Inhibition of Aggregation of Heat-Denatured Taka-Amylase A by Substrate

This article deals with the aggregation of Taka-amylase A (TAA) on heat inactivation and denaturation, in the absence and presence of substrate, starch. TAA, if substrate is present, is highly protected from heat inactivation[1]. This protective effect of starch is shown in Figure 1. Curve 1 and 1a show activity-incubation temperature relations (incubated for 10 min) in the absence and presence of starch, respectively. The presence of substrate gives rise to remarkable resistance against heat inactivation and shifts the activity-incubation temperature curve towards higher temperatures. This effect of substrate is mainly attributed to protection of the secondary structure of enzyme proteins from heat inactivation by the formation of enzyme-substrate and -product complexes which are thought to conjugate closely with the enzymatic activity.

The destruction of enzyme structure caused by heat treatment leads to inactivation and denaturation, and makes it possible for denatured enzyme molecules to combine to aggregates. For this reason, the amylase solution becomes turbid by the occurrence of heat denaturation of enzyme molecules. The turbidity is a measure of aggregation of denatured enzyme molecules.

The turbidity measured by the optical density at 500 nm runs parallel to the inactivation as shown in Figure 1, curve 2 and 2a. Moreover, the turbidity is also inhibited by the presence of substrate. The formation of enzyme-substrate and -product complexes prevents the formation of the aggregation of enzyme molecules.

The dependence of turbidity of amylase solution, heat-treated for 10 min at 60 °C in the absence and presence of substrate, on pH is shown in Figure 2. In alkaline regions, the turbidity is nearly zero but increases with decreasing pH in acid regions. It is of interest to note that curve 2, the difference between curve 1 and 1a, has a peak at the pH of the optimum activity (pH 5.6).

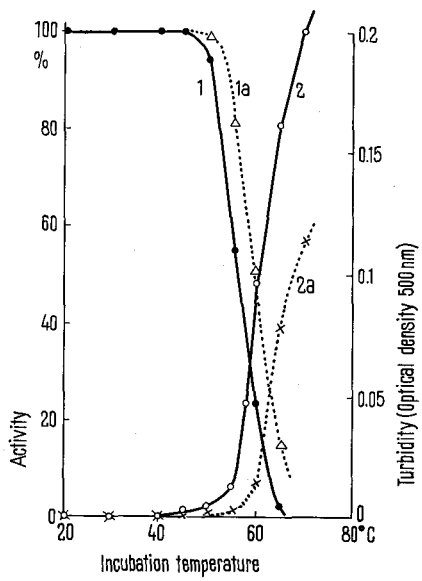

Fig. 1. Effect of incubation temperature on turbidity and activity. Curve 1 and 1a, the activity of heat-treated TAA in the absence and presence of starch, respectively; concentrations of TAA and starch, 0.036 and 6 mg/ml, respectively. Curve 2 and 2a, the turbidity measured by the optical density at 500 nm in the absence and presence of starch, respectively; concentrations of TAA and starch, 0.089 and 7.5 mg/ml, respectively. The heat-treatment was made for 10 min at pH 5.6 and the activity was measured at pH 5.6 (acetate buffer).

In conclusion, the results obtained above are considered to show that the enzyme protein in its optimum activity is most effectively stabilized by the formation of enzyme-substrate or -product complex and most highly protected from heat inactivation and denaturation, and also aggregation.

Native TAA can form at 0 °C an insoluble and stable complex with starch within the limited range of enzyme/substrate ratio, as in the case of pancreatic α-amylase and macro-dextrin[2]. But, under the present experimental conditions, the amylase/starch ratio is too small to form the insoluble complex in the native state and only soluble enzyme-substrate complex is formed. Therefore, the insoluble complex does not take part in the present experiment.

TAA was obtained from 'Taka-diastase Sankyo' by AKABORI's method[3] and the activity was measured by the blue value method[4] at 40 °C at pH 5.6 (acetate buffer).

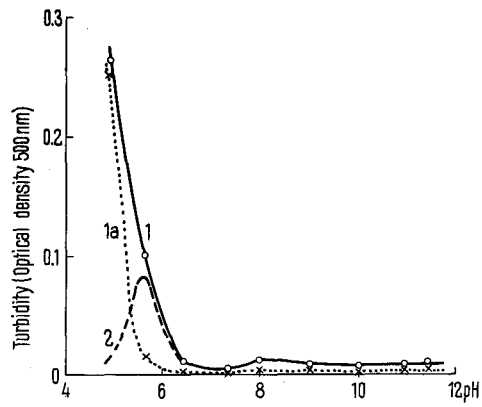

Fig. 2. Effect of pH on turbitity. Curve 1 and 1a, the turbidity measured by the optical density at 500 nm in the absence and presence of starch, respectively; curve 2, difference between curve 1 and 1a; concentrations of TAA and starch, 0.089 and 7.5 mg/ml, respectively. The heat-treatment was made for 10 min at 60 °C. TAA solutions were prepared in 0.02 *M* acetate, veronal or glycine buffers containing potassium chloride added to the final ionic strength of 0.1.

Zusammenfassung. Das Substrat beschützt stark die Taka-Amylase A nicht nur gegen Wärme-Inaktivierung und -Denaturierung, sondern auch gegen Wärme-Aggregation. Diese Schutzwirkung resultiert aus der Stabilisierung der Protein-Struktur durch Bildung der Enzym-Substrat- und -Produkt-Komplexe.

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December 24, 1965.

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