Chromatography of trypsinogen

The use of trypsin as a specific hydrolytic reagent in studies on the chemical structure of proteins has emphasized the need for preparations of this enzyme possessing a high degree of enzymic homogeneity. Attempts in this laboratory to purify trypsin by chromatography on the polycarboxylic acid resin IRC-50 (XE-64), in the manner accomplished for other basic proteins[1], have not been successful. Therefore, the chromatographic behavior of trypsinogen was studied, with the results reported in this communication.

The three samples of trypsinogen investigated had all been prepared by the method of KUNITZ AND NORTHROP[2], but only one contained no detectable amount of tryptic activity, as shown in Table I. The chromatographic behavior of the three samples was also found to be different.

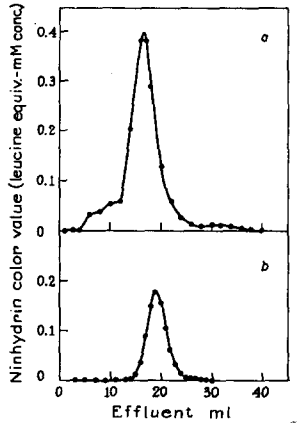

Fig. 1. Chromatography of trypsinogen on a 0.9 × 15 cm column of the sodium form of IRC-50 (XE-64) at 4°. Elution was performed with a 0.13 *M* phosphate buffer (0.15 *M* in respect to Na^+), pH 6.0, at a rate of 4 ml/h. A similar chromatogram was obtained by the use of a 0.2 *M* phosphate buffer, pH 5.8. The effluent was collected in 1 ml fractions and analyzed with ninhydrin[3]. *a*, 10 mg of once-crystallized trypsinogen (Worthington Biochemical Corp.), containing about 50 % $MgSO_4$. *b*, rechromatography of the fractions at the peak points of a chromatogram similar to that shown in *a*.

TABLE I

ENZYMIC ACTIVITY OF TRYPSINOGEN SAMPLES

Sample	*Trypsin units*	*Trypsinogen units*
1	< 0.05	1.55
2	0.16	1.52
3	0.23	1.06

Enzymic activity was determined with benzoyl-L-arginine amide (BAA) as substrate. Duplicate reaction mixtures were prepared, consisting of 0.2 ml trypsinogen solution, 0.5 ml trypsin solution for activation, and 0.05 ml *N* NaOH. The trypsinogen (1.37 mg containing about 50 % $MgSO_4$) was dissolved in 1.0 ml 0.13 *M* phosphate buffer, pH 6.0. The trypsin (0.004 mg) was dissolved in 1.0 ml 0.05 *M* *tris*-(hydroxymethyl)aminomethane buffer, pH 8.0, containing 0.3 *M* $CaCl_2$. Activity was determined in one reaction mixture immediately and in the other after standing at 4° for 23 h; in each case, the solution was warmed to 25°, 0.5 ml BAA solution (0.034 *M*, neutralized with NaOH and dissolved in 0.05 *M* *tris*-(hydroxymethyl)aminomethane buffer, pH 8.0) was added, and 0.05 ml aliquots were removed at 5 min intervals and pipetted into test tubes containing 0.1 ml 0.05 *N* HCl. The acidified aliquots were analyzed with ninhydrin[3], and the photometer readings were plotted against time for each reaction. Units of activity are given by the slope of the initial part of the curve, expressed as change in reading/min. Activity before activation is assumed to be a result of the presence of trypsin; the difference in activity before and after activation is attributable to trypsinogen.

Preliminary experiments showed that successful chromatography of trypsinogen at 25° was not possible, presumably owing to the instability of the zymogen, and all subsequent experiments were carried out at 4°. Curve *a* in Fig. 1 shows the results obtained with Sample 1 (Worthington Biochemical Corp., Freehold, New Jersey). The peak at 20 ml accounted satisfactorily for all of the ninhydrin color and activatable zymogen added to the column. Under the same chromatographic conditions, however, trypsinogen Samples 2 and 3 (from different sources) did not give a peak at about 20 ml. With Sample 2, 60 % of the applied ninhydrin color appeared as a long plateau starting at the column volume and terminating in a peak at 76 ml. With Sample 3, a tailing peak just behind the column volume and a large peak at 80 ml (which, however, did not react as a protein in the procedure of LOWRY *et al.*[4]) accounted for 64 % of the applied ninhydrin color; a total recovery of 6 % of the applied activatable zymogen was obtained in the effluent fractions at the position of trypsinogen Sample 1. That the difference in chromatographic behavior of the

three samples is not a result of the presence of trypsin was demonstrated by chromatographing a mixture of trypsinogen Sample 1 and trypsin; the position of the trypsinogen peak was not altered.

In order to determine the chromatographic behavior of trypsinogen subjected to a minimum of chemical manipulations, fresh beef pancreas was extracted with 0.25 N H_2SO_4 in the manner described by KUNITZ AND NORTHROP[2]. The extract was dialyzed in the cold against 0.01 N HCl, first in a Visking 23–32 casing overnight and then in a 20–32 casing for 5 h, in an attempt to remove trypsin inhibitor. Apparently, however, inhibitor was still present, for the existence of trypsinogen in the dialyzed extract could not be demonstrated by the method described in Table I. The dialyzed extract was lyophilized, the dry powder was made up in about 1/10 the original volume of distilled water, and a portion of this solution was chromatographed. Activatable zymogen in an amount equivalent to 8 units/g fresh tissue (see Table I for the definition of a unit) was found in the effluent at the position assumed by trypsinogen Sample 1. Since the total amount of zymogen placed on the column could not be determined, it is not known whether all of the trypsinogen of the pancreas is of the type represented by Sample 1, or whether there is also present some of the varieties represented by Samples 2 and 3.

The chromatographic results, considered in conjunction with the data in Table I, demonstrate clearly that more than one trypsinogen can exist. If, as seems likely, only a single molecular species is elaborated by beef pancreas, the other trypsinogen(s) could only have arisen as a result of secondary changes, probably induced by enzymes. It appears that there may be a group of trypsinogens and trypsins analogous to the several chymotrypsinogens and chymotrypsins already known to exist[5,6].

The chromatographic conditions described in this communication are useful for the purification of trypsinogen of the type exemplified by Sample 1. Chymotrypsinogen, the most probable contaminant, is retarded much more by the column. The purification of trypsinogen Sample 1 is illustrated in Fig. 1, *b*. Activation of such purified trypsinogen should provide trypsin free from other proteolytic enzymes*.

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* The chromatographic purification of trypsinogen on IRC-50 has also recently been accomplished independently by P. J. KELLER and H. NEURATH (personal communication from Professor NEURATH).

Effect of chloramphenicol on protein and nucleic acid synthesis in isolated thymus nuclei*

Chloramphenicol has become an important tool in the study of the possible relationship of protein and nucleic acid syntheses. In some systems[1,2] chloramphenicol inhibits protein synthesis without affecting nucleic acid synthesis, while in others[3,4] both protein and nucleic acid syntheses are strongly inhibited. A completely different effect has been reported by ALLFREY *et al.*[5] in isolated calf-thymus nuclei where chloramphenicol (at a concentration of $3.2 \cdot 10^{-4} M$) failed to inhibit the incorporation of ^{14}C-amino acids into nuclear protein. As this effect suggests that protein synthesis in nuclei might differ somehow from that in other systems, we have investigated in more detail the effect of chloramphenicol on both ^{14}C-amino acid incorporation into protein and ^{32}P-orthophosphate incorporation into the nucleic acids of isolated calf-thymus nuclei.

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