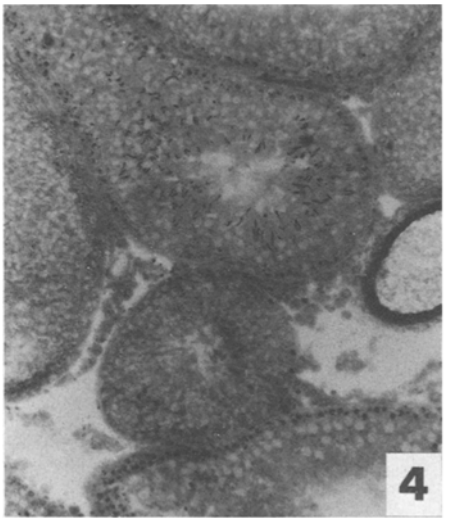

Figure 4. Arginine-aminopeptidase in the testis of a 6-week-old rat. High activity of arginine-aminopeptidase was observed in spermatids in the later stages of their development. × 80.

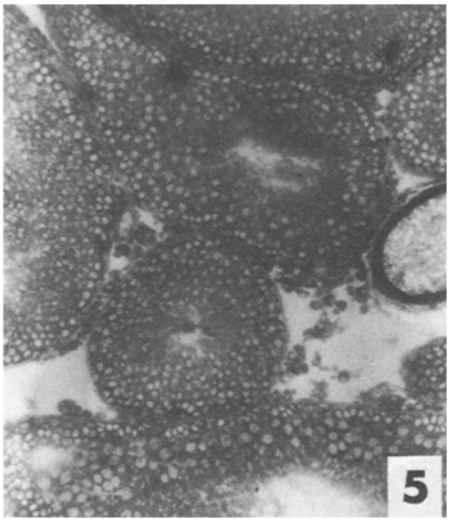

Figure 5. Leucine-aminopeptidase of section parallel to that in fig. 4. Spermatids hardly showed the enzyme activity. × 80.

is protamine rich[8,9]. Protamine, short-chain polyamine, contains mainly arginine and relatively few other amino-acids. These facts and the data obtained strongly suggest that testicular arginine-aminopeptidase is related to the transition of histone occurring through the later development of the spermatid. It is still unknown why arginine-aminopeptidase was higher in the testes of 9-day-old rats than in those of 3-week-old ones in spite of the absence of spermatids. One possible explanation may be that this high activity of arginine-aminopeptidase depends on the activities of different molecular forms or isoenzymes of the enzyme in the spermatid.

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Cleavage of p-nitroanilides of N-acylated tri- and tetrapeptides by alanine endopeptidase from the brush border membranes of rat enterocytes[1]

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Summary. The activity of the alanine endopeptidase from the intestinal brush border was studied using chromogenic substrates of the general fomula Sc-Ala_2-X-pNA, Sc-Y-Z-Ala-pNA and W-Ala_3-pNA respectively. Substrates with C-terminal Leu or Nle are hydrolyzed more readily than Ala-analogues. At least one Ala-residue in one of the positions adjacent to the C-terminus is necessary for the enzyme activity. An N^{α}-substituent has no effect on the activity.

The alanine endopeptidase of the enterocyte brush border has been described in man[3] and in the rat[4]. This enzyme cleaves succinyl-alanyl-alanyl-alanine-4-nitroanilide, previously found to be suitable for pancreatic elastase[5,6], between the 1st and 2nd alanine residues from the C-terminus. In the 2nd step Ala-pNA is split by aminopeptidase (scheme). This enzyme has been found in the kidney, liver, and brain[7]. Its physiological significance, however, has not been explained up to now. The enzyme is similar to the neutral metallo-endopeptidase described by Kerr and Kenny[8] in the kidney, and by Danielsen[9] in the intestinal mucosa. Sogawa[10] compared the catalytic properties of the renal and intestinal enzymes with the use of natural substrates. This study deals with the effect of the substitution in the positions P'_1, P_1, P_2 and P_3 (nomenclature of Schechter and Berger[11]) of the synthetic peptide substrates on the activity of intestinal alanine endopeptidase.

