Note

Enhanced Stability of a Microbial Lipase Immobilized to a Novel Synthetic Amine Polymer

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Since lipase assumes water-insoluble esters and glycerides as substrates, immobilization of the enzyme is attractive for their syntheses,¹⁾ transesterifications,²⁾ and hydrolyses. Of several kinds of immobilization techniques, covalent immobilization is considered preferable because it often results in stabilization of the enzyme by multi-point bondings to a solid support. However, so far as lipase is concerned, only a few reports are found describing covalent immobilization with a high activity yield involving an appreciable increase in enzyme stability.

We have immobilized *Geotrichum candidum* (ATCC 34614) lipase to a novel polyallyl amine beads leading to enzyme stabilization. One gram of the cross-linked polyallyl amine beads (PAA-B) supplied from Nitto Boseki Co. was suspended in 5 ml of water and the pH was adjusted to 5.6 with 1 N NaOH. After washing with 0.05 M acetate buffer, pH 5.6, the polymer beads were treated with 0.5% glutaraldehyde dissolved in 5 ml of the same buffer for 16 hr at 15°C.

Pure *Geotrichum candidum* lipase was obtained as described previously.³⁾ Five milligrams of the lipase dissolved in 3 ml of 0.05 M acetate buffer, pH 5.6, was incubated in 10 μ l of tricaproin for 5 min at 25°C to mask the active site with the substrate. To the enzyme solution was added 2.5 g of the wet glutaraldehyde-treated polymer beads. After deaeration in a desiccator for 5 min, the mixture was shaken for 16 hr at 15°C. The polymer beads were then separated from the supernatant using a glass filter and washed with the same buffer. Unless otherwise stated, the hydrolytic activity of the enzyme was assayed using olive oil as the substrate as reported elsewhere.⁴⁾ More than 80% of the total enzyme used was found to be attached to the polymer support and the immobilized enzyme had a 30 ~ 50% activity yield.

First, the effects of the immobilization on the pH dependence of the activity were examined. The immobilization not only brought about a shift of the optimal pH from 6.0 to 8.2, but allowed, even at pH 13, the exertion of 20% of the activity at the optimal pH. The former observation is rather unexpected because immobilization

to a polycationic support often lowers the optimal pH owing to a rise in the pH of the microenvironment around the enzyme molecule.⁵⁾ The stable pH ranges of the free and immobilized enzymes after incubation for 16 hr at 25°C were pH 4.5 ~ 10 and 3.5 ~ 12, respectively. Thus the pH stability was apparently enhanced by the immobilization.

The thermal stability of the enzyme was examined by a rate-assay using a pH-stat at pH 8.5. The olive oil was emulsified with the aid of 2% polyvinyl alcohol for the assay. Fatty acids released were continuously titrated with 0.05 N KOH. From Fig. 1, we can see that the immobilization makes the enzyme much more thermostable. The enhanced thermal stability was further demonstrated by the same method using emulsified beef tallow as the substrate at 45°C. Beef tallow has its melting point at around 45°C. Although the free lipase was completely inactivated after 30 min, the immobilized enzyme retained its full activity even after 60 min.

FIG. 1. Rate-assay at Elevated Temperatures with Emulsified Olive Oil as Substrate.

Fatty acids released during the assays were continuously titrated with 0.05 M KOH using a pH-stat. Two units of the free and immobilized enzymes were used for the assays at indicated temperatures. -----, free enzyme; —, immobilized enzyme.

TABLE I. ENZYME ACTIVITY IN THE PRESENCE OF ISOCTANE

Enzyme activities at different isooctane concentrations (% v/v) were expressed as percentages of that in the absence of isooctane. Each activity was obtained by incubating the assay mixture for 1 hr at 30°C.

	Isooctane concentration (%, v/v)					
	0	3	8	18	40	60
Free enzyme	100	82	66	50	38	34
Immobilized enzyme	100	90	78	63	50	45

Inhibition of the enzyme by a water-immiscible organic solvent was examined using isooctane. As can be seen from Table I, the immobilized enzyme was less inhibited by the solvent than the free enzyme was.

With the free and immobilized enzymes, we tried to synthesize glyceride from glycerol and oleic acid. Thirty units of the free or immobilized enzyme was put into a mixture of 4 ml of glycerol and 0.1 g of oleic acid saturated with 1 M phosphate buffer, pH 7.5. The whole mixture was incubated for 16 hr at 30°C with stirring at 200 rpm. The amount of oleic acid incorporated into glyceride was measured by titration of the remaining oleic acid with 0.2N KOH. The free enzyme could not catalyze the glyceride synthesis well, probably due to the inhibition caused by oleic acid. By contrast, 58% of the total oleic acid were consumed for glyceride synthesis when the immobilized enzyme was used.

Thus, *Geotrichum candidum* lipase immobilized to the cross-linked polyallyl amine beads was endowed with higher pH- and temperature stability as well as less inhibition by an organic solvent, isooctane.

REFERENCES

- 1) G. Bell, J. A. Blain, J. D. E. Patterson and C. E. L. Shaw, *FEMS Microbiol. Lett.*, **3**, 223 (1978).
- 2) C. Marlot, G. Rangland, C. Triantaphylides and J. Baratti, *Biotech. Lett.*, **7**, 647 (1985).
- 3) Y. Tsujisaka, M. Iwai and Y. Tominaga, *Agric. Biol. Chem.*, **37**, 1457 (1973).
- 4) J. Fukumoto, M. Iwai and Y. Tsujisaka, *J. Gen. Appl. Microbiol.*, **10**, 257 (1964).
- 5) H. Suzuki, Y. Ozawa and H. Maeda, *Agric. Biol. Chem.*, **30**, 807 (1966).