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HUMAN PANCREATIC ENZYMES: PROPERTIES OF TWO MINOR FORMS OF CHYMOTRYPSIN

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SUMMARY

Two minor forms of chymotrypsin have been isolated from activated extracts of acetone powders of human pancreas. Except for charge differences both appear to be identical in physical and chemical properties. Comparison of these enzymes with human δ-chymotrypsin indicates a high degree of similarity in both amino acid composition and in the method of activation. However, differences in specific esterase activity would suggest that each of the three enzymes was derived from a separate, distinct zymogen.

Examination of the proteins in both human pancreatic juice[1,2] and in activated extracts of human pancreas[3] have shown the existence of multiple forms of chymotrypsinogen and chymotrypsin. In a recent communication[3] we described both the fractionation of chymotrypsin activity in activated extracts of human pancreas into two distinct forms as well as the purification and properties of the major chymotrypsin component (chymotrypsin II). In this report we wish to summarize our results on the resolution of chymotrypsin I, the minor chymotrypsin fraction, into two homogeneous species which we have designated chymotrypsins IA and IB, respectively. Some of the properties of these enzymes are also described, particularly with respect to those of chymotrypsin II.

All chymotrypsin esterase assays and all analytical techniques were performed by methods previously described[3]. Chymotrypsins IA and IB were prepared by further fractionation of the chymotrypsin I fraction obtained during the preparation of both human trypsin and human chymotrypsin II[3].

Purification of enzymes. The chymotrypsin I fraction obtained initially from 20 g of acetone powder was dialyzed against 5 mM piperazine-*N*,*N*′-bis-(2-ethanesulfonic acid) (PIPES)–HCl buffer, pH 6.75, and then applied to a DEAE-Sephadex column equilibrated against the same buffer. After washing out inactive protein with starting buffer the column was developed with a linear gradient from zero to 0.1 M NaCl in the PIPES buffer. This resulted in the resolution of chymotrypsin esterase activity into two components as shown in Fig. 1. The fractions outlined under each

Abbreviation: PIPES, piperazine-*N*,*N*′-bis-(2-ethanesulfonic acid).

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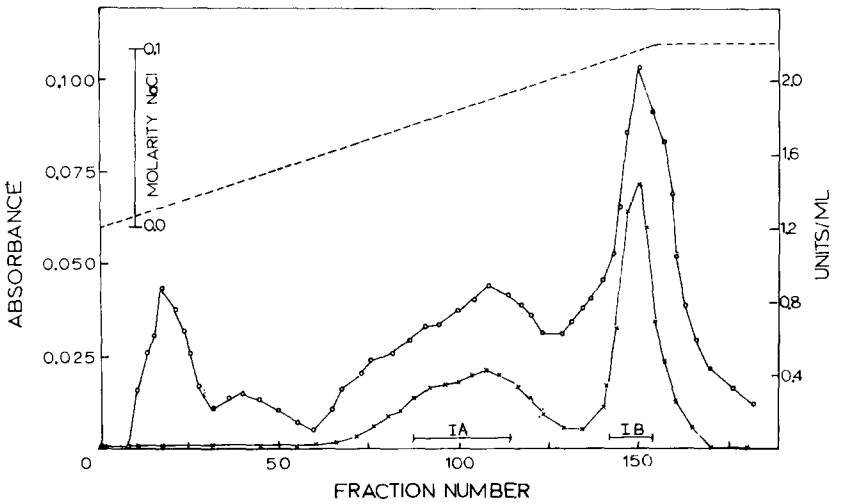

Fig. 1. DEAE-Sephadex chromatography of pooled human chymotrypsin I. The column was equilibrated with 5 mM PIPES-HCl buffer, pH 6.75, and eluted with a linear gradient from zero to 0.1 M NaCl as indicated. Curves are: absorbance at 280 nm (○—○), left ordinate; activity against *N*-acetyl-L-tyrosine ethyl ester (×--×), right ordinate.

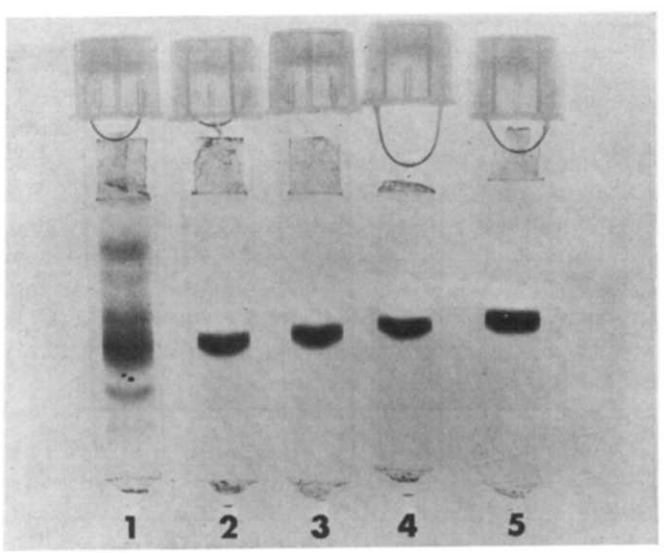

Fig. 2. Polyacrylamide disc electrophoresis of human pancreatic proteases. Direction of migration is from anode (top) to cathode (bottom). pH 4.30, 7.5% gel; 1, whole homogenate; 2, human trypsin; 3, human chymotrypsin IA; 4, human chymotrypsin IB; 5, human chymotrypsin II.

peak had constant specific activity and were pooled, dialyzed against 1 mM HCl, and lyophilized. The yield of chymotrypsin IA was 8 mg and that of chymotrypsin IB was 11 mg.

