Reversibility of Heat-Inactivation of Bacterial α -Amylase

Sir:

Taka amylase (α -1,4-glucan-4-glucanohydrolase of *Asp. oryzae*) inactivated by heat¹⁾ and by acid²⁾ has been known to be reactivated under certain condition. Isemura and Imanishi³⁾ have reported renaturation of *Bac. subtilis*' α -amylase denatured by urea.

Recently, we have found that inactivation of *Bac. subtilis*' α -amylase by heating is also reversible. To the experiment was used the crystalline bacterial α -amylase which had been freed from proteolytic enzyme⁴⁾ and, after dissolving in N/1000 sodium hydroxide solution, dialyzed against distilled water for two days, in order to remove any excess calcium ions in the crystalline enzyme. The amylase was completely inactivated when heated for 10 min. at temperatures higher than 85°C. However, when allowed to stand at room temperature, the inactivated enzyme restored its activity, the reactivation degree depending on the temperature at which the enzyme was heated (Fig. 1). The enzyme reaction for activity assay was carried out at unusually high temperature, 50°C, otherwise the enzyme activity immediately after heating was somewhat inconstant. The best reversible inactivation was obtained with the enzyme which was heated in a solution of pH 8.6 adjusted only by sodium hydroxide or in borate buffer solution of pH 8.6 (prepared without addition of sodium chloride) of low concentration. Heating at pH values less than 7.0 with the presence of acetate or phosphate in concentrations more than M/100 resulted in irreversible inactivation of the enzyme.

The degree of reversibility was also dependent upon concentration of enzyme and, when the

FIG. 1. Change of amylase activity after heating at various temperatures for 10 min.

—, Activity immediately after heating;
 ----, Activity after incubating the heated enzyme at 25°C for 150 min.
 ●, M/400 acetate buffer (pH 6.05);
 △, M/400 borate buffer (pH 8.60);
 □, M/20, NaCl containing M/100 Ca-acetate (pH 6.10).

enzyme concentration was more than 0.1%, the reversibility became less with the concentration.

Restoring of enzyme activity was best when the heat-inactivated enzyme was incubated at 30°C, and the higher or the lower the temperature of incubation, the less reactivation was obtained. The addition of inorganic salts (20%) or organic solvents (25%) to the enzyme immediately after heating resulted in no reactivation, although removal of the additions by dialysis caused reactivation to some extent. Also, the addition of proteinase to the heat-inactivated enzyme caused hydrolysis of amylase protein. However, later addition of proteinase resulted in decrease in the hydrolysis degree by the extent for the amylase to restore its activity (Fig. 2). The amylase reversibly inactivated by heating

1) E. Ohlson and T. Sweitchin, *Bull. Soc. Chim. Biol.* **11**, 333 (1929), E. Ohlson and O. Edfeldt, *ibid.*, **15**, 470 (1933).

2) S. Akabori, S. Hayashi and K. Kashimoto, *J. Chem. Soc. Jap.*, **61**, 1035 (1940). S. Akabori and K. Okahara, *ibid.*, **12**, 55 (1937).

3) T. Isemura and A. Imanishi, *J. Biochem. (Japan)*, **51**, 172 (1962).

4) T. Yamamoto and J. Fukumoto, *This Jour.*, **24**, 16 (1960).

FIG. 2. Susceptibility of the heat-inactivated amylase to proteinase (a solution of 0.34 mg amylase per ml of M/240 borate buffer (pH 8.6) was heated at 85°C for 10 min. and on subsequent incubation at 30°C proteinase was added at the times indicated by arrow.).

—, amylase activity without addition of proteinase.
 - - - - -, " " " " with the addition of proteinase.

also showed a reversible change in the absorption spectra at around 280 $m\mu$ (Fig. 3). These results clearly show that the reversible inactivation by heating is quite consistent with reversible denaturation of the enzyme.

FIG. 3. Difference absorption spectra of the heat-inactivated amylase (a solution of amylase of 0.55 mg per ml of M/400 borate buffer (pH 8.6, $[E]_{280\text{ m}\mu} = 1.40$) was heated at 85°C for 10 min. and, at varied times of subsequent incubation at 30°C, its absorbancy was determined against the unheated amylase solution.

Takehiko YAMAMOTO
 Akimasa NISHIDA
 Juichiro FUKUMOTO

Osaka City University
 Faculty of Science

Received July 21, 1964