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Production of L-DOPA by Tyrosinase Immobilized on Modified Polystyrene

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Abstract

Mushroom tyrosinase was immobilized on modified polystyrene—polyamino styrene (PSNH) and polymethylchloride styrene (PSCL)—to produce L-DOPA from L-tyrosine. Glutaraldehyde was used as an activating agent for the PSNH to immobilize the tyrosinase, and 10% (w/v) glutaraldehyde was optimal in conferring the highest specific activity (11.96 U/g) to the PSNH. Methylchloride on the PSCL was directly linked with the tyrosinase, and 1.5 mmol of Cl/g was optimal in attaining the specific activity of 17.0 U/g. The temperature and optimal acidity were, respectively, 60°C and pH 5.5 for the PSNH, and 70°C and pH 3.0 for the PSCL. In a 50-mL batch reactor working over 36 h, the L-DOPA production rate at 30°C was 1.44 mg/(L·h) for the PSNH and 2.33 mg/(L·h) for the PSCL. The production rate over 36 h was 3.86 mg/(L·h) for the PSNH at 60°C and 5.54 mg/(L·h) for the PSCL at 70°C. Both of the immobilized enzymes showed a remarkable stability with almost no change in activity after being stored wet. The operational stability study indicated a 22.4% reduction in L-DOPA production for the PSNH and an 8.63% reduction for the PSCL over seven runs (each run was for 144 h at 30°C) when the immobilized enzymes were used under turnover conditions. The immobilized tyrosinase was more stable on the PSCL than on the PSNH.

Index Entries: L-DOPA; tyrosine; tyrosinase; enzyme immobilization; modified polystyrene.

Introduction

L-DOPA is the drug of choice for treatment of Parkinson disease. Although most of the current supply of L-DOPA is produced chemically,

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recent research has centered on microbiologic *(1,2)* and enzymatic production *(3–6)*. The microbiologic production of L-DOPA proved to be quite expensive for removing impurities *(2)*. On the other hand, the enzymatic production of L-DOPA using tyrosinase would yield the desired product and be easy to separate from the reaction medium, making it more economical.

Tyrosinase (EC 1.14.18.1) is found throughout the animal and plant kingdoms. This enzyme possesses the ability to catalyze two different reactions: the hydroxylation of monophenols to *o*-diphenols (monophenol + O_2 → *o*-diphenol + H_2O) and the oxidation of *o*-diphenols to *o*-quinones (2 *o*-diphenol + O_2 → 2 *o*-quinone + 2 H_2O), both using molecular oxygen. Tyrosinase also catalyzes other intermediate reactions in the process of producing melanin. To prevent diphenol (L-DOPA) from undergoing the subsequent reaction to dopaquinone and polymerizing into melanin, reductants such as ascorbate, NADH, and NH_2OH can be used to convert dopaquinone to L-DOPA *(7)*.

It is well known that immobilized enzymes offer considerable advantages over soluble enzymes. The immobilized enzyme can be reused and retained within the reactor without introducing fresh enzyme into the feed stream. In addition, immobilized enzymes may keep their activity longer than soluble enzymes. Tyrosinase has been immobilized on enzacryl-AA *(3)*, nylon 6, 6 *(4)*, chitosan flakes *(5)*, and zeolite *(6)* to produce L-DOPA from L-tyrosine.

Polystyrene (PS) is inexpensive, readily available in different particles, and possesses mechanical rigidity and stability over a wide range of pH values. This material can be easily activated to immobilize enzymes, such as glucoamylase *(8,9)*, glucose isomerase *(8)*, and proteins *(10)*.

In the present study, two kinds of modified PS were used, polymethylchloride styrene (PSCL) and polyamino styrene (PSNH), as supports for immobilizing the tyrosinase and then for producing L-DOPA from tyrosine. These two kinds of immobilized enzymes were compared regarding enzyme characteristics, optimal pH and temperature for the production of L-DOPA, time course of L-DOPA accumulation, and enzyme stability for storage and operation.

Materials and Methods

Chemicals and Equipment

Mushroom tyrosinase (T7755) with specific activity (5350 U/mg, determined by the supplier), L-DOPA, ascorbic acid, and a protein assay kit (P 5656) were obtained from Sigma (St. Louis, MO). Styrene, divinylbenzene (DVB), methylchloride styrene (MCS), polyvinylpyrrolidone (PVP), 2,2'-azobis (2-methylpropionitrile) (AIBN), and D-glucosamine hydrochloride (98%) were obtained from Acros (Geel, Belgium). Glutaraldehyde aqueous solution (25%) was purchased from Fluka (Buchs, Switzerland). Fluorescamine was obtained from Lancaster (Eastgate, England). All other

reagents and solvents were obtained commercially and were of standard reagent grade. Fresh mushrooms were purchased from a local farm to prepare the acetone powder for extraction of tyrosinase. A fluorescence spectrophotometer (Hitachi F-4500) was used to determine the amino group content of the PSNH beads. The activity of the enzymes was measured with an ultraviolet (UV)/visible spectrophotometer (Jasco V-530), and the production of L-DOPA was measured by high-performance liquid chromatography (HPLC) (Jasco PU-986).

