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Conducting Polymer-Silica Composites for Immobilization of Enzymes

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A new enzyme immobilization method based on hydrophobic interaction between supporting material and enzyme has been successfully developed. The efficacy of the new technique has been investigated by loading a horse radish peroxidase (HRP) enzyme on the surface of conducting polymer-silica composites and by measuring the enzyme activity and leaching property of HRP loaded within polymer-silica composites. The immobilized HRP enzyme showed activity profiles similar to that of free HRP in phosphate buffer (pH 6). Above all, HRP adsorbed on the polymer-silica composites has showed excellent stability over 10 days, compared to HRP adsorbed on the pristine silica. It is thought that with appropriate optimization works, the present method would be used as a cost-effective and facile route for the immobilization of biomolecules.

Key Words : Polymer-silica composites, Immobilization, Biomolecules

Introduction

Since the discovery of mesoporous silica by Mobile researchers, a number of mesoporous materials have been synthesized and applied in many areas, such as adsorption, host-guest complexes, separation, energy materials, and catalysis.[1-7] To endow them with new physicochemical properties, many silica composites have been synthesized.[4-13] Organic functional groups have been introduced onto the surface of silica through various synthetic routes.[8-10] On the other hand, polymer-based composites have been prepared by free radical or oxidative polymerization within mesoporous materials.[11-13] In many cases, however, the resulting materials showed poor mesoporous properties due to pore filling by polymer. To achieve uniform polymerization, Ryoo and co-worker reported a new method toward silica composites possessing accessible mesopores.[14] They presented new scientific possibilities for the application of mesoporous materials in that the location of polymers in mesoporous silica can be systematically controlled with structures of the silica framework and the polymerization conditions. Especially, the size of complementary pores of silica pore walls proved to be critical for the formation of thin polymers onto the internal surface of silica without causing pore blockage. In general, synthesis of polymer-silica composites proceeded in a two-step process including impregnation of reactants into mesoporous materials and polymerization within mesoporous materials by radicals or oxidizing agents.

Recently, a novel one-pot synthetic route toward high quality polymer-silica composites, which can be applied to all silica materials regardless of the structure of complementary pores of silica walls, has been reported.[15] It is thought that the polymerization method would provide new possibilities for many application areas due to the following unique aspects. First, polymerization confined on the surface with acidic proton occurs, resulting in locating polymers in the specific sites. This makes it possible to do a fine tuning of the pore size of mesoporous materials, maintaining well-defined mesoporosity. Second, degree of polymerization and amount of polymers produced are controlled by the surface concentration of acidic protons on the surface (*e.g.* silanol). Third, a wide variety of conducting polymer-based composites can be fabricated through a facile surface modification of solid materials with acidic group (*e.g.* inorganic oxides, CNT derivatives with acidic group).

In this study, we immobilized a horse radish peroxidase (HRP) enzyme in the surface of polymer-silica composites and examined the enzyme leaching property in order to demonstrate the usefulness of the new immobilization method.

Experiment Details

Mesoporous silica/polypyrrole (ppy) composites were prepared through the selective polymerization method described elesewhere.[15] Mesoporous silica (MSU-H) and pyrrole were used as received from Aldrich. First, 200 mg of silica was dispersed in 100 mL distilled water by stirring for two hours and 0.7 mL pyrrole was added. For the adsorption of ppy in the mesopores, the resulting suspension was stirred for several hours and 0.69 g of $NaNO_2$ was added to initiate polymerization. After one day of stirring, the ppy/MSU-H composites were obtained by filtering the solution using membrane filter with 0.2 micron pore size. For purification, the composites were re-dispersed in 100 mL triple distilled water and filtered. The products were dried under vacuum at 50 °C for 72 hours for their characterization.

Horseradish peroxidase (HRP, Sigma P8250) provided by Sigma was used as an enzyme to be immobilzed on the surface of ppy/MSU-H composites. HRP-immobilized ppy/MSU-H composites were prepared by adding HRP to an aqueous solution containing the ppy/MSU-H composites at room temperature. 30 mL HRP (30 mg of HRP) was added to 50 mL aqueous solution containing the resulting ppy/MSU-H and stirred 1 day. The HRP-adsorbed ppy/MSU-H composites were collected by filtering the solution using membrane filter (pore size = 0.2 μm) and washing with triple distilled water three times. The products were finally dispersed in 0.1 M phosphate buffer solution (pH 6.0). For control experiments, HRP adsorbed silica (HRP-silica) was prepared by adding HRP to silica-dispersed solution. The catalytic activity of HRP was determined by measuring the conversion rate of pyrogallol to purpurogallin in the presence of hydrogen peroxide.[16] Briefly, 0.1 mL enzyme solution was added to 2.9 mL mixture solution which consists of 0.1M phosphate buffer (pH 6, 0.32 mL), 5% pyrogallol solution (0.32 mL), 0.5% H_2O_2 solution (0.16 mL) and H_2O (2.1 mL). The increase in absorbance of purpurogallin at 420 nm was recorded immediately after adding H_2O_2 solution at 20 °C for 5 minutes. The change in absorbance per 20 seconds (ΔA_{420nm}/20 sec) over the maximum linear portion of the curve was used to calculate the activity of the enzyme. The enzyme concentration was modified so that the value of ΔA_{420nm}/20 sec is in the range of 0.16 to 0.28.

