THE AMINO ACID SEQUENCE OF A HITHERTO UNOBSERVED SEGMENT FROM PORCINE PEPSINOGEN PRECEEDING THE *N*-TERMINUS OF PEPSIN

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1. Introduction

Ong and Perlmann [1] have analysed the amino acid sequence of the first 41 residues in porcine pepsinogen. This fragment is cleaved off during the irreversible transformation of pepsinogen into pepsin (EC 3.4.4.1). Amino acid analyses suggested that this *N*-terminal fragment was directly followed by the peptide chain of the active pepsin, but no overlapping sequence was analysed by Ong and Perlmann, and the question of the continuation of the *N*-terminal fragment was left open. By activation of porcine pepsinogen with an *Aspergillus* proteinase Stepanov et al. [2] found that the *N*-terminal isoleucine of pepsin in the pepsinogen was preceeded by leucine. This, however, gave no connection to the *N*-terminal amino acid sequence determined by Ong and Perlmann.

At this laboratory we have recently analysed an amino acid sequence of bovine pepsinogen overlapping the *N*-terminus of bovine pepsin [3]. In order to be able to compare these results with porcine pepsinogen, we have searched for an overlapping sequence from this zymogen. Amino acid analyses and studies on the primary structure of porcine pepsinogen, summarized by Perlmann [4] show that the basic amino acids in porcine pepsinogen are clustered at the *N*- and *C*-termini, thus a tryptic digestion restricted to lysyl- and arginyl bonds only should give no cleavage between lysine no 36 and an arginine group 20 residues from the *C*-terminus. This means that a tryptic digest of porcine pepsinogen should produce a series of low molecular weight peptides and one large fragment beginning with the last part of the activation fragment and continuing with the *N*-terminus of the pepsin. On basis of this strategy we have prepared such a fragment and analyses substantiate the homology with bovine pepsinogen.

2. Experimental

Crystalline porcine pepsinogen (Worthington) was reduced and S-carboxymethylated as described by Hirs [5] except for the use of a 10 times excess of dithiothreitol instead of a 100 times excess of mercaptoethanol. The amount of iodoacetate was correspondingly reduced. Fifty mg of the dialyzed and freeze-dried preparation was digested with TPCK*-treated trypsin (Worthington), enzyme:substrate ratio (mole per mole) 1:50 in 0.01 M $CaCl_2$ at 11°C for 10 min, pH was kept constant at 7.8 with 0.05 M NaOH [6]. The reaction was terminated by addition of basic pancreatic trypsin inhibitor (Trasylol®, Bayer), and the digest was submitted to gel filtration on a Sephadex G-100 column (1.6 × 91 cm). Eluent 0.05 M NH_4HCO_3, pH 8.0; flow rate 8 ml/hr. Measured by E_{280} one major peak emerged followed by some minor peaks.

Homogeneity of the major peak was ascertained by parallel analysis of three fractions from the ascending, the central and the descending part of the peak. These fractions were examined by disc electrophoresis in polyacrylamide gel (7.5%, pH 8.5) [7]. After staining with Coomassie brilliant blue one predominant band and only faint traces of impurities were observed.

* Abbreviations: SDS = sodium dodecyl sulfate; TPCK = tocyl phenylalanyl chloromethyl ketone; Dns = 1-dimethylamino-naphthalene-5-sulfonyl.

Table 1
The amino acid sequence overlapping the activation fragment and the pepsin moiety of porcine pepsinogen.

	36	37	38	39	40	41	42	43	44	45	46	47
Porcine	LYS–	TYR–	Phe–	Pro–	GLU–	ALA–	ALA–	Ala–	LEU–	Ile–	Gly–	Asp
	→	→	→	→	→	→	→	→	→	→	→	→
										↑		
Bovine	LYS–	TYR–	Ile	–Arg–	GLU–	ALA–	ALA–	Thr–	LEU–	Val–	Ser–	Glu
										↑		

→ Indicates that the residue has been located by sequential Edman degradation–dansylation. For comparison the corresponding sequence of bovine pepsinogen [3, 13] is also shown. Identical residues are in capitals. The numbering is from the *N*-terminus according to the sequence of Ong and Perlman [1]. ↑ Indicates the peptide bonds hydrolyzed by normal acid activation of the zymogens.

Samples containing 20–35 nmoles were subjected to sequence analyses by sequential Edman degradation and dansylation in SDS*-containing solution according to Weiner et al. [8]. Identification of Dns*-amino acids was performed by thin layer chromatography on (5 × 5 cm) polyamide layer sheets [9, 10]. Interfering contents of Dns-O-tyrosine were removed by microgel filtration on Sephadex G-25 [11].

3. Results and discussion

The results of the sequencing are shown in table 1. The amino acid residues are numbered according to the *N*-terminal sequence found by Ong and Perlman. These authors reported residue no 36 to be lysine and 37 to be tyrosine. Consistent with this we found *N*-terminal tyrosine in the large tryptic fragment throughout the major peak of the gel filtration. Ong and Perlmann have pointed out that some uncertainty existed about residues no 40 and 41. Our results show the opposite order of these two residues, and three more have been found before the known *N*-terminal sequence of porcine pepsin. From our experiments we are not able to decide whether residue no 40 is glutamine or glutamic acid, but Ong and Perlmann digested a nonapeptide containing this residue with aminopeptidase-M and found only glutamic acid. As shown in table 1 the present sequence unrestraindedly fits in homology with bovine pepsinogen (the previously published homology [3] would require one deletion or insertion between residue no 26 and 41).

After completion of this work our attention has been drawn to a preliminary communication of Tang et al. [12] in which it is reported that commercial crystalline pepsin contains minor amounts of pepsin with additional Ala–Leu at the *N*-terminus. This indicates that although cleavage after Leu no 44 normally occurs by acid activation of porcine pepsinogen, cleavage after Ala no 42 is also possible.

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