Preparation of Polymer Beads

The methods of Wu et al. *(11)* were modified to prepare the PS beads with an average diameter range of 37–250 μm (400 mesh to 60 mesh). AIBN (1.15 g) (initiator) and PVP (3 g) (stabilizer) were dissolved in 100 mL of ethanol and then mixed with the monomer solution of 0.495 mol of styrene and 0.005 mol of DVB in a 500-mL conical flask. Polymerization was performed by stirring at 250 rpm at 60°C for 24 h. The resultant PS beads were washed for 10 min each in 1,4-dioxane (twice), methanol (twice), and deionized water. The slurry of the PS beads was sieved with a mesh using deionized water. Beads in this range are easy to filter out from the reaction medium. The PS beads of desired size range were then dried in a vacuum oven at room temperature.

The PS beads were nitrated and hydrogenated to form PSNH. For the nitration of the PS, 10 g of the dried PS beads was suspended in 20 mL of anhydrous acetic acid and gently stirred for 20 min at 60°C. A mixture of 65% (v/v) nitric acid (30 mL) and 70% (v/v) sulfuric acid (30 mL) was prepared in an ice bath and then added to the flask of PS beads. After a reaction time of 5 h, the product was diluted with 0°C deionized water, and the acidic liquid was drained off, washed with 0.1 *N* NaOH, and then finally washed with deionized water until reaching a neutral pH.

Nitrated PS (8 g) was suspended in 15 mL of anhydrous acetic acid and mixed with 50 mL of 12 *M* HCl containing 20 g of $SnCl_2 \cdot 2H_2O$. The reaction mixture was stirred for 72 h to obtain the amino-containing product. This product was then washed with 0.1 *N* NaOH and deionized water, dried in a vacuum oven, and stored at 4°C for future use.

PSCL beads were prepared using the same method as for the preparation of the PS beads except that the monomers were replaced with styrenes, MCS, and DVB. The mol fraction was kept at 2% for DVB and was adjusted for MCS in order to obtain different chloride levels in the PSCL.

Amino Group Content of PSNH

The amino group content of the PSNH beads was based on the fluorescence measurements. Fluorescamine solution containing 1.0 mg/mL was prepared in acetone. The reaction mixture contained a 100 m*M* sodium phosphate buffer (pH 9.0), fluorescamine, and 0.1 g of the PSNH beads. The fluorescence of the resulting solution was measured at 480 nm after excitation at 389 nm. D-Glucosamine hydrochloride was used as standard.

Chloride Content of PSCL

Chloride was released by quaternization with pyridine and estimated by Volhard titration in the following procedures. The PSCL beads (0.1–0.2 g) were heated with 2 mL of pyridine in 100°C water for 60 min. The solution with the resin was transferred to an Erlenmeyer flask with 50 mL of 20% acetic acid, and then three drops of a saturated ferric ammonium sulfate indicator, 5 mL of concentrated nitric acid, 5 mL of 0.1 *N* silver nitrate, and 3 mL of toluene were added. After 10 min, the mixture was backtitrated with 0.1 *N* potassium thiocyanate to calculate the chloride content.

Characterization of Beads

Morphology of the beads was determined by scanning electron microscopy (SEM) (Jeol CX, Temscan, Tokyo, Japan). Samples of the beads were sputtered with an Au/Pd film in an argon atmosphere. Scans were carried out at magnifications between 3700 and 5500. The porosity and specific surface area of the beads were determined by Brunauer-Emmett Teller (BET) sorptometer (Porous Materials Inc., NY).

Preparation of Enzyme Solution

Fresh mushrooms (500 g) were minced with a knife, placed in 5 L of acetone, and then cooled to 0°C. The suspension was homogenized with a tissue homogenizer and the pulp was separated by filtration. The resultant paste was squeezed between two layers of filter paper to drain off the acetone, and then it was broken into small pieces and dried at 4°C. This acetone powder was stored at –30°C.

The acetone mushroom powder (30 g) was suspended in 150 mL of a 0.1 *M*, pH 6.0 (the optimal pH to extract enzyme; data not shown here) sodium phosphate buffer, followed by incubation at 4°C for 24 h. The sodium dodecyl sulfate polyacrylamide gel electrophoresis of the mushroom tyrosinase showed that its mol wt was about 110 kDa. The suspension of the acetone mushroom powder was filtered, and the effluent was centrifuged through a diafiltration cup (Vivaspin concentrators, materials with a mol wt <100 kDa were removed) at 17,000*g* and 0°C for 30 min. The upper broth was further purified through a HiTrap DEAE FF column (Amersham Pharmacia Biotech, Buckinghamshire, England) and then centrifuged through the diafiltration cup to desalt. The enzyme solution was adjusted to the desired pH and used immediately.

