# ON PORCINE CHYMOTRYPSINOGEN B

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Whereas three zymogens with potential * ATEE **-splitting activity (chymotrypsinogens A and B, and one of the subunits (fraction II) of procarboxypeptidase A) are known to be present in bovine pancreas, only two have so far been identified in porcine pancreas. These zymogens are: a cationic chymotrypsinogen A accounting for about 70% of the ATEE-splitting activity of the tissue [1,2] and an anionic zymogen, designated chymotrypsinogen C and probably related to bovine fraction II (ref. [3]). The purification and some properties of a second anionic chymotrypsinogen of porcine origin, probably similar to bovine chymotrypsinogen B, are reported below.

Complete purification of porcine chymotrypsinogen B was achieved in three steps starting from 15 g lots of lyophilized pancreatic juice. The first step was a chromatography on DEAE-cellulose in a Tris-acetate buffer (pH 6.0), 10 mM in Tris. After the emergence of the cationic proteins, the column was eluted with a linear concentration gradient of NaCl (0–0.35 M). This resulted in the separation of a number of already known anionic enzymes (lipase, deoxyribonuclease, procarboxypeptidases A and B) and of two peaks with potential ATEE-splitting activity. One emerged at a 0.27 M NaCl concentration and corresponded to chymotrypsinogen C. The other (at 0.20 M NaCl) was located between deoxyribonuclease and the procarboxypeptidases which were the main contaminants.

In the second step, the proteins under this latter peak were filtrated through Sephadex G-100 at low ionic strength (sodium citrate buffer (pH 6.0), 30 mM in citrate). Two peaks were obtained, one at the end of the second retention volume of the column, containing chymotrypsinogen B and one form of procarboxypeptidase A, the other at the beginning of the third retention volume, containing procarboxypeptidase B and another, apparently lighter form of procarboxypeptidase A. The last step of the purification was a filtration through Sephadex G-100 at high ionic strength (Tris-acetate buffer (pH 6.0), 0.1 M in Tris and 0.4 M in NaCl). Under these new conditions, chymotrypsinogen B emerged at the beginning of the third volume and it was well separated from procarboxypeptidase A which remained in the second volume The ionic strength dependence of the monomer-polymer equilibrium of chymotrypsinogen B at pH 6.0 permitted the complete elimination of procarboxypeptidases A and B by means of two successive Sephadex filtrations.

The preparations thus obtained were found homogeneous by ultracentrifugation and disc electrophoresis. The yield was 60–70 mg of pure product from 15 g lyophilized juice containing 4 g proteins.

When 6 or 10 mg/ml solutions of a pure sample of the zymogen were filtrated through Sephadex G-100 at high ionic strength (Tris-acetate buffer (pH 6.0) 0.1 M in Tris and 0.4 M in NaCl), elution occurred at nearly the same volume as with bovine chymotrypsinogens A and B. Therefore, the molecular weight of the monomer was assumed to be about 26,000. Polymerization was again observed at lower ionic strength and it was found to be strongly concentration-dependent.

Using the value 26,000 for the molecular weight of the monomer, the following amino acid composition was derived from an analysis of the protein by

* Activity appearing after activation by trypsin.

** The following abbreviations are used: ATEE, acetyl-L-tyrosine ethylester; STI, trypsin inhibitor from Soya beans.

the automatic technique of Spackman, Stein and Moore [4]: $Ala_{22}$, $Arg_8$, $Asx_{20}$, $Cys_{10}$, $Glx_{15}$, $Gly_{22}$, $His_3$, $Ile_{11}$, $Leu_{16-17}$, $Lys_6$, $Met_2$, $Phe_8$, $Pro_{14}$, $Ser_{28}$, $Thr_{17}$, $Trp_{13}$, $Tyr_4$, $Val_{24-25}$ (total number of residues, 243–245). This composition is quite similar to the ones already determined for porcine chymotrypsinogen A (ref. [2]), bovine chymotrypsinogen A (ref. [5]) and bovine chymotrypsinogen B (ref. [6]). However, it is markedly different from the composition indicated for porcine chymotrypsinogen C by Folk and Schirmer [3] and for bovine fraction II by Brown et al. [7]. The zymogen contains a single peptide chain with an N-terminal half-cystine and a C-terminal asparagine.

After full activation with 10% trypsin for 15 min at 0° pH 7.9 in the presence of 0.1 M β-phenyl propionate, a sample of porcine chymotrypsinogen B was oxidized by performic acid and the short chain formed during the process (chain A) was extracted with water and purified by filtration through Sephadex G-50 in 5 mM HCl and electrophoresis-chromatography on paper [2,6,8]. Its amino acid composition ($Ala_1$, $Arg_1$, $Cys_1$, $Gly_2$, $Ile_1$, $Leu_2$, $Pro_3$, $Ser_2$, $Val_2$) was identical with that found for chain A of porcine chymotrypsinogen A (ref. [2]) and consequently very similar to the composition of the corresponding chain in bovine chymotrypsinogens A and B (refs. [6,8]). Moreover, the first two residues in the insoluble oxidized material left behind after extraction of chain A were isoleucine and valine.

These results are consistent with the view that the activation by trypsin of the new porcine chymotrypsinogen is brought about by the specific splitting of the 15th bond ($Arg_{15}$–$Ile_{16}$ bond) of the molecule. Hence, this zymogen appears to be closely related to the other known chymotrypsinogens, with the possible exception of porcine chymotrypsinogen C and bovine fraction II. It was provisionally designated here chymotrypsinogen B because of its anionic character. But the real extent of its homology with bovine chymotrypsinogen B will only be ascertained when its full sequence is known.

## References

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