TEMPERATURE-DEPENDENT ASSOCIATION-DISSOCIATION OF *STREPTOCOCCUS LACTIS* INTRACELLULAR PROTEINASE[1, 2]

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Association-dissociation reactions of enzymes have been studied using dextran gel-filtration. (Kakiuchi *et al*, 1964; Winzor and Scheraga, 1963) Using this method, Winzor and Scheraga (1963) studied the rapidly reversible association of alpha-chymotrypsin. The strongly temperature-dependent exothermic association of B-lactoglobulin A at pH 4.65 has been well characterized by Townend *et al* (1960). During purification of the intracellular proteinase of *Streptococcus lactis*, evidence suggestive of an association-dissociation interaction of the enzyme was observed. The present communication reports on this association-dissociation.

The crude enzyme was purified by means of column chromatography on Sephadex G-50, DEAE Sephadex, and Sephadex G-100. The purified enzyme was found to be homogeneous to free-boundary electrophoresis, sedimentation velocity ultracentrifugation and to chromatography, excepting for the two forms to be reported on here. The detailed procedure will be published elsewhere. After purification, the enzyme was stored in a refrigerator at 3°. The enzyme was chromatographed on a 2.5 x 40.0 cm column containing Sephadex G-100. Elution was accomplished with 0.05 M phosphate, pH 7.5. I-Figure 1 represents the elution profile of the enzyme which was stored

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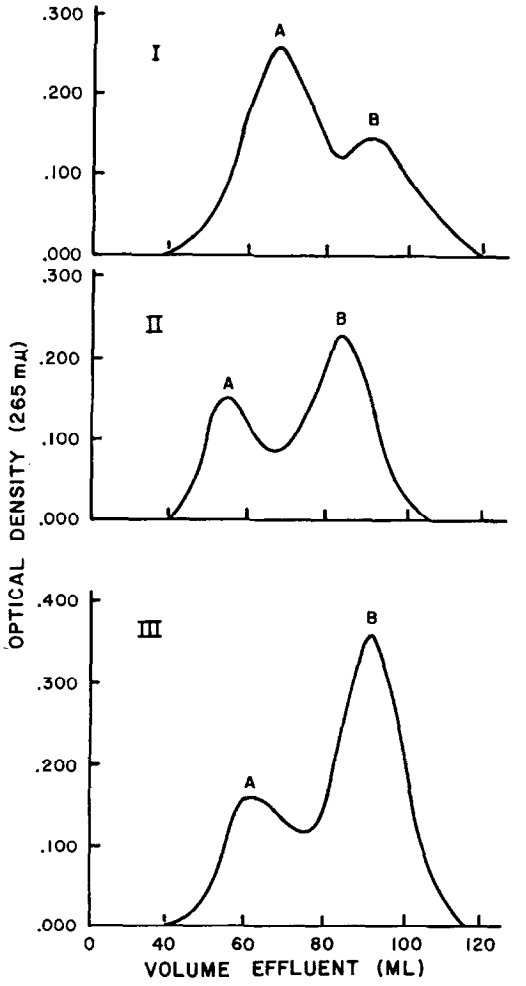

Figure 1. Elution profiles of S. lactis intracellular proteinase on Sephadex G-100. Eluting buffer 0.05 M phosphate, pH 7.5. I. Enzyme stored at 3°; conc. 5 mg/ml. II. Enzyme equilibrated 10 hr at 25°; conc. 5 mg/ml. III. Enzyme equilibrated 24 hr at 25°; conc. 5 mg/ml.

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at 3° until it was applied to the column equilibrated at room temperature (25°). The enzyme profile shows a fast-moving fraction (Fraction A) and a small amount of the slower-moving fraction (Fraction B). An aliquot of the enzyme stored at 3° was allowed to equilibrate 10 hr at 25° before chromatographing on Sephadex G-100. As shown in II-Figure 1, dissociation of the faster-moving fraction (A) had occurred, resulting in formation of a definite peak for the slower-moving fraction (B). Equilibration for 24 hr at 25°

resulted in even greater diminution of Fraction A and an increase in the slower-moving fraction (B) (III-Figure 1). Archibald approach-to-equilibrium experiments at 3° and 22° gave molecular weight values of 16,000 and 8,000 respectively. These values correspond to the predominating species observed in the elution profiles.