Immobilization of Enzyme

The PSNH beads were activated with glutaraldehyde and then covalently coupled with tyrosinase. One gram of the PSNH beads was soaked in 30 mL of a 0.1 *M*, pH 8.0 phosphate buffer (containing glutaraldehyde) for 2 h at 30°C. Glutaraldehyde at concentrations between 1 and 15% (w/v) was used to study the effects on the activity of immobilized

tyrosinase. After incubation, the activated PSNH beads were washed with a 0.1 *M*, pH 7.0 phosphate buffer.

Polymer beads (either PSCL or activated PSNH) (0.5 g) were immersed in 15 mL of enzyme solution (total: 52.8 mg of protein with 920 U) at 4°C for 24 h to immobilize tyrosinase. The final beads were washed with a 0.1 *M*, pH 7.0 phosphate buffer until there was no tyrosinase activity detected in the draining buffer. The immobilized tyrosinase was stored in a 0.1 *M*, pH 7.0 phosphate buffer at 4°C for further experiments.

Enzyme Activity Assay

The activity of free and immobilized tyrosinase was determined spectrophotometrically with the direct measurement of the increase in light absorption at 475 nm caused by the formation of dopachrome from the L-DOPA *(12)*. The experiment was conducted at 25°C in 2.5 mL of 10 m*M* L-DOPA in a 50 m*M* phosphate buffer (pH 7.0). After adding 100 µL of enzyme solution (or 100 µL of the phosphate buffer and 20 mg of the immobilized enzyme), the increase in absorbance at 475 nm was monitored for 2 min. The enzyme activity from this procedure was calibrated against the activity reported by the manufacturer (i.e., activities were normalized with respect to the manufacturer's units; one unit = ΔA_{280} of 0.001/min at pH 6.5 and 25°C in 3 mL of reaction mixture containing L-tyrosine).

Determination of Immobilized Protein

The amount of immobilized protein was estimated by subtracting the amount of protein determined in the supernatant after immobilization from the amount of protein used for immobilization. The protein content in the solution was determined by the modified Lowry method *(13)*.

Optimal pH and Temperature

Using 2.5 m*M* L-tyrosine and 2.5 m*M* ascorbic acid contained in a 10 mL, 0.1 *M* sodium phosphate buffer as substrate, mixed with 0.5 g of immobilized enzyme in a 50-mL batch reactor, the reaction was achieved at 30°C for 24 h to find the optimal pH value for producing L-DOPA. Under the optimal pH, L-DOPA was produced using PSNH or PSCL at different temperatures for 24 h to determine an optimal production temperature.

L-DOPA Production Analysis

The production of L-DOPA was processed at 150 rpm, under atmosphere in 10 mL of a 0.1 *M* phosphate buffer containing 2.5 m*M* L-tyrosine and 2.5 m*M* ascorbic acid as substrate, and finally mixed with 0.5 g of the immobilized enzyme in a 50-mL batch reactor.

The concentration of L-DOPA was determined by HPLC with a UV detector (Jasco model UV-1575) corresponding to maximum absorbance at 270 nm. HPLC was performed on a Polaris C-18A column with a 25 m*M*,

pH 2.5 phosphate buffer as the mobile phase at a constant flow rate of 1.0 mL/min.

Storage and Operation Stability

To examine the stability of the immobilized tyrosinase stored in a 0.1 *M*, pH 7.0 phosphate buffer at 4°C, L-DOPA production was measured periodically at 30°C. The operation stability was tested by reusing the immobilized enzyme to produce L-DOPA.

Results and Discussion

Characterization of PS Beads

Figure 1 shows photomicrographs of the PSNH beads (Fig. 1A) and the PSCL beads (Fig. 1B). SEM photomicrographs showed that these beads were pearl-spread-like on the surface. The BET also indicated the total pore volume and specific surface area for both the modified PS beads to be 0.004 cm^3/g and 1.801 m^2/g, respectively. These results imply that the PS beads were almost nonporous and that immobilization of the enzyme occurred on the outer surface of the beads. The density of PS is about 1.06 g/cm^3. For the sphere PS beads with particle size in the range of 75–150 μm, the calculated specific surface area is 0.076–0.038 m^2/g. The pearl-spread-like surface of the beads supplies more specific surface area than the sphere beads to immobilize the enzyme.