In order to examine the reactivity of a substrate to HRP enzyme immobilized on the ppy/MSU-H composites, absorbance change with reaction time was recorded at 420 nm. For control experiments, free HRP in phosphate buffer solution was used. Then, enzyme leaching properties of both HRP-loaded MSU-H and HRP-loaded ppy/MSU-H (HRP-ppy/MSU-H) were determined by measuring the activity of enzyme released from silica or ppy-silica with time using UV spectrometer.

The mesoporous silica (MSU-H), ppy/MSU-H and HRP-ppy/MSU-H were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), nitrogen sorption experiments and thermogravimetric analysis (TGA). SEM studies were performed using a FE-SEM (JEOL JSM-7500F). XRD patterns were recorded using a Rigaku D/Max 2500 diffractometer equipped with a Cu K_α radiation. The nitrogen sorption isotherms were obtained using a Micromeritics TriStar 3000 at liquid N_2 temperature. Thermogravimetric analysis was done using a TA Instruments-SDT 2960 with a heating rate of 10 °C min^{-1}.

Results and Discussion

Scanning electron microscopy (SEM) investigation showed similar images before and after polymerization (no apparent difference) as shown Figure 1, implying that bulk polymers were not formed on the external surface of the silica particles. As seen in the XRD patterns for the silica and the ppy/silica composites, the intensity of the (100) reflection peaks from the two-dimensional hexagonal meso-

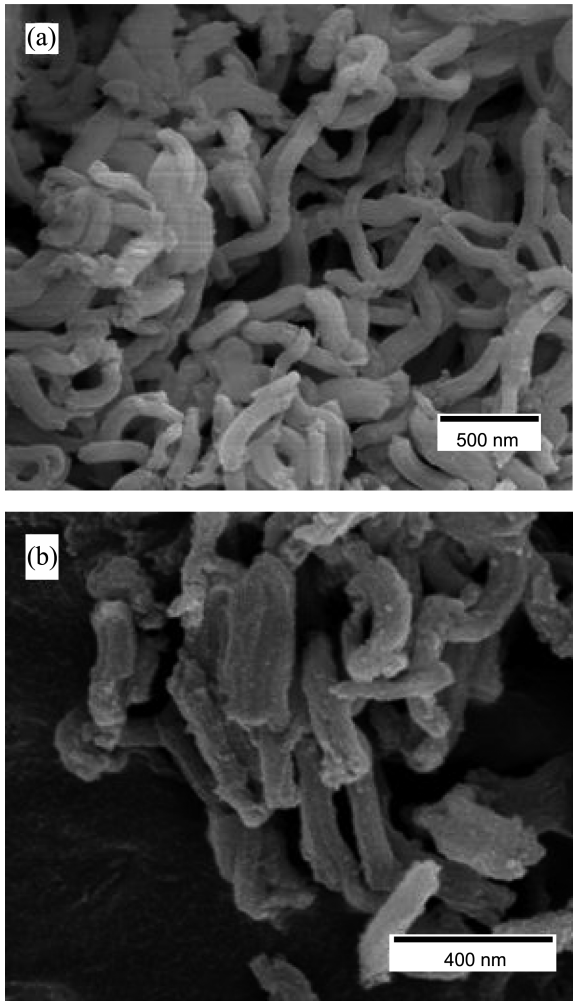

Figure 1. SEM images for MSU-H (a) and ppy/MSU-H composites (b).