Each chymotrypsin component was found to be homogeneous by high speed sedimentation equilibrium experiments[4] at pH 2.0 and by disc electrophoresis at

pH 4.3 in 7.5% gels[5]. The latter are shown in Fig. 2 in comparison with those of human trypsin and human chymotrypsin II.

The amino acid compositions of chymotrypsins IA and IB were found to be very nearly identical and differed only slightly from that of chymotrypsin II (Table I).

TABLE I

COMPARATIVE AMINO ACID COMPOSITIONS OF HUMAN CHYMOTRYPSINS

Amino acid	*IA*	*IB*	*II*
Tryptophan	7	7	5
Lysine	17	17	16
Histidine	4	4	4
Arginine	7	7	8
Aspartic acid	25	25	23
Threonine	17	17	19
Serine	23	23	21
Glutamic acid	17	17	18
Proline	13	14	15
Glycine	24	24	25
Alanine	20	21	24
Half-cystine	8	8	8
Valine	25	25	22
Methionine	2	2	2
Isoleucine	12	12	11
Leucine	20	21	18
Tyrosine	3	3	3
Phenylalanine	8	8	7
Total	252	255	249

Similarly, the molecular weights determined from the sedimentation equilibrium data, as well as the amino and carboxyl terminal residues were essentially the same in all three cases. As can be seen in Table II the only major difference between the

TABLE II

COMPARATIVE PROPERTIES OF HUMAN CHYMOTRYPSINS

Type	*Mol. wt.*	*Amino terminal*	*Carboxyl terminal*	*Specific activity*
IA	26 300	Ile, Cys	Asn, Ser	10.8
IB	26 240	Ile, Cys	Asn, Ser	7.5
II	25 800	Ile, Cys	Asn, Ser	30.0

three enzymes was found to be in their relative specific activities towards the chymotrypsin esterase substrate *N*-acetyl-L-tyrosine ethyl ester. From the amino and carboxyl terminal residues found it can be concluded that all three proteins are composed of only two polypeptide chains. This was confirmed by gel electrophoresis in sodium dodecyl sulfate of the DFP-treated enzymes. Two peptides were detected in each case, having molecular weights of 25 000 and 1000, respectively. The latter moved as a diffuse band with the tracking dye and stained very poorly.

Examination of peptide maps after digestion of the aminoethyl derivative of the A chain of insulin with each of the three chymotrypsins indicated essentially

identical specificities towards this polypeptide substrate except for the presence of one extra component found in chymotrypsin IB digests which could only barely be detected in the other two samples.

Finally, immunodiffusion of the three chymotrypsins against rabbit anti-human chymotrypsin II yielded solid precipitin lines in all three cases with no evidence of spur formation (Fig. 3).

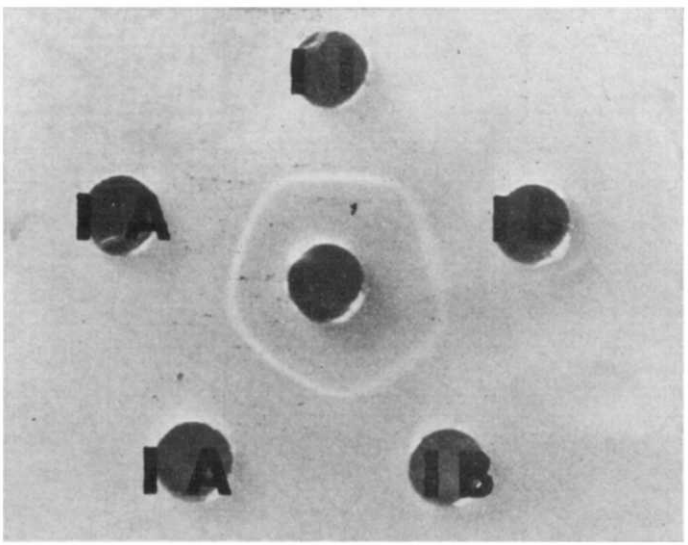

Fig. 3. Immunodiffusion of human chymotrypsins. Center well, rabbit antihuman chymotrypsin II; outer wells as depicted.

Experiments designed to determine the types of proteolytic enzymes in human duodenal juice have resulted in the detection of the same three chymotrypsin species described here and previously[3]. Thus, it is unlikely that our preparations are artifacts of the activation or isolation process. The similarity in properties between chymotrypsins IA and IB is quite striking. Both have essentially identical molecular weights, amino acid compositions, and amino and carboxyl terminals. The two proteins are, however, easily separated by ion-exchange chromatography, indicative of a significant difference in charge. Since the amino acid compositions are so much alike it is likely that differences exist in the degree of amidation of aspartic and glutamic acid residues in the individual proteins.

We have previously proposed that chymotrypsin II is analogous to bovine δ-chymotrypsin both because of its high specific activity as well as its two-chain structure. Chymotrypsins IA and IB are also composed of two chains and have similar amino and carboxyl terminals as chymotrypsin II. Yet, their specific activities against esterase substrates are significantly lower. Possibly, the slight differences in amino acid composition between these two proteins and chymotrypsin II are responsible for structural differences resulting in the increased activity of the latter.

Chymotrypsin C, isolated from porcine pancreas[7], is an enzyme with low activity towards *N*-acetyl-L-tyrosine ethyl ester but with significant activity towards

benzoyl-L-leucine ethyl ester. We have attempted to demonstrate this activity in both crude activated extracts of human pancreas as well as with each of the purified chymotrypsins. In no case could we detect hydrolysis of this substrate, suggesting the absence of this enzyme in the human species. The results presented would account for the presence of three different zymogens in unactivated pancreatic juice. Such an interpretation would support the pattern reported by Figarella[2] except for their contention that one of these components represents chymotrypsinogen C.

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