Immobilization of Tyrosinase on PS

The amino group content on the PSNH was 0.131 mmol/g according to the fluorescence measurement. The Cl content of the PSCL was found to be the same as the mol ratio of the MCS monomer initially added. The effects of the glutaraldehyde concentration and Cl content on the specific activity of the PS are shown in Fig. 2. The optimal glutaraldehyde concentration for activating the PSNH was 10% (w/v) and thus conferred the PSNH with a specific activity of 12.0 U/g and immobilized tyrosinase of 3.15 mg/g. It was found that 1.5 mmol/g of Cl was enough for the PSCL to have a maximal specific activity of 17.0 U/g. The residual Cl on the PSCL was blocked with ethylamine to avoid the interaction of $–CH_2Cl$ with the substrates. The consumed ethylamine was found to be <0.04 mmol/g of PSCL by fluorescence measurement. Therefore, in subsequent experiments, the residual Cl on PSCL was uncapped. Meanwhile the amount of immobilized tyrosinase was unchanged (4.17 mg/g of PSCL) when the Cl content was >1.5 mmol/g. This phenomenon indicates that steric hindrance inhibits immobilization of the enzyme on the PSCL. There were more binding sites on the PSCL than on the PSNH to immobilize the enzyme, so that a multipoint attachment of tyrosinase on the PSCL could form. In later experiments, PSCL with a capacity of 1.5 mmol of Cl/g was used.

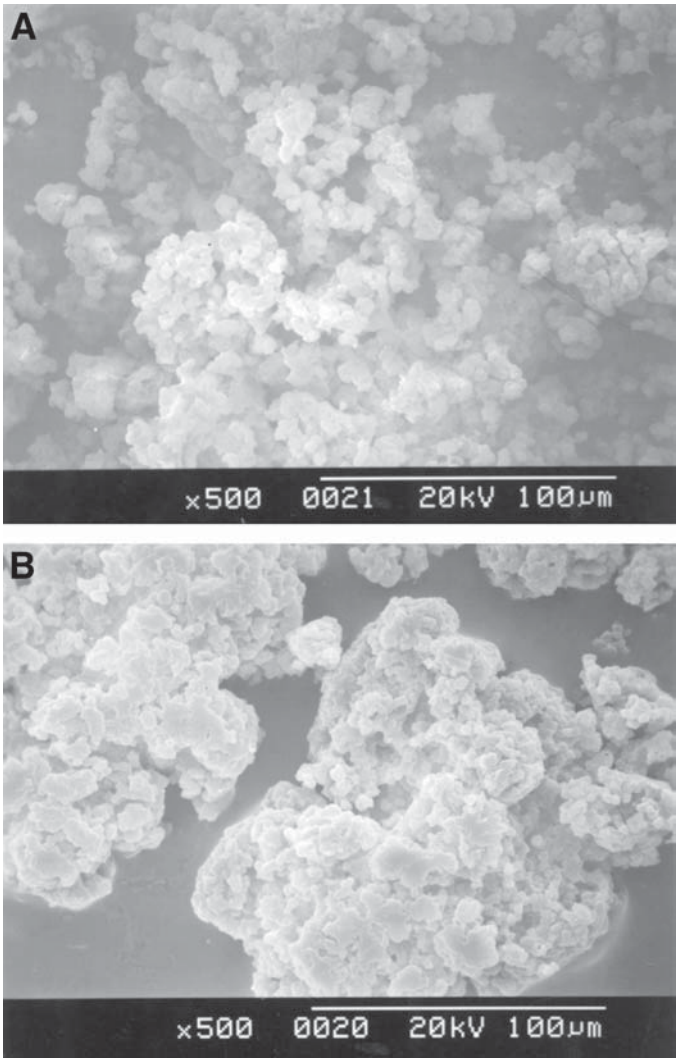

Fig. 1. Photomicrographs of **(A)** PSNH beads and **(B)** PSCL beads.

The activity of the enzyme solution was 61.3 U/mL with a protein content of 3.52 mg/mL. The specific activity was 17.4 U/mg of protein for the free enzyme, 3.80 U/mg of protein for the PSNH, and 4.08 U/mg of protein for the PSCL. The residual activity of tyrosinase (expressed as a percentage of the specific activity of the enzyme solution) for the immobilized PSNH and PSCL was 21.8 and 23.4%, respectively. The structural changes in the enzyme introduced by the immobilization procedure and the lower accessibility of the substrate to the active sites of the immobilized enzyme caused an activity depression. As shown in Fig. 3, the immobilizing rate of tyrosinase on the PSCL was slightly higher than on the PSNH.

