-methoxyaniline) is as follows:

Typically, in 1:1 molar ratio of *o*-methoxyaniline (0.023 mmol and SPS (0.023 mmol) (based on monomer repeat unit) were added to 7.5 mL 0.01 M sodium phosphate buffer solution (pH 4.3) at room temperature under constant stirring. The mixing was followed by the addition of a catalytic amount of the enzyme (1 mg HRP). To initiate the reaction, 2.3 mL of diluted hydrogenperoxide (0.02 M) was added dropwise under vigorous stirring over a period

of 1 h and immediately after the first few drops, the reaction solution turned bluish-violet. The reaction was then left to stir 24 h at the ambient temperature. The resulting dark violet solution was transferred to a cellulose tube and dialyzed (cut off molecular 3000) overnight to remove any unreacted monomers and oligomers.

RESULT AND DISCUSSION

After the addition of the first drop of hydrogen peroxide, the reaction media of *o*-methoxyaniline developed a violet colour within several seconds indicating a very fast *o*-methoxyaniline oxidation without induction period.The poly(*o*-methoxyaniline) in complex with SPS exhibits nominal structure that is shown in Figure 2.

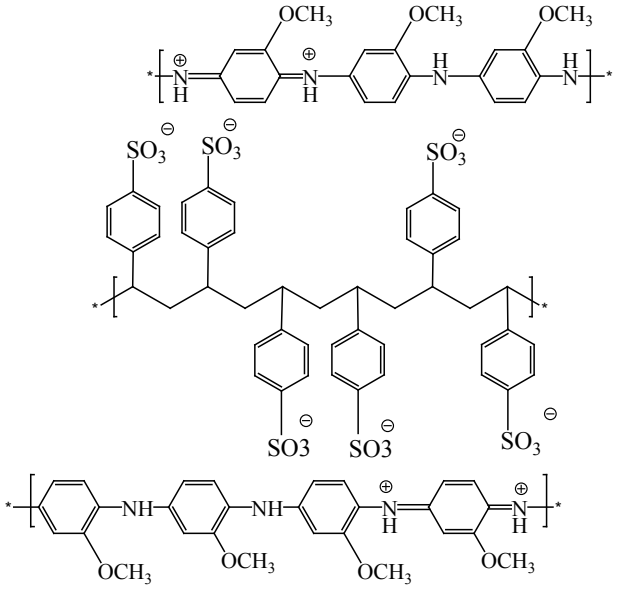

Figure 2. The structure of poly(*o*-methoxyaniline)/SPS complex.

Catalytic mechanism of HRP

HRP is classified as an oxidoreductase due to the cyclic reduction and oxidation of the heme group which gives rise to its enzymatic activity. In brief, HRP reduces hydrogen peroxide to form a complex which can oxidize a variety of organic and inorganic substrates [23].

HRP catalyzedthe oxidation reaction of *o*-methoxyanilinewith H_2O_2 in phosphate buffer solution. The processes of the enzyme-catalyzed reaction can be expressed as Figure 3 [24]. The catalytic mechanism of HRP can be seen as two one-electron reduction steps that generate radical species. The generation of radical species creates a complex profile of reaction *o*-methoxyaniline, resulting in the *o*-methoxyaniline cation radical. This cation radical attacks other cation radicals of the monomer to form a dimmer, with elimination of two protons [25,26].Primary dimer can also act as peroxidase substrates which in turn produce additional radical species that take part in further coupling reactions. As a result, the oxidation of *o*-methoxyaniline by HRP produces poly(*o*-methoxyaniline) [27].

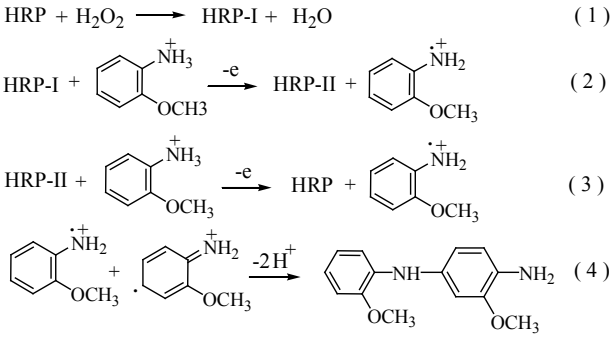

Figure 3.The catalytical cycle of HRP in reaction with *o*-methoxyaniline. HRP-I and HRP-II are the intermediates of HRP.