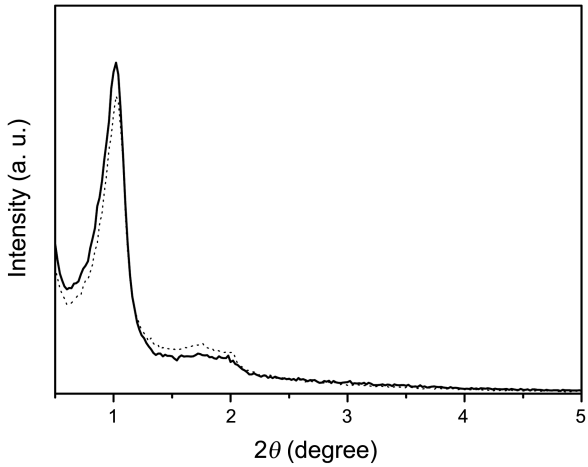

Figure 2. XRD patterns for MSU-H (—) and ppy/MSU-H composites (····).

phase were very similar to each other (Fig. 2), indicating that ppy/MSU-H composites maintained mesoporosity of silica after polymer incorporation. Generally, it has been well known that the intensity of the (100) reflection decreases in case of random or nonselective incorporation causing meso-

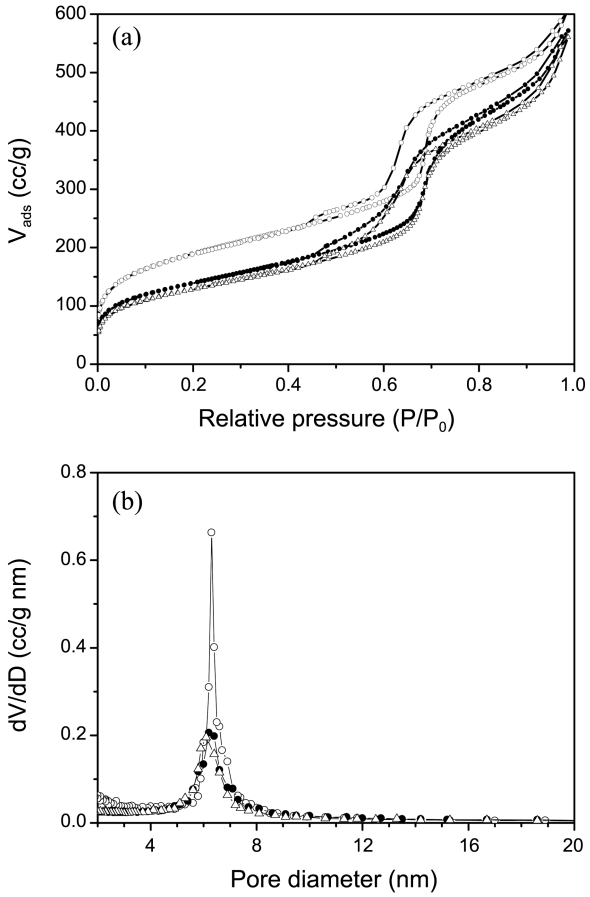

Figure 3. (a) Nitrogen adsorption and desorption isotherms and (b) pore size distribution curves of MSU-H (○), ppy/MSU-H composites (●) and HRP-loaded ppy/MSU-H composites (△).

pore filling in the interior of mesopores.[14] Structural changes of the silica after polymerization were revealed by N_2 adsorption/desorption experiments. The ppy-silica composite exhibited type IV isotherm with a narrow H1-type hysteresis loop (Fig. 3(a)), which is characteristic of mesoporous materials according to the IUPAC nomenclature, similar to that of the pristine silica. It is generally accepted that such an H1-type hysteresis loop with sharp change around P/P_0 = 0.6-0.7 is observed in uniform mesopores with open cylindrical geometry.[14,17] This indicates that the cylindrical mesopore walls of the silica were uniformly coated with ppy. It is thought that electrostatic interaction between ppy with positive charge and silica walls with negative charge greatly contribute to the formation of the stable ppy coating layer. The pore size distribution curves of the silica, determined by BJH analysis, showed two kinds of mesopores: large pores (4-10 nm) of the cylindrical channels and small pores (< 4 nm) of the pore walls that interconnect adjacent mesoporous channels (Fig. 3(b)).

After polymer incorporation, differential pore volume was decreased greatly in the both pores but median pore size was slightly changed. The detailed structural parameters of the materials are listed in Table 1. Interestingly, total pore volume slightly decreased from 0.94 to 0.88 mL/g even though BET surface areas significantly decreased from 641 to 472 m^2/g. Overall results indicate that the mesopore walls of the silica (MSU-H) was quite uniformly deposited with ppy, maintaining mesoporous structure (> 4 nm), which would be crucial for providing much more easily accessible catalytic sites. Polymer content of the ppy-silica composite determined by thermogravimetric analysis (TGA) was about 9.0 wt %, which means that very small portion of the monomers (< 2%) was converted to polymers (Fig. 4). HRP enzyme content incorporated within ppy/MSU-H composites, estimated based on TGA data, was 3.4 wt %.