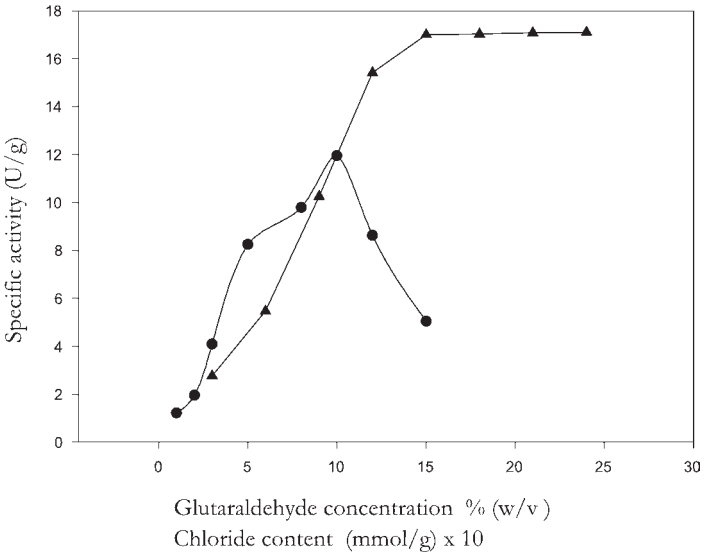

Fig. 2. Effect of glutaraldehyde concentration for activating PSNH (●) and chloride content of PSCL (▲) on specific activity of immobilized tyrosinase in 50 m*M*, pH 7.0 phosphate buffer containing 10 m*M* L-DOPA as substrate.

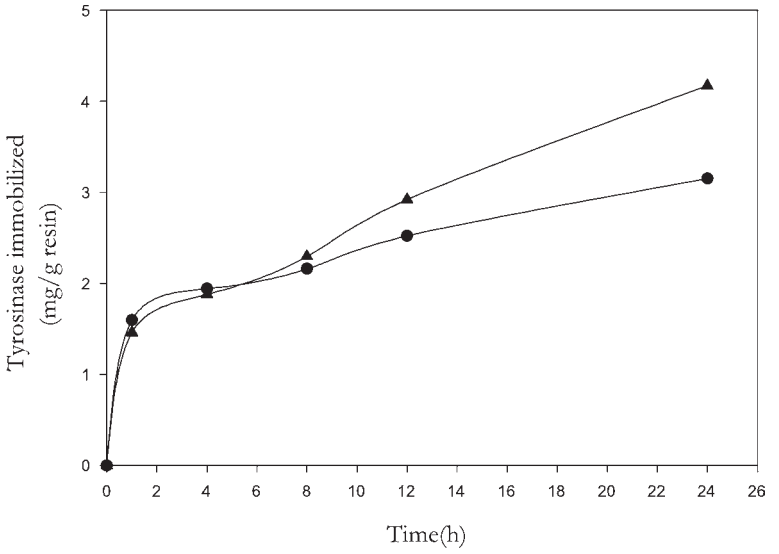

Fig. 3. Immobilizing rate of tyrosinase on PSNH (●) and PSCL (▲).

Optimal pH and Temperature for L-DOPA Production

The profiles of L-DOPA production with a different pH are shown in Fig. 4. Here it is clearly seen that the production rate increased up to pH 5.5 for PSNH and 3.0 for PSCL. For free tyrosinase the optimal pH to convert to diphenol from monophenol was 6.0 *(14)*. This shift phenomenon can be

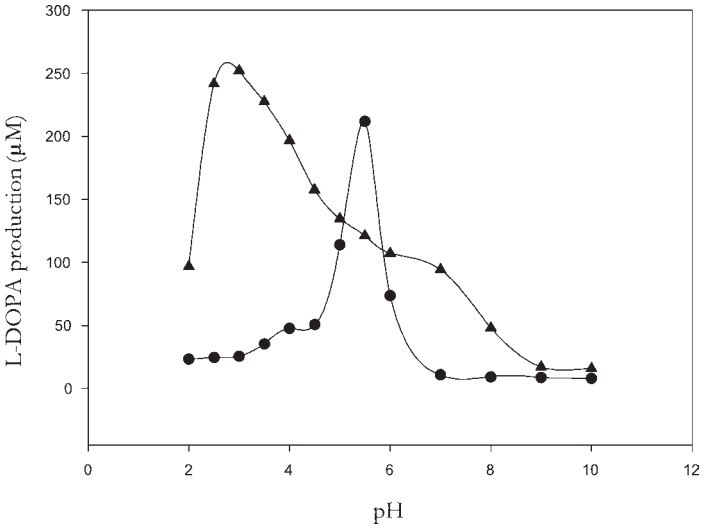

Fig. 4. Effects of pH on production of L-DOPA for PSNH (●) and PSCL (▲).

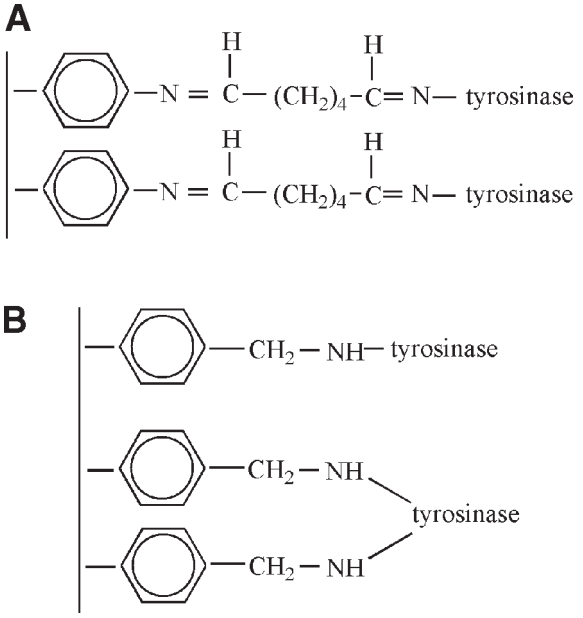

Fig. 5. Chemical structure of immobilized tyrosinase on **(A)** PSNH beads and **(B)** PSCL beads.

