Note

Thermal Stability of Free and Immobilized Invertase Studied by Activity Assay and Calorimetry

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Most biocatalysts are not very thermostable, so attempts have been made to increase their thermostability by chemical modification, immobilizing them in or on solid supports, and by screening for thermophile enzymes, microorganisms, or both. The effects of immobilization on enzyme activity have been studied,^{1~6)} but evaluations of the thermal stability of immobilized enzymes by calorimetry are few.

We developed a poly(vinyl alcohol) (PVA) fibrous support that can easily incorporate various functional groups by acetalization. This support consists of a bundle of superfine filaments (SFF) with a diameter of 1 μ m or less; the bundle has a large surface area.⁷⁾ A fibrous support can be formed into various shapes, such as thread, fabric, and filter paper. Some enzymes are immobilized on SFF by ionic bonds.^{8~10)} Here we investigated the thermal stability of invertase by both activity assay and calorimetry, and tried to evaluate the stability of invertase by thermal analysis.

Invertase (β -D-fructofuranoside fructohydrolase, EC 3.2.1.26) from *Candida utilis* (Seikagaku Kogyo Co., Ltd.) was dialyzed against distilled water for 48 hr and was then lyophilized. About 64 mg of dimethylaminated SFF was put into 50 ml of invertase solution (about 0.67 g of enzyme/liter) and shaken at 37°C for 1.5 hr. The amount of immobilized invertase was estimated to be around 0.26 g of enzyme/g of SFF from the optical densities of the enzyme solution before and after adsorption with SFF.⁸⁾ Sample tubes with 15 ml of free invertase solution (FI, about 0.78 g of enzyme/liter) and with 15 ml of HCl solution (pH 4.5), into which the invertase immobilized on SFF (IIS) was dipped, were heated from 25°C to various temperatures at a heating rate of 1°C/min in a thermobath equipped with a temperature controller (Chino Works, Ltd.) and then cooled rapidly in an

ice bath. Both the invertase and substrate solution were adjusted to pH 4.5 by adding HCl. Samples treated with heat or not were studied by activity assay and thermal analysis.

The activity assay was described elsewhere.⁷⁾

Two kinds of differential scanning calorimeters (DSC) were used to study these FI and IIS samples, at a scan rate of 1°C/min. We used a DASM-1M (Privalov) calorimeter with 1 ml cells for FI and SSC/560U (Seiko Instruments & Electronics Ltd.) with 70 μ l silver cells for IIS.

One milliliter of the enzyme solution (about 0.78 mg of invertase) and about 35 mg of wet SFF containing about 2.7 mg of invertase were placed in the cells for DSC analysis. The denaturation temperature is not affected by the amount of enzyme used in DSC analysis, but is affected by the enzyme concentration for FI and by enzyme loading (g of enzyme/g of SFF) for IIS.

DSC curves for the thermal denaturation of FI and IIS are shown in Fig. 1. The peak of the curves represents the denaturation temperature, T_d . The value of T_d for FI was 72°C and for IIS, 77°C. The results show that the invertase immobilized on SFF is more thermostable than in solution. The alteration of higher-order structure starts at 63°C for FI and at 67°C for IIS. The transition is complete at 76°C for FI and at 83°C for IIS.

An increase in thermal stability was also observed when invertase was immobilized in PVA film.¹¹⁾ The T_d of invertase increases, as the concentration of invertase increases.¹¹⁾ The enzyme immobilized on a support is limited spatially to the surface of the support. Polyhydric alcohols and carbohydrates have a stabilizing effect on enzymes.^{12,13)} PVA is a kind of polyhydric alcohol and yeast invertase contains about 50% carbohydrate.^{14,15)} Consequently, the increase in thermal stability shown in Fig. 1 is an effect of enzyme concentration and the interaction between enzyme and the PVA support.

Figure 2 shows the thermal stability of FI and IIS in a plot of relative activity and relative denaturation enthalpy against the temperature of the heat treatment. The relative values are defined as the ratios of activity and denaturation enthalpy for treated samples to those for untreated samples.

When FI was heated over 60°C, enzyme activity decreased rapidly with the increase in temperature. Sixty percent of the original activity remained in the sample heated to 70°C and 6% in the sample heated to 72.6°C. IIS heated to 75°C and 77°C maintained 50% and 25% of its original activity, respectively. At 70°C, 3.5% of IIS changed its conformation (see Fig. 1), but this change seems to be reversible, because IIS kept its original activity even after heating. These data also show that the invertase immobilized on SFF is more thermostable than that in solution. The results from thermal analysis agree with those from the activity assay.

The IIS sample heated to 70°C had a relative activity of about 110%. Some enzymes have higher activity after a very small amount of a denaturing agent is added.¹⁶⁾ In a

FIG. 1. DSC Curves for Invertase Immobilized on SFF (a) and in Solution (b) at pH 4.5.

FIG. 2. Thermal Stability of Free and Immobilized Invertase.

○, free; △, immobilized. Open symbols are results of activity assays and closed symbols are results from thermal analysis.

similar way, the enhanced activity of the enzyme heated to 70°C might be caused by a slight increase in the internal motility of the immobilized invertase.

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