Table 1. Structural parameters for MSU-H, ppy/MSU-H composites and HRP-loaded ppy/MSU-H composites

Materials	S_{BET}[a]/$m^2\ g^{-1}$	D_{BJH}[b]/nm	V_{tot}[c]/$cm^3\ g^{-1}$
MSU-H	641	6.3	0.94
ppy/MSU-H	472	6.2	0.88
HRP-ppy/MSU-H	440	6.1	0.86

[a]BET surface area calculated in the range of relative pressure (P/P_0) = 0.05-0.20. [b]Mesopore diameter calculated from the N_2 adsorption branches using the BJH method. [c]Total pore volume measured at P/P_0 = 0.99.

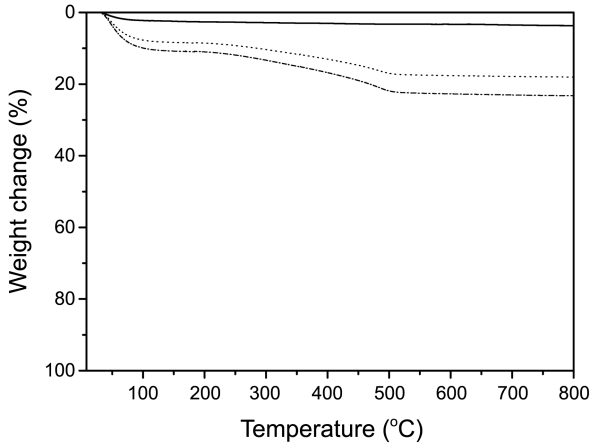

Figure 4. TGA curves for MSU-H (—), ppy/MSU-H composites (····) and HRP-loaded ppy/MSU-H composites (–··–).

Enzyme immobilization studies have attracted worldwide interest due to many benefits of enzyme immobilized on a solid support in terms of enhanced stability, easy separation, reusability and protection of products from enzyme contamination.[18] So far, various methods have been presented to immobilize enzymes, which can be classified to the following three types: first, carrier binding through physical adsorption, ionic bonding and covalent bonding; second, cross linking based on the formation of covalent bonds between enzyme molecules; finally, physical entrapment within polymer matrix and semi-permeable membrane.[18] Physical adsorption method has a drawback in that enzymes become rapidly desorbed from the support when enzyme-loaded samples are dispersed in solution.

To overcome these issues, in this work, enzyme was immobilized on the surface of polymer-silica composites. N_2 isotherm results for HRP/ppy-silica show that the materials still maintained mesoporosity of the ppy-silica, indicating

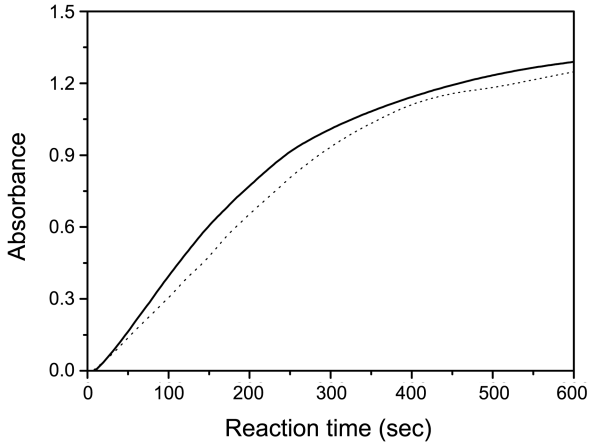

Figure 5. Absorbance change with reaction time at 420 nm: free HRP in phosphate buffer (—) and HRP-loaded ppy/MSU-H composites (····).

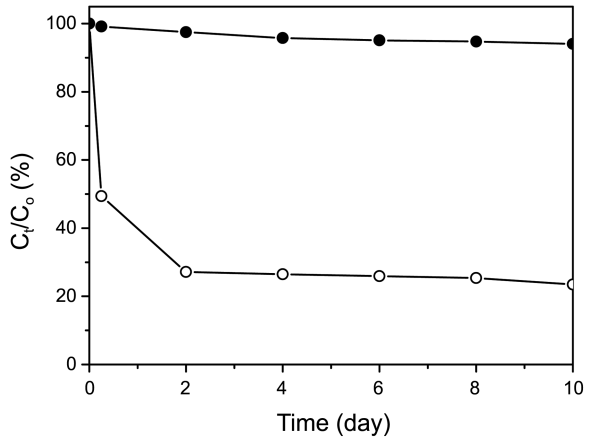

Figure 6. HRP leaching properties of HRP-loaded MSU-H (○) and HRP-loaded ppy/MSU-H composites (●). C_t/C_0 means the enzyme content remaining in the host structure at time t.

that HRP exists on the outer surface of the ppy-silica. Free HRP and the HRP-loaded ppy/MSU-H showed very similar absorbance changes of purpurogallin at 420 nm with reaction time (Fig. 5), indicating that catalytic reaction of HRP immobilized in ppy-silica is as fast as that of free HRP in solution and the catalytic sites of enzymes are exposed.