described by the partition effect. As shown in Fig. 5, a nitrogen atom (electron donor) was left on the surface of both supports after immobilization. These nitrogen atoms could adsorb positive ions and repel hydrogen ions to diffuse into tyrosinase. The surface of the PSCL (capacity: 1.5 mmol/g) possessed more nitrogen atoms than the PSNH (capacity: 0.131 mmol/g).

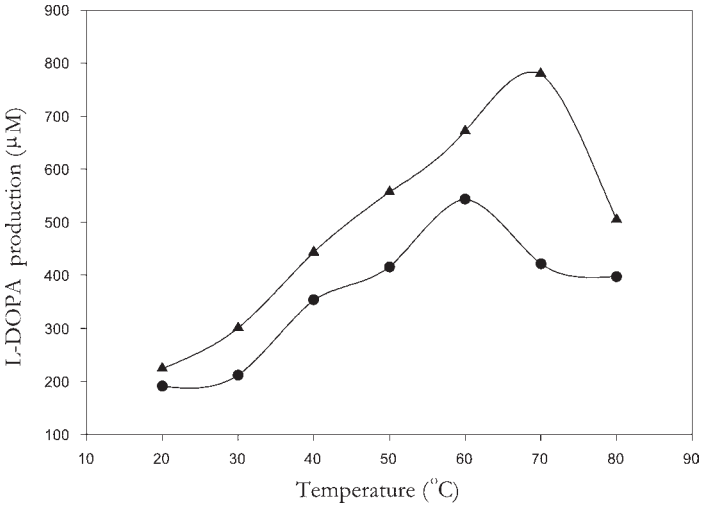

Fig. 6. Effect of temperature on L-DOPA production over 24 h under optimal pH for PSNH (●) and PSCL (▲).

Furthermore, the spacer length between the immobilized tyrosinase and the support was shorter on the PSCL (one nitrogen atom) than on the PSNH (two nitrogen atoms and four carbon atoms). The high density of positive charges on the surface of the immobilized tyrosinase (adjacent to PSCL) caused a high concentration of hydrogen ions to migrate to the active site of the PSCL. Thus, a large shift in the pH (from 6.0 to 3.0) was exhibited. The lower density of the positive charges on the PSNH and bigger spaces between the PSNH and the tyrosinase caused a slight shift in the pH (from 6.0 to 5.5).

L-DOPA production in 24 h at a temperature range of 20–80°C for the PSNH and the PSCL under the optimal pH is shown in Fig. 6. The optimal temperature for the PSNH to produce L-DOPA was 60°C and 70°C for the PSCL. It increased 2.57 times for the PSNH and 2.60 times for the PSCL in L-DOPA production at 30°C. The optimal temperature for free tyrosinase was found to be 45°C *(15)* in an aqueous environment. The immobilized tyrosinase exhibited higher thermostability than the free enzyme. The higher temperature endurance of tyrosinase reflected the greater stability of the enzyme on the PSCL. This observation fitted the hypothesis of the multipoint attachment of tyrosinase on PSCL giving PSCL a higher temperature endurance.

Production of L-DOPA

As shown in Fig. 7, three sizes were used to detect the effect of particle size on the production of L-DOPA and on the amount of tyrosinase immobilized. The production of L-DOPA increased as particle size decreased for the PSNH, and the optimal size for the PSCL was 75–150 μm. The PSCL beads were more hydrophobic than the PSNH. When the size of the PSCL

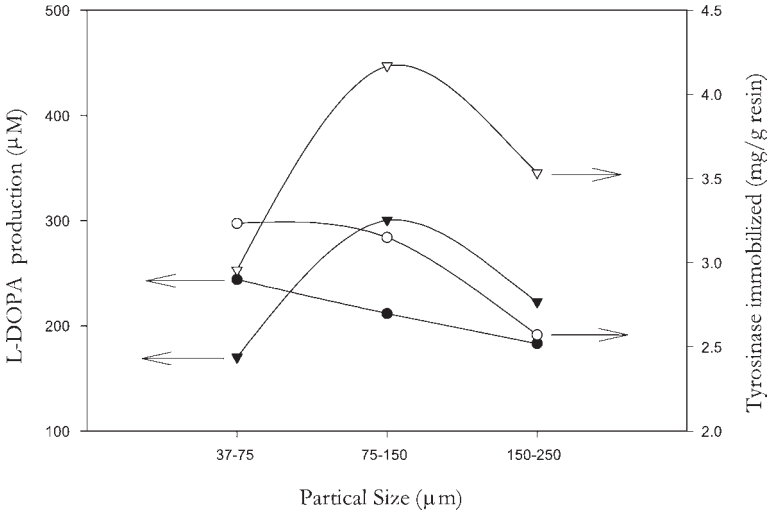

Fig. 7. Effects of particle size on production of L-DOPA for PSNH (●) and PSCL (▼), and on amount of tyrosinase immobilized for PSNH (○) and PSCL (▽).

