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CHEMICAL MODIFICATION OF LIPASE WITH FERROMAGNETIC MODIFIER —— A FERROMAGNETIC-MODIFIED LIPASE ——

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SUMMARY

A ferromagnetic modifier was prepared by reacting ferrous(Fe^{2+})- and ferric(Fe^{3+})-ions with polyethylene glycol having two carboxyl groups (MW:2000) at pH 8.0-8.5. Lipase from Pseudomonas fragi 22-39B was coupled with the modifier using water-soluble carbodiimide. The modified lipase, which was dispersed into buffered solutions in the size range of 30-70 nm, exerted the hydrolytic activity of 8.0 U/mg. In a magnetic field of 250 Oe, the ferromagnetic-modified lipase was readily recovered from the colloidal solution.

INTRODUCTION

Chemical modification of enzymes, for example with the amphipathic and non-immunogenic polymer, polyethylene glycol, is an available technique to improve their properties(1,2) such as stability, immunogenicity, substrate specificity, and solubilization into organic solvents. In the present study, we have developed a new modifier consisting of dicarboxypolyethylene glycol and magnetite(ferromagnetic modifier) for the chemical modification to render enzymes ferromagnetic.

In biotechnology, the method of enzyme-recovery is often critically important. A most useful approach is magnetic separation, which has recently received fresh technical interest(3-5). The ferromagnetic

modifier will be useful for the preparation of the modified enzyme which can be magnetically separated.

MATERIALS AND METHODS

Lipase(EC 3.1.1.3) from Pseudomonas fragi 22-39B and dicarboxypolyethylene glycol(DCPEG) with average molecular weight of 2000 were kindly donated from Sapporo Breweries Ltd.(Shizuoka, Japan) and Kawaken Fine Chemicals Co. Ltd.(Tokyo, Japan), respectively. Water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide chloride, was purchased from Wako Pure Chemical Industry Ltd.(Osaka, Japan) Other reagents were of analytical grade.

Ferromagnetic modifier was prepared by mixing 10 ml of 50%(w/w) DCPEG with 2.4 ml of an aqueous solution containing 720 mg $FeCl_3 6H_2O$ and 290 mg $FeCl_2 4H_2O$. While shaking, the mixture was adjusted to pH 8.0-8.5 by the addition of 28% aqueous ammonium solution and was heated to 60 °C for 10 min. After the reaction, the resulting magnetic material was washed with phosphate buffered saline(PBS, pH7.0) to obtain ferromagnetic modifier.

The chemical modification of lipase with the ferromagnetic modifier was carried out as follows. The mixture of 5 mg lipase and 19 mg ferromagnetic modifier in PBS(pH 7.0) was sonicated, followed by adding 500 mg of water-soluble carbodiimide. The reaction mixture was kept at 37 °C for 90 min. The ferromagnetic-modified lipase thus prepared was washed with PBS(pH 7.0) and water.

The enzymic activity of lipase for hydrolysis of olive oil was measured as follows. To the mixture of 0.5 ml of emulsified olive oil, 0.4 ml of 0.2 M Tris-HCl(pH 9.0) and 0.1 ml of 110 mM $CaCl_2$ were added 0.1 ml of 0.15 mg/ml ferromagnetic-modified lipase solution. The reaction mixture was incubated at 37 °C for 60 min. After that, to the reaction mixture(1.1 ml)was added 2 ml of the mixture of acetone and ethanol(1:1) to stop the reaction. The mixture was titrated with 0.05 N NaOH in the presence of phenolphtalein dissolved in ethanol. One unit(U) of enzyme was defined as the amount which liberates one micromole of fatty acid per minute.

Magnetic field of 250 Oe, generated by two permanent magnets placed 2 cm apart from each other, was used to recover the ferromagnetic-modified lipase form the colloidal solution.

