ENZYMATICALLY ACTIVE COMPONENTS IN TRYPSIN AUTOLYSATES[1]

Sir:

The finding of a low molecular weight protein with enzymatic properties is of considerable interest for a study of the interrelationship of protein structure and function. Autolysis experiments with several enzyme preparations[2,3,4] have suggested the existence of enzymatically active intermediates which are considerably smaller than the original protein. The evidence, however, is only convincing in the case of pepsin.[5] The following experiments were performed to establish whether or not enzymatically active intermediates are formed during the autolysis of trypsin.

Commercial trypsin preparations[6] (I) of low specific activities and highly purified trypsin[7] (II) were used. Enzyme solutions, 5 mg./ml., were autolyzed at 25°, *p*H 9.1, in 0.05 *M* sodium borate buffer. After 1.75 hours the reaction was stopped by addition of acetic acid and calcium chloride solutions to give a final concentration of 0.01 *N* acetic acid, 0.03 *M* calcium chloride, and 0.03 *M* borate ion. Diffusion experiments were then carried out with these solutions at 25°. The properties of the 18/32 and 20/32 Visking cellophane membranes and the diffusion technique employed have been studied extensively by Craig.[8,9] The enzymatic activity of the material that had diffused through the membranes was measured by the azo-casein[10] and the more sensitive dye-fibrin method.[11]

It can be seen in Fig. 1 that the proteolytically active component of I, II and autolysate II, diffuses through the 20/32 membrane (permeable[9] to trypsin, mol. wt. 20,000) at the same rate and as a single component. Under identical experimental conditions of temperature, buffer concentration, *p*H, and enzyme concentration (as measured by the azo-casein method), the proteolytically active material of autolysate I diffuses through this membrane as two components. The minor component contains about 2% of the enzymatic activity of autolysate I. Experiments with 18/32 membranes (impermeable to ribonuclease,[9] mol. wt. 13,000) were also consistent with the presence, in autolysate I, of an enzymatically active component which diffuses at a faster rate than trypsin. Five per cent. of the proteolytic activity of the minor component of autolysate I was found to diffuse through this membrane in 12 hours at 0°. Under identical experimental conditions, but during a 24-hour period, these membranes were found to be impermeable to the proteolytically active component of I, II and the autolysate of II.

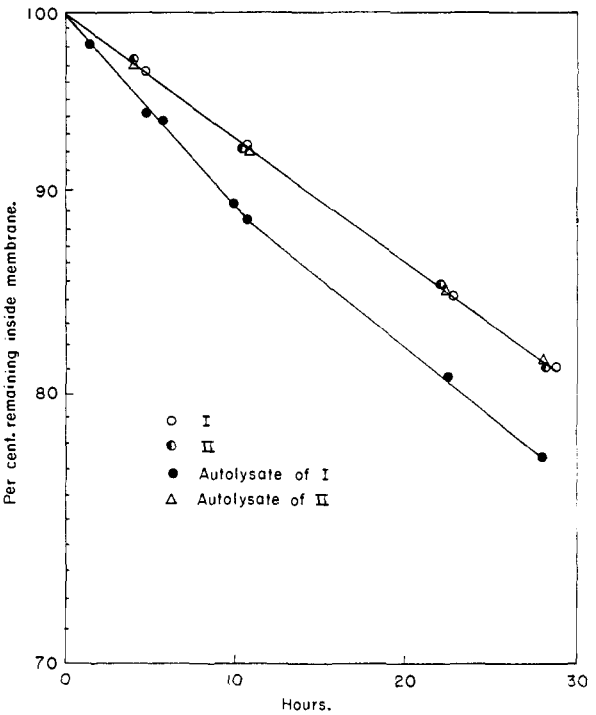

Fig. 1.—Diffusion curves through 20/32 Visking cellophane in 0.01 *N* acetic acid, 0.03 *M* calcium chloride, 0.03 *M* borate ion, at 25°. The difference in the observed initial rates of diffusion of I and the autolysate of I corresponds to the presence of about 2% enzymatically active material.

Diffusion experiments with I indicated that the low specific activities of these preparations were due to the presence of an enzymatically inactive protein which diffuses at the rate of trypsin. During autolysis this protein was found to hydrolyze twice as fast as trypsin. This suggests that this inert protein competes as substrate with the minor enzymatic component leading to accumulation of the latter, and would explain why only one active component is found in autolysate II. In support of these conjectures, it appears that autolysis experiments with equal mixtures of II and heat inactivated II lead to trace amounts of enzymatically active material that diffuses through the 18/32 membranes. These data would also support the hypothesis that the minor enzymatic component is a degradation product of trypsin I and not an impurity in this preparation.

The significance of these findings is that a proteolytic enzyme smaller in size than trypsin is produced through the autolysis of trypsin I, though in small quantities.

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(6) Once crystallized trypsin: Worthington Biochemical Corporation, Freehold, New Jersey; 0.9–1.3 *mM* *p*-toluenesulfonyl-L-arginine methyl ester (TAMe) hydrolyzed/min./mg. enzyme nitrogen.

(7) 1.9 m*M* TAMe/min./mg. enzyme nitrogen. Ratio of rates of hydrolysis L-tyrosine ethyl ester/TAMe is 1:400.

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THE STRUCTURE OF PHOTOSANTONIC ACID

Sir:

Renewed interest in the irradiation products of santonin (I) and other dienones is evident from the