decreased to 37–75 μm, the particles aggregated and adhered to the vessel wall in the enzyme-immobilizing step, so the efficiency of the enzyme immobilization was slowed down; that is why the production of L-DOPA was decreased. Therefore, in later experiments, particle sizes ranging from 75 to 150 μm were used.

The production-time profiles of L-DOPA for both cases of immobilized tyrosinase are shown in Fig. 8. At 30°C L-DOPA production increased steadily but production rate reduced slowly. At high temperatures (60°C for PSNH and 70°C for PSCL), L-DOPA production reached maximum and then declined. As can be seen from the sigmoidal curves in Fig. 8, the PSCL initially exhibited a faster L-DOPA-producing rate to reach maximum, and afterward the decreasing rate of L-DOPA was faster on the PSCL than on the PSNH.

There are two competitive reactions for tyrosinase to catalyze: hydroxylation of tyrosine to the L-DOPA and oxidation of the L-DOPA to dopaquinone. In the presence of ascorbic acid, dopaquinone can be reduced to L-DOPA, leaving it as the only product. When ascorbic acid is consumed, the dopaquinone cannot be reduced to L-DOPA any more, and the L-DOPA is then catalyzed in the process of producing melanin. In the experiments at high temperature, it was found, after 36 h for the PSCL and 48 h for the PSNH, that the ascorbic acid disappeared (detected by HPLC) and the reaction medium turned brown. This phenomenon may be the result of melanin formation. It is reasonable to assume that the faster accumulation of L-DOPA causes the quicker consumption of ascorbic acid. Furthermore, tyrosinase is an oxidase of ascorbic acid, and the oxidizing rate is linearly proportional to the activity of the tyrosinase *(16)* with the result that the consumption rate of the ascorbic acid is faster in the PSCL (higher-activity) system than in the PSNH (lower-activity) system.

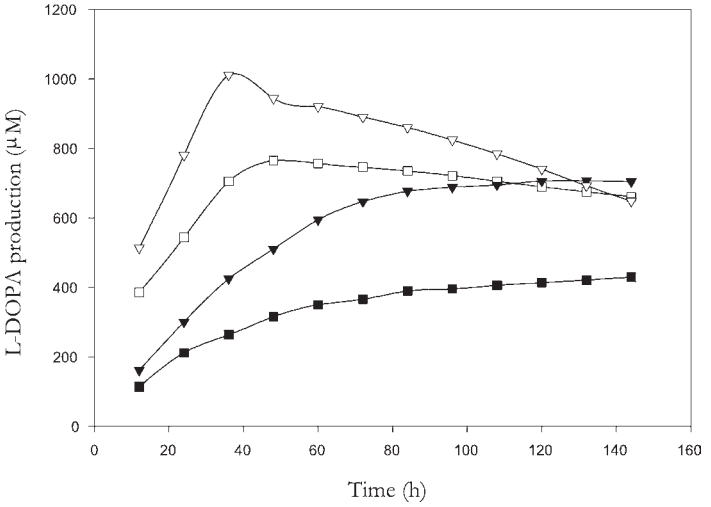

Fig. 8. Production-time profiles of L-DOPA for PSNH (■) and PSCL (▼) at 30°C, PSNH (□) at 60°C, and PSCL (▽) at 70°C with optimal pH.

Table 1
L-DOPA Production on Different Supports

Support (tyrosinase source)	Production rate (mg/[L·h])	Duration (h)	Total L-DOPA produced (mg)
Enzacryl-AA (frog)[a]	27.6	8	4.5
Nylon 6,6 (mushroom)[b]	1.70	170	143
Chitosan flakes (mushroom)[c]	44.86–54.0	—	—
Zeolite (mushroom)[d]	31–36	7	111–135
PS (mushroom)			
PSNH (30°C)	1.44	36	0.52
PSNH (60°C)	3.86	36	1.39
PSCL (30°C)	2.33	36	0.84
PSCL (70°C)	5.54	36	1.99

[a]Ref. *3*.
[b]Ref. *4*.
[c]Ref. *5*.
[d]Ref. *6*.

