A Second Activation Peptide from Bovine Cationic Trypsinogen

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1. Although only one activation peptide of bovine cationic trypsinogen has been reported previously, the peptide fraction obtained from activation mixtures shows several bands on paper electrophoresis at pH 6.5. 2. The major band was the peptide previously described. The band second in intensity of staining with ninhydrin (10–20% of that of the main band, as judged by eye) had an electrophoretic mobility consistent with its being related to the main peptide. It appeared on activation both of bulk commercial samples of trypsinogen and, as the Appendix shows, of samples prepared from pancreases obtained at the local abattoir. 3. The second peptide proved to be Phe-Pro-Val-Asp-Asp-Asp-Lys, and we conclude that it is another activation peptide. We discuss briefly the genetic and phylogenetic implications of our findings.

The sequence of activation peptides liberated during the conversion of trypsinogen into trypsin has been determined for several mammalian species and a few fishes [see Guy *et al.* (1976) for a summary of these sequences]. So far, the structure of the mammalian peptides appears to be of the form X-Asp-Asp-Asp-Lys, where X represents either one or three further residues. Apart from the possible significance of such peptides in mechanistic and phylogenetic studies, one has been shown (Abita *et al.*, 1973) to inhibit gastric secretion.

So far, three different activation peptides from bovine trypsinogens have been sequenced, namely Val-Asp-Asp-Asp-Lys from cationic trypsinogen (Davie & Neurath, 1955), and Ser-Asp-Asp-Asp-Lys and Phe-Pro-Ser-Asp-Asp-Asp-Lys from anionic trypsinogens (Louvard & Puigserver, 1974). Only one activation peptide has been reported from bovine cationic trypsinogen, although closely related species such as red deer (*Cervus elephas*) (Bricteux-Grégoire *et al.*, 1971b), roe deer (*Capreolus capreolus*) (Bricteux-Grégoire, 1970), sheep (*Ovis aries*) (Bricteux-Grégoire *et al.*, 1966) and goat (*Capra hircus*) (Bricteux-Grégoire *et al.*, 1971a) possess two, namely Val-Asp-Asp-Asp-Lys and Phe-Pro-Val-Asp-Asp-Asp-Lys.

In the course of other work (Webster & Offord, 1972) we had to prepare trypsin from chromatographically purified bovine cationic trypsinogen. We noticed that samples of the activation mixture from which the trypsin had been removed showed several peptide bands on paper electrophoresis at pH 6.5. We found that the major band was, as its strongly anodic mobility suggested, the known activation peptide Val-Asp-Asp-Asp-Lys. The band that was next

in order of intensity of staining (about 10–20% of the intensity of the major band, as judged by eye) migrated less rapidly to the anode than the main band, but still at a fairly high rate for a component of a tryptic digest. This observation led us to suspect that the second peptide might have within itself a region related to the remarkably acidic sequence of the main activation peptide. The present paper describes the confirmation of this hypothesis. The sequence of the peptide is Phe-Pro-Val-Asp-Asp-Asp-Lys, and we are led to conclude that bovine cationic trypsinogen produces a second activation peptide.

Materials and Methods

Bovine cationic trypsinogen (once-crystallized) was purchased from Worthington, Freehold, NJ, U.S.A. and from Miles-Seravac, U.K., Holyport, Maidenhead, Berks. The zymogen was purified by ion-exchange chromatography as described by Webster & Offord (1972).

The trypsinogen was activated by the method of Maroux & Desnuelle (1969). The material was used in 0.5 g portions, and after activation the mixture was applied to a column (95 cm × 3.4 cm) of Sephadex G-25 in 0.05 M-sodium citrate buffer, pH 3.7 (measured by glass electrode at 18°C). When the effluent was monitored at 240 nm, three peaks were seen to emerge, at 0.40–0.56, 0.56–0.76 and 0.8–1.2 column-volumes. These peaks proved to be trypsin, the activation peptides and salt respectively. The material from the second peak was freeze-dried, dissolved in as small a volume of water as possible and applied to a second column (96.5 cm × 2.3 cm) of Sephadex G-25 in water. Two large peaks were monitored at 240 nm, which were eluted at 0.6–0.7 and 0.7–1.0 column-volumes. The first of these proved to

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contain the peptides and the second the salts. The eluate containing the peptides was freeze-dried, and the resulting material fractionated by high-voltage paper electrophoresis at pH 6.5. This technique, and all other paper and sequence methods, were carried out as described by Gonzalez & Offord (1971).

Results and Discussion

Two peptides were found to be present in sufficient quantity to permit their isolation. The major peptide had an electrophoretic mobility (relative to aspartic acid = -1.0) of -0.9 at pH 6.5. End-group and amino acid analysis confirmed that it was Val-Asp-Asp-Asp-Asp-Lys, the activation peptide of Davie & Neurath (1955). The mobility of this peptide, predicted by the method of Offord (1966), is -0.89. The second peptide, which stained with ninhydrin with 10–20% of the intensity of the major band (as judged by eye), had a mobility value of -0.7. At least two other peptides were seen, with even smaller negative mobilities, but they were not present in sufficient quantities to permit their characterization. It is therefore possible that there are more than two activation peptides.

