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The Denaturation of Trypsin

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The denaturation of α- and β-trypsin in alkaline and neutral solution was studied. The denaturation of α-trypsin was a strict second-order reaction at neutrality. However, the denaturation of β-trypsin was not a pure second-order reaction at the same pH. Calcium ion retarded the rate of β-trypsin denaturation to a greater extent than that of α-trypsin. In alkaline solution, trypsin has very short half life ($t_{\frac{1}{2}}$ < 30 minutes). On the other hand, the denaturation of immobilized trypsin in alkaline solution is a first-order reaction as is immobilized chymotrypsin. The rates of these two denaturations are similar. Calcium ion does not affect the rate of trypsin denaturation in alkaline solution.

Pancreatic proteases are unstable, even at neutral pH because of a cannibalistic denaturation (autolysis) involving two molecules of enzyme (1,2). Chymotrypsin has been studied previously (1) but trypsin is an enzyme which has been investigated thoroughly both in structure as well as catalytic function (3,4). It has been a model for those enzymes involved in the cascade reactions of blood clotting and complement binding (5,6). For example, many of the enzymes in blood clotting such as thrombin (7) and plasmin (8) are related to trypsin both in catalytic mechanism and in specificity. The transformation of malignant to normal cells has also been shown to occur by trypsin (9). The denaturation of trypsin is thus of importance in determining the concentration of active enzyme in many important physiological processes.

The denaturation of trypsin (EC 3,4,21,4) in solution has been studied for decades, but the conclusions are still ambiguous. The reason for these problems can be attributed to: (1) impure enzyme samples; (2) multiple conformation transitions of the enzyme molecules; and (3) changes in enzyme activity

This paper is dedicated to Prof. David Shemin on his seventieth birthday.

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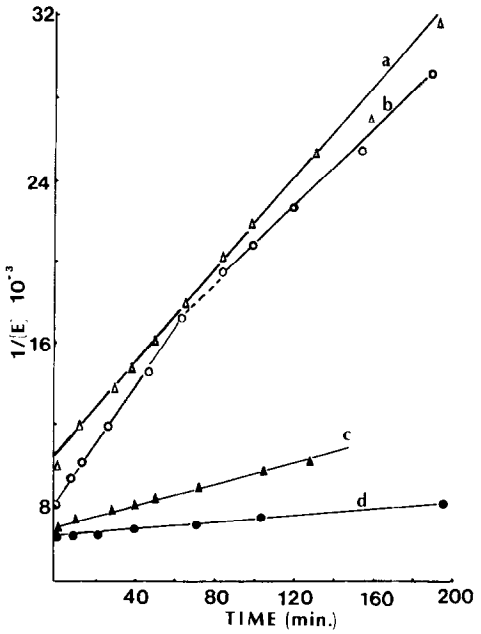

Fig. 1. The Denaturation of α- and β-Trypsin with and without Ca^{++}. α- and β-Trypsin (7.5 mg) dissolved in 1.5 ml of barbital buffer pH 8.2 were incubated at 25°. Trypsin samples (50 µl) were removed and titrated with *p*-Nitrophenyl-*p*-guanidinobenzoate as described in methods. (a) α-Trypsin and (b) β-Trypsin in 0.1M barbital buffer. (c) α-Trypsin and (d) β-Trypsin in 0.1M barbital buffer containing 0.1M Ca^{++}.

during measurement. Here we use purified α- and β-trypsin to study the stability of both enzymes at different pH's both in the presence and in the absence of calcium ion.

The denaturation of α-trypsin gave us a perfect second-order plot at neutral pH but the denaturation of β-trypsin at the same pH showed a definite curvature in the plot, indicating the transformation from one species to another (Fig. 1). The denaturation of β-trypsin was reported to be a second-order process previously (1). Since β-trypsin has one chain whereas α-trypsin has two, the curvature may reflect the transformation of β-trypsin to α-trypsin or denaturation. SDS gel electrophoresis of denatured β-trypsin indicates the formation of a significant amount of α-trypsin in addition to other denatured products. Thus both α- and β-trypsin showed a cannibalistic reaction, α-trypsin showed a pure one and β-trypsin showed a combination of a conformational change and a cannibalistic denaturation.

β-trypsin denaturation decreased about twenty-fold in the presence of 0.2M Ca^{+2}; however, in the presence of 0.2M calcium ion the denaturation of α-trypsin

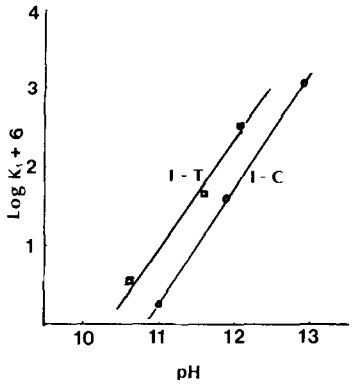

Fig. 2. I-T (immobilized trypsin) and I-C (immobilized chymotrypsin) were incubated in 0.1M carbonate buffer for 3 to 12 hours until 70% enzyme activity disappeared. Every 30 to 120 minutes, a small amount of the enzyme suspension was removed to measure the residual enzyme activity. The first-order rate constants (K_1) were obtained by plotting log [E] against time. The slope of (log K_1) + 6 (+6 is a constant to make the numbers in the figure positive) against pH is 1.1 ± 0.1 which indicates that the denaturation of immobilized enzymes in alkaline solution is first-order in [E] as well as [OH^-].

was decreased by 4.5 fold (see also Fig. 1). Apparently calcium ion is less effective in the denaturation of α-trypsin than in β-trypsin, probably because calcium ion binds to β-trypsin more tightly than to α-trypsin.

In alkaline solution α-trypsin loses activity very quickly and protein precipitation was noticed within 5 minutes. The kinetics of α-trypsin denaturation at pHs above 11 was thus complicated by the aggregation of protein molecules and which thus leads to loss of enzyme activity. To obviate this problem, we looked at the denaturation of immobilized trypsin where protein-protein interactions can be eliminated (9) and where we find very slow strictly first-order denaturation (Fig. 2). The absolute value of the immobilized trypsin denaturation at alkaline pH is very similar to that of immobilized chymotrypsin (Fig. 2). The calcium ion does not affect the rate of denaturation of immobilized trypsin in alkaline solution.

Materials and Methods: α-Chymotrypsin (Lot CDAG 35-A365) and trypsin (Lot TRL 31H971) were obtained from Worthington Biochemical Corp. α-Trypsin and β-trypsin were purified according to Schroeder and Shaw (11). Sulfoethyl-Sephadex was purchased from Sigma Chemical Co. *N*-Acetyl-L-tryptophan-*p*-nitrophenylate was obtained from Vega Biochemicals Co. and recrystallized. Tosyl-L-arginine methyl ester was obtained from Nutritional Biochemical Co. All other chemicals were reagent grade. Immobilized enzymes on Sepharose-CL-4B were prepared by the cyanogen bromide method following Porath et al. (12) Trypsin was titrated with *p*-nitrophenyl-*p*'-guanidinobenzoate as described by Ford et al. (13) The method of measuring immobilized enzymes activity was described in a previous paper (10).

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