When the ascorbic acid was consumed, the descending rate of L-DOPA was faster in the PSCL system than in the PSNH system (Fig. 8). This result agrees with the fact that the oxidation rate of L-DOPA to dopaquinone is faster than the hydroxylation rate of the tyrosine to L-DOPA *(7)*. With a high L-DOPA concentration even with the same ascorbic acid still present, the accumulation rate of the L-DOPA is reduced. This phenomenon shows that the converting rate of tyrosine to L-DOPA by tyrosinase slows down, proving that L-DOPA in fact inhibits the hydroxylation of tyrosinase.

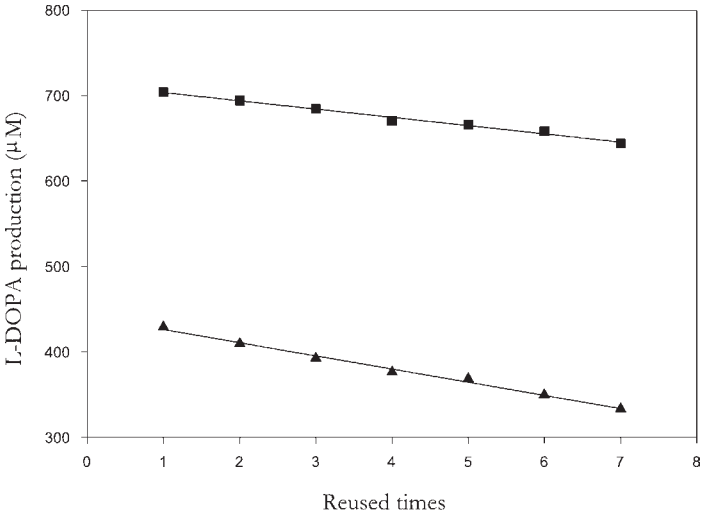

Fig. 9. Reduction of L-DOPA production by repeatedly using PSNH (▲) and PSCL (■) in fresh substrates for runs of 144 h each.

The L-DOPA production rates were compared to the production rates observed by other researchers (Table 1). Vilanova et al. *(3)*, who used Enzacryl-AA polyacryl-amide support to immobilize frog epidermis tyrosinase, obtained an L-DOPA production rate of 27.6 mg/(L·h) in a batch reactor. Pialis et al. *(4)*, Freire et al. *(5)* and Saville and Seetharam *(6)* immobilized mushroom tyrosinase on a nylon membrane, chitosan flakes, and modified zeolite, and obtained an L-DOPA production rate of 1.70, 44.86–54.0, and 31–36 mg/(L·h), respectively. The L-DOPA production rates in this study are 1.44 to 5.54 mg/(L·h) depending on temperature and supports.

Storage and Operational Stability

When stored in a 0.1 *M*, optimal pH phosphate buffer at 4°C for 40 d, the production activity of the L-DOPA was the same as with the freshly immobilized enzyme.

The production of L-DOPA by reusing the immobilized enzyme is shown in Fig. 9. After reusing the enzyme seven times at 30°C (each run was 144 h), L-DOPA production with the seventh run was 0.66 mg for the PSNH and 1.27 mg for the PSCL. Compared with L-DOPA production of the first run (0.85 mg for PSNH and 1.39 mg for PSCL), the operation stability tests indicated 22.4% reduction in activity for the PSNH and 8.63% reduction for the PSCL under turnover conditions over 42 d.

During the oxidation of diphenols to *o*-quinones there is an irreversible enzymatic inactivation reaction *(17)*. This inactivation process has been interpreted as being the result of a direct attack of an *o*-quinone on a nucleophilic residue (His) near the active-enzyme center *(18)*. The multipoint attachment of the enzyme with support enhances the stability of the immo-

bilized enzyme *(19)* and depresses the inactivation of the PSCL. From another viewpoint, if the target of *o*-quinone does not near the active site, the attack will not cause the inactivation of tyrosinase. The higher immobilized tyrosinase of the PSCL may scatter the attack by the dopaquinone reaching inactivation to a lesser extent. This may be why the PSCL is less inactivated than the PSNH.

Conclusion

Our results show that the nonporous modified PS beads with a pearl-spread-like surface are suitable for immobilizing the tyrosinase, with the optimal coupling agent concentration being 10% (w/v) glutaraldehyde to activate the PSNH and 1.5 mmol of Cl/g being enough for the PSCL to link with the tyrosinase. In addition, the optimal pH to produce L-DOPA was 5.5 for the PSNH and 3.0 for the PSCL. The optimal temperature for the production of the L-DOPA was 60°C for the PSNH and 70°C for the PSCL. The immobilization of the tyrosinase on the modified PS increased the thermal stability. Furthermore, the productivity of the L-DOPA was faster in the PSCL system than in the PSNH system. In comparison with other supports used to immobilize tyrosinase, PSCL has the advantages of low cost, middle production rate, ease of preparation, ready immobilization of the enzyme without activating it, and the capacity of the methylchloride groups to be easily regulated. Finally, the tyrosinase immobilized on PSCL was more stable than when immobilized on PSNH.

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