RESULTS AND DISCUSSION

The reaction of ferrous(Fe^{2+})- and ferric(Fe^{3+})-ions at pH 8.0-8.5 in the presence of dicarboxypolyethylene glycol(average molecular weight of 2000; DCPEG) yielded a colloidal solution of DCPEG-coated ferromagnetic(DCPEG-Fe_3O_4). The DCPEG was irreversibly adsorbed on the magnetite particles. This adsorption may be attributed to hydrogen-bonds (or complex) between carboxyl group of DCPEG and hydroxyl group (or iron ions)on the magnetite particles, although its exact mechanism is unclear.

The colloidal solution(2 mg/ml) of the ferromagnetic modifier(DCPEG-Fe_3O_4) was slightly turbid, and was not precipitated by centrifugation at 1700 x g for 5 min. The ferromagnetic modifier was readily separated from the colloidal solution in a magnetic field of 250 Oe in 2-5 min.

Coupling of lipase with the ferromagnetic modifier(DCPEG-Fe_3O_4) using water-soluble carbodiimide(formation of acid-amide bonds between amino groups of the enzyme and carboxyl group of the DCPEG coated on the Fe_3O_4 particles) was performed to prepare a ferromagnetic-modified lipase. The ferromagnetic-modified lipase exerted the enzymic activity for hydrolysis of olive oil(8.0 U/mg enzyme). The colloidal solution of the ferromagnetic-modified lipase was slightly turbid, stable in the PBS(pH 7.0) and in Tris-HCl buffer(pH9.0), and not precipitated by the centrifugation at 1700 x g for 5 min. Fig. 1 shows the electron micrograph of the ferromagnetic-modified lipase. The size of the ferromagnetic-modified lipase ranged from 30 to 70 nm, which is large enough to be separated magnetically from the reaction mixture in a relatively low magnetic field around several hundreds oerstead(Oe)(Ref. 6). Table 1 shows the result of the magnetic separation: all of the ferromagnetic-modified lipase was recovered from the solution(500 μg/ml) in

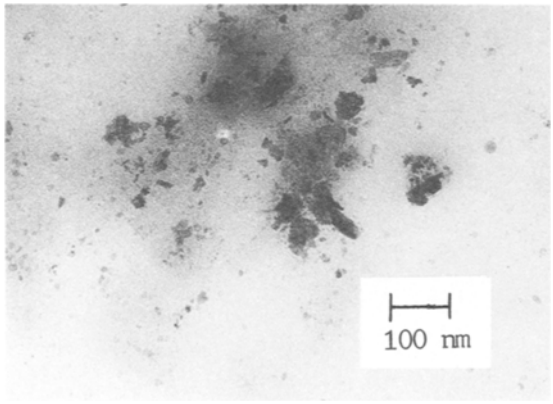

Fig. 1. Electron micrograph of the ferromagnetic-modified lipase.

Table 1. Magnetic separation of ferromagnetic-modified lipase

Suspension	Total Unit of Enzyme(U)
Colloidal solution of Ferromagnetic-modified lipase	7.96
Supernatant after magnetic separation	0.00
Ferromagnetic-modified lipase recovered by magnetic separation	8.06
Ferromagnetic modifier washed after mixing with enzyme	0.03

Ferromagnetic-modified lipase was dispersed into 2 ml of 0.2 M Tris-HCl(pH 9.0) at the concentration of 0.5 mg/ml. Magnetic field of 250 Oe was applied to the separation for 3 min.

3 min by the magnetic field of 250 Oe. This result was obtained by measuring the enzymic activity before and after removing the modified lipase from the solution. The enzyme was not magnetically recovered from the mere mixture of the modifier and the lipase(Table 1), whereas the ferromagnetic-modified lipase was readily recovered even when its concentration was lower(15 μg/ml). These results indicate that the lipase is strongly bound to ferromagnetic modifier and that the ferromagnetic-modified lipase exerts the hydrolytic activity. The relatively low magnetic field that is required can be easily generated with a popular rotating drum magnetic separator, and high gradient magnetic separators(HGMS), generally used to separate much smaller magnetic particles from the solution, are not necessary.

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