UV-vis Spectroscopy

UV–vis spectrum of the poly (*o*-methoxyaniline)/ SPS complex were obtained in 0.1M sodium phosphate buffer at pH4.3 is shown in Figure 4. The peak at 320 nm is due to the π–π* transition of the benzenoid rings of poly(*o*-methoxyaniline)[28]. The characteristic peaks at 580 and 760 nm

were assigned to polaron bands and confirmed the presence of a conductive form of poly(*o*-methoxyaniline). Because of SPS contained an electron-withdrawing sulfonic group in the complex, the polaron band appeared below 800 nm [29]. As it is shown in this figure, the UV-vis spectrum of polyaniline is exactly the same as previous reports [30].

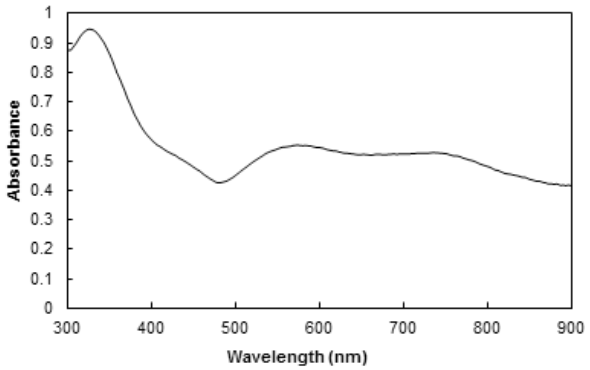

Figure 4.UV-Vis spectra of enzymatically synthesized poly(*o*-methoxyaniline)

FT-IR Spectroscopy

The FT-IR spectra of poly(*o*-methoxyaniline)/SPS complexes are presented in Figure 5. The characteristic peaks of poly(*o*-methoxyaniline) at 1590 and 1510 cm^{-1} are assigned to C=C stretching vibrations of the quinoid and benzoid rings, respectively [31]. The peak at 1284cm^{-1} is assigned to the $C-N^+$ stretching in the polaron structure of poly(*o*-methoxyaniline) [32]. The peak at 1214 cm^{-1} is assigned to secondary aromatic amine stretching [33]. Two other peaks at 835 and 758 cm^{-1} are related to the CH out-of-plane bending of 1, 2, 4-trisubstituted benzene ring [34]. Also, the peaks observed at 1005 and 1035 cm^{-1}, corresponding to symmetric and asymmetric S=O stretching, confirm the presence of SPS in the complex [35]. These observations indicate that poly(*o*-methoxyaniline)/SPS complexes consisting mainly head-to-tail coupling.

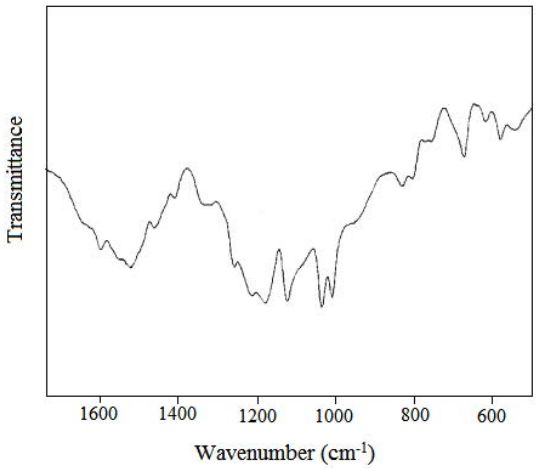

Figure 5. FTIR spectrum of the poly(*o*-methoxyaniline)/SPS complex synthesized with the HRP catalyst.

CONCLUSION

The *o*-methoxyaniline was polymerized in the ambient condition at pH 4.3 by enzyme HRP as a catalyst in the presence of SPS as a template. UV–vis and FT-IR indicated that the conducting form of poly(*o*-methoxyaniline) was synthesized with HRP. This method is simple and economic for the preparation of water-soluble conducting poly(*o*-methoxyaniline).

REFERENCES

1. Y. Liao, C. Zhang, Y. Zhang, V. Strong, J. Tang, X.-G. Li, K. Kalantar-zadeh, E.M.V. Hoek, K.L. Wang, R.B. Kaner, Nano Lett., 2011, 11, 954–959.
2. W.A. Gazotti Jr., G. Casalbore-Miceli, S. Mitzakoff, A. Geri, M.C. Gallazzi, M.A. De Paoli, Electrochim. Acta, 1999, 44, 1965–1971.
3. V. Cherpak, P. Stakhira, Z. Hotra, O. Aksimentyeva, B. Tsizh, D. Volynyuk, I. Bordun, J. Non-Cryst. Solids, 2008, 354, 4282-4286.
4. W.F. Alves, E.C. Venancio, F.L. Leite, D.H.F. Kanda, L.F. Malmonge, J.A. Malmonge, L.H.C. Mattoso, Thermochim. Acta, 2010, 502, 43-46.
5. S. Cattarin, L. Doubova, G. Mengoli, G. Zotti, Electrochim. Acta, 1988, 33, 1077- 1084.
6. J. Jiang, L.-H. Ai, A.-H. Liu, Synth. Met., 2010,160, 333-336.
7. P.A. Kilmartin, L. Trier, G.A. Wright, Synth. Met., 2002, 131, 99-109.

