Note

Enzyme Immobilization on Glycosylated Edible Proteins

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Most of the supports for enzyme immobilization are artificial materials, and chemical reactions are also required to immobilize the enzymes in most cases.1] These support materials and chemicals for immobilization must not be allowed to contaminate final products, especially foods or drugs. The requirements to achieve this are tedious, costly, and may have the wrong effects on enzyme activities.

The other solution is presented here. Inexpensive edible proteins were used as supports, and the glycosylation products formed on these proteins were used as the binding site for immobilization.

Nonenzymatic glycosylation of proteins, which occurs in the initial stage of the Maillard reaction, has been reported for various proteins. In the later stages of the reaction, glycosylated protein are known to undergo complex chemical changes such as impairment of some amino acid residues, discoloration, development of fluorescence and crosslinking.2,3] The mechanisms for these crosslinking reactions have been studied by many workers.4~6] These studies indicated the pronounced ability of the glycosylation products to make crosslinks between proteins. These crosslinking abilities can be applied to enzyme immobilization.

Trypsin (Sigma, from bovine pancreas, Type 3) and \( \alpha \)-amylase (Sigma, crude from the Bacillus species) were used throughout this work. Casein granules (Nacalai Tesque) and dried egg white (food grade) were used as support proteins, and Chitopearl was obtained from Fuji Boseki Co. Benzoyl-L-arginine-\( \beta \)-nitroanilide (BAPA)7] was used for trypsin activity (TU) measurements, while the amylase activity8] (AU) was measured by using soluble starch as a substrate.

Dried egg white and casein were used as the edible protein supports. The egg white was dissolved in water (10%) and coagulated by heat treatment to make it insoluble, the coagulated protein then being crushed and lyophilized. The casein was used without heat denaturation because the granule preparation was very slowly soluble.

Five grams of each protein was dispersed in 50 ml of a 50% glucose solution (wide-range buffer,9] pH 2–12). The dispersion was incubated at 80°C for 5–25 hr, before the protein granules were washed with 1 l of water and lyophilized. The glycosylation reaction was faster at higher pH values, although the proteins treated above pH 5 started to dissolve. Therefore, the support proteins were treated with a glucose solution at pH 5 in the subsequent experiments.

The glycosylated proteins (0.5 g) were dispersed in 50 ml of the wide-range buffer (pH 5), and 1 ml of the trypsin solution (aqueous, 0.05 g/ml) or of the \( \alpha \)-amylase solution (aqueous, 0.5 g/ml) was added. The mixtures were incubated at 4°C for 72 hr with shaking. The immobilized enzymes were successively washed with 300 ml of 1 mM HCl, 300 ml of 1 M NaCl and again 300 ml of 1 mM HCl, and then lyophilized. When the supports were incubated above pH 5, autolysis of the protease and digestion of the support proteins were observed in the trypsin case. Therefore, the enzymes were immobilized at pH 5.

Figures 1 and 2 show the effects of glycosylation time on the immobilized enzymes.

Fig. 1. Effect of the Glycosylation Period of the Protein Support on the Immobilized Trypsin Activity.

Fig. 2. Effect of the Glycosylation Period of the Protein Support on the Immobilized \( \alpha \)-Amylase Activity.
on the immobilizing activities. Trypsin (Fig. 1) was immobilized even on untreated supports, whereas only a small amount of α-amylase (Fig. 2) was immobilized under similar conditions. The support glycosylated for 10 hr had the maximum ability to immobilize both the enzymes in the casein case. On the other hand, the egg white support glycosylated for 15 hr had the maximum binding activity. The immobilizing ability of both the supports increased with increasing incubation time, although this was decreased by over-glycosylation. This may have arisen from the loss of binding sites by the formation of crosslinks between the adjacent support proteins.

Only small differences from the pH–activity profile of the free enzyme were observed for the optimum pH of the immobilized trypsin on both supports. On the other hand, the pH profiles of the immobilized amylase became narrower than that of the free enzyme.

The optimum temperatures for the trypsin and α-amylase immobilized on both supports were 50°C and 60°C, respectively. There were similar values for the free enzymes. Therefore, the enzyme conformation may not have been affected by the immobilization, which indicates that the method is milder than the other chemical methods.

The highest activities in these experiments for trypsin and α-amylase were 12.7 TU and 23.1 AU, respectively, these values being higher than the activities for trypsin (1.5 TU) and α-amylase (12.0 AU) immobilized on Chitopearl by the ordinary method.10

The method presented here for enzyme immobilization has many advantages for use in food or drug production. We can choose inexpensive proteins for the support materials, which comprise the major running cost of immobilized enzyme reactor systems. The immobilizing process is very easy, needing only the high-temperature incubation of the support with glucose or, more conveniently with starch hydrolysate. The immobilized enzyme system is also essentially safe, because edible proteins can be chosen for supports and the crosslinking reaction is similar to the one occurring in cooking processes.

References
1) I. Chibata, "Im mobilized Enzyme," Kodansha, Tokyo, 1975, pp. 9–86.