The ppy-silica exhibited a dramatic increase in HRP loading stability, compared to that of the silica as shown in Figure 6. Less than 6% enzyme was released out of the ppy-silica composite over 10 days. On the other hand, the enzyme leaching of about 75% occurred in the pristine silica. It is thought that the strong hydrophobic interaction between HRP enzyme and ppy coated on the mesoporous silica contributed to the significantly enhanced stability.

Conclusion

A simple and effective method to immobilize enzymes has been developed by placing polymer onto the surface of mesoporous silica (MSU-H). Here, polypyrrole (ppy) was uniformly coated on the mesopore surface by selective polymerization method. The immobilized HRP enzyme showed high activity profiles with reaction time similar to that of free HRP in phosphate buffer (pH 6). Moreover, HRP enzymes adsorbed in polymer-silica composite compared to HRP loaded on silica have showed the remarkably enhanced stability that less than 6% enzyme was released over 10 days. It is thought that the high stability is caused by the strong hydrophobic interaction formed between HRP enzyme and ppy coated on the mesoporous silica. We believe that the present approach would be used as a simple and facile route for the immobilization of biomolecules.

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References and Notes

1. Thomas, J. M.; Raja, R. *Acc. Chem. Res.* **2008**, *41*, 708.
2. Melde, B. J.; Johnson, B. J.; Charles, P. T. *Sensors* **2008**, *8*, 5202.
3. Vallet-Regi, M.; Balas, F.; Arcos, D. *Angew. Chem. Int. Ed.* **2007**, *46*, 7548.
4. Lee, H. I.; Kim, J. H.; Kim, J. M.; Kim, S.; Park, J.-N.; Hwang, J. S.; Yeon, J.-W.; Jung, Y. *J. Nanosci. Nanotechnol.* **2010**, *10*, 217.
5. Ahuja, T.; Mir, I. A.; Kumar, D.; Rajesh. *Biomater.* **2007**, *28*, 791.
6. Vinu, A.; Miyahara, T. M.; Ariga, K. *J. Nanosci. Nanotechnol.* **2006**, *6*, 1510.
7. Jung, Y.; Lee, H. I.; Kim, J. H.; Yun, M.-H.; Hwang, J.; Ahn, D.-H.; Park, J.-N.; Boo, J.-H.; Choi, K.-S.; Kim, J. M. *J. Mater. Chem.* **2010**, *20*, 4663.
8. Melde, B. J.; Holland, B. T.; Blanford, C. F.; Stein, A. *Chem. Mater.* **1999**, *11*, 3302.
9. Asefa, T.; MacLachlan, M. J.; Coombs, N.; Ozin, G. A. *Nature* **1999**, *402*, 867.
10. Clark, J. H.; Macquarrie, D. J. *Chem. Commun.* **1998**, 853.
11. Moller, K.; Bein, T. *Chem. Mater.* **1998**, *10*, 2950.
12. Wu, C.-G.; Bein, T. *Science* **1994**, *264*, 1757.
13. Nguyen, T. Q.; Wu, J. J.; Doan, V.; Schwartz, B. J.; Tolbert, S. H. *Science* **2000**, *288*, 652.
14. Choi, M.; Kleitz, F.; Liu, D. N.; Lee, H. Y.; Ahn, W. S.; Ryoo, R. *J. Am. Chem. Soc.* **2005**, *127*, 1924.
15. Jung, Y.; Spray, R. L.; Kim, J. H.; Kim, J. M.; Choi, K.-S. *Chem. Commun.* **2010**, *46*, 6566.
16. Kwak, S.-S.; Kim, S.-K.; Lee, M.-S.; Jung, K.-H.; Park, I.-H.; Liu, J.-R. *Phytochem.* **1995**, *39*, 981.
17. Kruk, M.; Jaroniec, M. *Chem. Mater.* **2001**, *13*, 3169.
18. Wang, Y.; Caruso, F. *Chem. Mater.* **2005**, *17*, 953.