8. P. Pawar, A.B. Gaikwad, P.P. Patil, Electrochim. Acta, 2007, 52, 5958-5967.
9. S. Patil, M.A. More, R.B. Gore, S.V. Rao, P.P. Patil, Mater. Sci. Eng.,B, 1999, 65, 145-149.
10. S. Chaudhari, A.B. Gaikwad, P.P. Patil, Curr. Appl Phys., 2009, 9, 206-218.
11. S.Tanwar,J. A. A. Ho, Molecules, 2015, 20(10), 18585-18596.
12. P.Xu,A.Singh, D. L. Kaplan, Adv. Polym. Sci., 2006, 194, 69−94.
13. M. R. Nabid, Z. Zamiraei, R. Sedghi,Iran. Polym. J., 2010, 19(9), 699–706.
14. J. A. Akkara, K. J. Senecal, D. L. Kapalan, J. Polym. Sci. Part A: Polym. Chem., 1991, 29, 1561-1574.
15. J. S. Dordick, M. A.Marletta, A. M. Klibanov, Biotechnol. Bioeng., 1987, 30, 31-36.
16. K. S. Alva, J. Kumar, K. A. Marx, S. K. Tripathy, Macromolecules, 1997, 30, 4024-4029.
17. W. Liu, J. Kumar, S. K. Tripathy, K. J. Senecal, L.A. Samuelson, J. Am. Chem. Soc., 1999, 121(1), 71-78.
18. W. Liu, J. Kumar, S. K. Tripathy. Langmuir, 2002, 18, 9696-9704.
19. R. Ikeda, H. Uyama, S. Kobayashi, Macromolecules, 1996, 29, 3053-3054.
20. S. K.Sahoo, R.Nagarajan, L.Samuelson, J.Kumar, A. L.Cholli, S. K. Tripathy, J. Macromol. Sci., Part A: Pure Appl.Chem., 2001, 38, 1315−1328.
21. K. S.Alva, J.Kumar, K. A.Marx,S. K.Tripathy, Macromolecules, 1997, 30, 4024− 4029.
22. W.Liu, A. L.Cholli, R.Nagarajan, J.Kumar,S.Tripathy, F. F.Bruno, L. Samuelson, J. Am. Chem. Soc., 1999, 121(49), 11345−11355.
23. N. C. Veitch, Phytochemistry, 2004, 65, 249-259.
24. J. N. Rodriguez-Lopez, D. J. Lowe, J. Hernández-Ruiz, A. N. Hiner, F. Garcia-Cánovas, R. N. Thorneley, J. Am. Chem. Soc., 2001, 123, 11838-11847.
25. Sh. Zhang, K. Jiao, H. Chen, Electroanalysis, 1999, 11, 511-516.
26. M. R. Nabid, Z. Zamiraei, R. Sedghi, N. Safari, React. Funct. Polym., 2009, 69, 319-324.
27. Z. Zamiraei, M. R. Nabid, Chem. Biol. Interface, 2015, 5(2), 151-156.
28. M. R. Nabid, R. Sedghi, P. R. Jamaat, N. Safari, A. A. Entezami, J. Appl. Polym. Sci., 2006, 102, 2929–2934.
29. S.E. Bourdo, B.C. Berry, T. Viswanathan, J. Appl. Polym. Sci., 2005, 98, 29–33.
30. C.DeArmitt, S. P.Armes, J. Winter, F. A.Urbe, S.Gottesfeld, C.Mombourquette, Polymer, 1993, 34, 158-162.
31. Z. Yang, X.Wang, Y. Yang, Y. Liao, Y.Wei, X. Xie, Langmuir, 2010, 26, 9386–9392.
32. Q. Lue, C. Wang, X. Cheng, Microchim. Acta, 2010, 169, 233–239.
33. L. Zhang, H. Peng, J. Sui, C. Soeller, P.A. Kilmartin, J. Travas-Sejdic, J. Phys. Chem. C, 2009, 113, 9128–9134.
34. X. Wanga, S. Ray, R. P. Cooneya, P. A. Kilmartina, G.I.N. Waterhouse, A. J. Easteal, Synth. Met., 2012, 162, 1084–1089.
35. M. R. Nabid, R. Sedghi,A.A. Entezami, J. Appl. Polym. Sci., 2007, 103, 3724–3729.