The second peptide had the amino acid composition (18 h hydrolysis) Lys_{1.0}, Asp_{4.0}, Pro_{1.0}, Val_{1.1}, Phe_{0.9} (mobility predicted if there were no side-chain amides = -0.72). The sequence was determined as shown

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- (a) Phe-Pro-Val-Asp-Asp-Asp-Asp-Lys
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- (b) Val-Asp-Asp-Asp-Asp-Lys
- (c) Ser-Asp-Asp-Asp-Asp-Lys
- (d) Phe-Pro-Ser-Asp-Asp-Asp-Asp-Lys

Fig. 1. Sequences of the putative second activation peptide (a), the major activation peptide (Davie & Neurath, 1955) (b) and the activation peptides of bovine anionic trypsinogen (Louvard & Puigserver, 1974) (c and d)

→ indicates the result of a dansyl-Edman determination. ⇒ indicates that determination was confirmed by a semi-quantitative amino acid analysis of a sample of the truncated peptide. N^α-Dansyl(5-dimethylaminonaphthalene-1-sulphonyl)-lysine was seen at every stage (except after seven cycles of degradation, when the quantity of material had become too small to give any visible spots). Secondary 'dansyl' spots were seen after the first and second cycles of degradation, particularly after short hydrolysis of the dansylated peptides. These were taken to be dansyl-prolylvaline and dansylvalylaspartate respectively; these assignments were consistent with the behaviour of the spots on t.l.c.

in Fig. 1. It will be noted that the peptide cannot represent contamination by the longer activation peptide of anionic trypsinogen (Fig. 1d). Although it seemed most unlikely that the commercial zymogen could be contaminated with material from another species (an assumption confirmed for us beyond reasonable doubt, after enquiries within the company by one of the manufacturers), the similarity of the sequence to one of those found in the cationic trypsinogens of other species of the Artiodactyla ('even-toed ungulates') led to the check on the bovine origin of the peptide described in the Appendix. The Appendix shows that it was not easy to prepare sufficient of the peptide from a single pancreas, but that it appeared, in roughly the same amount relative to the main peptide as that found above, in material prepared from two pancreases. This suggests to us that the two activation sequences probably occur in one animal; the alternative hypothesis, that one of the pair of animals contributed 80% or more of the trypsinogen exclusively in the 'Val' form, and that the other contributed 20% or less, exclusively in the 'Phe' form, seems to be less likely, although we have not disproved it.

The ox (*Bos taurus*) therefore joins most of the other species of the Artiodactyla so far examined in having two activation peptides of its cationic trypsinogen, Val-Asp-Asp-Asp-Asp-Lys and Phe-Pro-Val-Asp-Asp-Asp-Asp-Lys. The relative amounts of the two are not the same in all the species; for example, the longer peptide predominates in the goat. It appears to be customary to assume that the two forms derive from trypsinogens coded for by different cistrons, rather than that they represent stages in the degradation of a single sequence. We assume that the reasoning behind this view is that the sequence -Pro-Val- is not commonly cleaved by peptidases.

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APPENDIX

The Production of the Second Activation Peptide from Trypsinogen Isolated from Individual Pancreases

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The need for a check on the bovine origin of the second peptide [see main paper, Webster & Offord (1977)] was met by isolating trypsinogen from ox pancreases taken at the abattoir (under the supervision of P. C. M.) from freshly killed animals. Trypsinogen was isolated from these pancreases by the method of Northrop *et al.* (1948), scaled down by the appropriate factor. For a single pancreas this factor was 12 and our preparation produced approximately the expected quantity of material. This unfortunately proved insufficient in our hands, when the method of Balls (1965) was used, to give enough crystalline material. However, two pancreases repeatedly gave sufficient material (usually about 30mg) for further study. The crystalline zymogen from such a preparation was activated as described by Maroux & Desnuelle (1969), and the peptide fraction was isolated by using a scaled-down version of the method described in the main paper (Webster & Offord, 1977).

In a typical experiment, 15 mg of activated trypsinogen (in 1.5 ml of activation buffer) was applied to a column (40 cm × 1.2 cm) of Sephadex G-10 packed in 1 mM-HCl and eluted with the same solvent. The effluent was monitored at 240 nm. On electrophoresis of the peptide fraction (0.6–0.75 column volumes) at pH 6.5, and after staining with ninhydrin, both peptides were visible in much the same relative amounts as seen on the purchased material mentioned in the main paper (Webster & Offord, 1977). The

quantity of material obtained after elution from the electrophoresis paper was insufficient to give a satisfactory estimate of the amino acid composition. However, it was at least possible to show that the minor peptide here probably contained the same *N*-terminal amino acid residue as that obtained from purchased material. On dansylation of the peptide fraction from the column by the method of Gray (1972), the only visible spots corresponded to *N*^ε-dansyl*-lysine, dansylvaline and dansyl-phenylalanine.

* Abbreviation: dansyl, 5-dimethylaminonaphthalene-1-sulphonyl.

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