A COMPARATIVE EVALUATION OF METHODS FOR DETERMINING THE ACTIVITY OF CHYMOTRYPSIN

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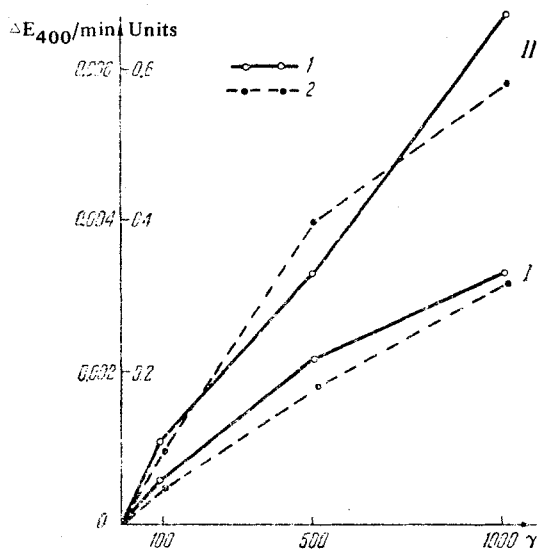
The characteristic difference between chymotrypsin and trypsin is the capacity of the former for coagulating milk. In addition to chymotrypsin, this capacity is possessed by pepsin, rennin, and bacterial proteinases. The milk-coagulating capacity is the basis of several methods for the quantitative determination of pepsin and chymotrypsin.

Enzyme	Concentration of the enzyme, γ/ml	SPOFA sample			Sample obtained in the laboratory		
		by the hydrolysis of APNA, ΔE_{400}/min	With respect to milk, units	According to Anson, TU	by the hydrolysis of APNA, ΔE_{400}/min	With respect to milk, units	According to Anson, TU
Chymotrypsin	1000	0.0032	0.33	8.28	0.0058	0.67	9.6
	500	0.0018	0.22	—	0.004	0.33	—
	100	0.0005	0.055	—	0.001	0.111	—
	80	0.0005	0.045	—	0.0006	0.062	—
	40	0	0	—	0.00025	0.037	—
	20	0	0	—	0.00008	0	—
	10	0	0	—	0	0	—
Trypsin	1000	0.00075	0.048	9.12	0	0	9.2
	500	0.00008	0.03	—	0	0	—
	250	0	0	—	0	0	—

At the present time, milk-coagulating methods are frequently used to determine pepsin [1-4], while protein substrates, hemoglobin and casein, and various synthetic esters of aromatic amino acids, tyrosine and phenylalanine [5, 6], have been proposed for the quantitative determination of chymotrypsin. Protein substrates are nonspecific for chymotrypsin and give values of the general proteolytic activity of a mixture of enzymes. The synthetic substrates are highly specific for chymotrypsin but are in rather short supply and not available for wide use.



Activity of chymotrypsin as a function of the concentration. I) Czech sample; II) laboratory sample. 1) Activity with respect to milk-acetate mixture; 2) activity with respect to APNA.

The present paper gives the results of a comparison of methods of determining chymotrypsin from its milk-coagulating capacity and from the hydrolysis of N-acetyl-D, L-phenylalanyl-p-nitroaniline (APNA).

Experimental

The experiments were carried out with crystalline trypsin and chymotrypsin, which we obtained by Northrop and Kunitz's method [7], and with commercial samples of these enzymes from Czechoslovakia (SPOFA products). The solutions were prepared in 0.0025 N hydrochloric acid.

N-Acetyl-D, L-phenylalanine p-nitroanilide was synthesized by the method of Tuppy et al. [5]. The method for determining activity was also taken from this paper. To 0.5 ml of the sample were added 3 ml of 0.1 m tris-citrate buffer with pH 8.0 and 0.04 ml of a solution of the substrate (33 mg of APNA in 10 ml of dimethylformamide). The mixture was incubated for 1 hr at 35° C and examined in a spectrophotometer (SF-4A) at 400 mμ against water.

The milk-coagulating capacity of the enzymes was determined by Kunitz's method [7] as modified by N. P. Pyatnitskii [8]. To 0.5 ml of the sample was added 5 ml of milk-acetate mixture (a mixture of equal volumes of defatted milk and 1 M acetate buffer with pH 5.0). Test tubes with the reaction mixture were placed in a water bath at 35° C. The time for the appearance of flocs of coagulated casein was measured on a stopwatch. The activity causing the coagulation of the milk-acetate mixture in 1 min was taken as unity.

A mixture was regarded as inactive if the coagulation time was more than 30 min (less than 0.033 units). The Anson activity was determined by the method given by N. G. Belen'kii [9].

The table gives the results of a comparison of the activities of two samples of chymotrypsin and two samples of trypsin determined by the methods mentioned (the concentration of the enzymes is expressed in γ/ml). It is evident that the factory sample of chymotrypsin had a lower activity than that obtained in the laboratory. If it is considered that the second sample approximates pure chymotrypsin, the sensitivity of the method based on the hydrolysis of APNA is 20 mg/ml. These results approximate to the data of Tuppy et al. [5]. The sensitivity of the milk-coagulation method is 40 γ of chymotrypsin per ml.

The figure shows curves characterizing the activity of chymotrypsin as a function of the concentration for both methods. As can be seen, they satisfactorily indicate a linear relationship within the limits of concentration of the enzyme studied.

The crystalline trypsin that we obtained did not hydrolyze APNA and did not cause the coagulation of milk-acetate mixture in a concentration of 1 γ/ml. The SPOFA trypsin possessed a slight chymotryptic activity (see table), obviously because of contamination of the sample with chymotrypsin. Thus, APNA and milk-acetate mixture react specifically only with chymotrypsin.

Summary

A method has been developed for determining the activity of chymotrypsin with respect to the coagulation of milk-acetate mixture in N. P. Pyatnitskii's modification. In specificity and sensitivity, this method approaches that based on the hydrolysis of N-acetyl-D, L-phenylalanine p-nitroanilide.

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