The dissociation of the enzyme was shown to be fully reversible. When the enzyme maintained at 22° was returned to 3°, an elution profile comparable to that of I-Figure 1 was obtained. Elution of the enzyme on Sephadex G-100 using as eluting agent 0.05 M phosphate buffer, pH 6.0, does not change the type of profile observed. Therefore, temperature appears to be a major contributing factor in determining the existing form.

Immediately following their separation, the enzymatic activity of each fraction was determined by incubating the enzyme with a casein substrate for 3 hr at 37° in 0.05 M phosphate buffer, pH 6.0. The liberation of tyrosine as a result of proteolysis was determined using the Folin phenol reagent. Under the conditions of assay one unit of activity was defined as a 0.1 optical density unit change at 650 mu, after 3 hr incubation. Protein concentration was determined by the Lowry method. Both fractions had a specific activity of 50 units per mg protein. The enzyme was also treated prior to chromatography with 2.0% EDTA (ethylenediaminetetraacetic acid) to test for metal ion effect. Elution profiles of EDTA-treated enzyme are the same as those presented here suggesting that metal ions are not involved.

In order to ascertain the equilibrium nature of the two forms of the enzyme, Fraction A was carefully separated from Fraction B. Immediately after separation the separate fractions (A and B) were chromatographed on an analytical Sephadex G-100 column (1.5 x 30 cm). These experiments, repeated five times, resulted in the elution of only that fraction (A or B) (I and III-Figure 2). These elution profiles provide evidence for homogeneity of the enzyme, since only the fraction applied to the column was eluted. The separated fractions were then stored at 3° for 24 hr and

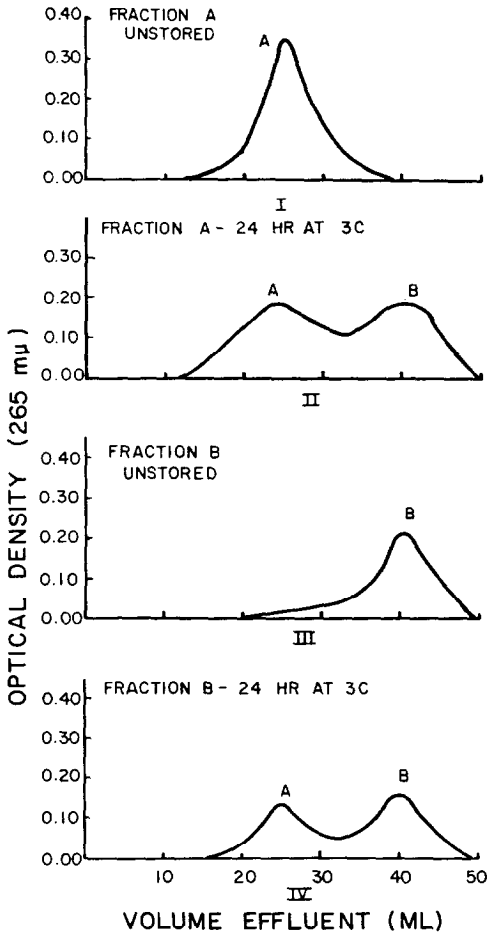

Figure 2. Elution profiles of separated fractions of Streptococcus lactis intracellular proteinase using 0.05 M phosphate buffer, pH 6.0.

following storage the fractions were rechromatographed under the same conditions as above. Rechromatography of the faster-moving fraction (A) showed the presence also of Fraction B (II-Figure 2). Similar results were observed when the slower-moving fraction (B) was rechromatographed (IV-Figure 2). These data provide strong evidence for an equilibrium and re-equilibration between the associated and dissociated forms of the enzyme.

Kauzmann (1959) has reported that hydrophobic bonds are more stable at room temperature than at 0°. It would appear, therefore, that forces other

than hydrophobic bonding are responsible for the association of the enzyme at low temperatures. Studies are currently in progress to further characterize this association-dissociation.

References

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