Material and methods. The enzyme preparation containing endopeptidase and aminopeptidase was obtained from solubilized brush border after centrifugation at

105,000 × g[4,12]. Both enzymes were separated by isoelectric focusing[4]. The substrates were prepared in a similar way to that described for Sc-Ala_3-pNA[6]. The analytical data will be presented separately. The incubation medium consisted of 100 µl of the enzyme preparation containing both endopeptidase and aminopeptidase, or 50 µl of each enzyme after electrofocusing and 2.5 mM of substrate solution (in dimethylsulphoxide, final concentration 7%); it was made up to the total volume of 1.5 ml with 0.1 M TRIS-HCl buffer pH 8.0. The absorbance was measured at 37 °C and 405 nm with the UNICAM SP-800 spectrophotometer ($\varepsilon = 8800$). Enzyme activity is given in µmoles per ml of substrate hydrolyzed per min and the relative (%) values indicate the ratio of activities of the substrate examined and Sc-Ala_3-pNA.

Scheme

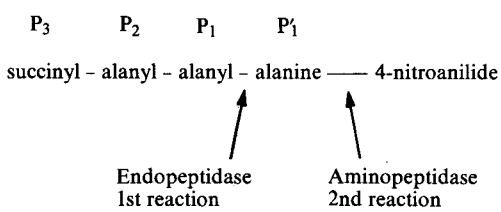

Table 1. Specific activity of the endopeptidase toward substrates Sc-Ala_2-X-pNA (X = Ala, Ile, Leu, Nle, Pro, Val)

Substrate	µmoles/min · ml	%
Sc-Ala_2-Ala-pNA	402.8	100.0
-Leu-	1256.4	311.9
-Nle-	1256.4	311.9
-Ile-	136.7	33.9
-Val-	59.1	14.7
-Pro-	22.1	5.5

Table 2. Specific activity toward substrates with substituted amino acid residues in positions P_1 and P_2 i.e. Sc-Y-Z-Ala-pNA (Y,Z = Ala, Gly, Leu, Pro, β-Ala)

Substrate	µmoles/min · ml	%
Sc-Ala-Ala-Ala-pNA	402.8	100.0
-Leu-Ala-	153.5	38.6
-Ala-Leu-	63.9	15.5
-Gly-Ala-	44.7	10.6
-Ala-Gly-	108.7	26.9
-β-Ala-Ala-	44.7	10.6
-Ala-β-Ala-	not hydrolyzed	
-Gly-Gly-		
-Leu-Leu-		
-Gly-Pro-		
-Pro-Gyl-		

Table 3. Specific activity of the endopeptidase toward substrates of the type W-Ala_3-pNA (W = Ac-His, Ac-Tyr, MBS-Lys, Sc, Sc-Asn)

Substrate	µmoles/min · ml	%
Sc -Ala_3-pNA	402.8	100.0
Ac-Tyr -	489.6	121.6
Ac-His -	428.4	106.4
Sc-Asn -	436.4	108.5
MBS-Lys-	476.8	118.2

Results and discussion. Position P'_1. The 2 substrates in which leucine or norleucine is located in this position are hydrolyzed the most quickly of the substrates Sc-Ala_2-X-pNA. The activity is three times higher in comparison with the substrate with alanine at P'_1. This substrate (Sc-Ala_3-pNA) is considered to be most suitable for pancreatic elastase[5,6]. The rate of cleavage decreased in the following order: Leu, Nle > Ala > Ile > Val > Pro (table 1). This is in agreement with the behavior of the human enzyme for which the order Nle > Leu > Phe > Ala has been described[3].

Position P_1 and P_2. Substrates of the general formula Sc-Y-Z-Ala-pNA are split, provided that at least 1 alanine residue occupies these positions. The substrate with alanine residues both at P_1 and P_2 is cleaved most quickly (table 2). Our results are similar to those reported by Varandani[13] for the cleavage of proinsulin C-peptide by renal neutral metallo-endopeptidase. Porcine peptide is split in 6 positions, of which 4 comprise alanine at the cleaved bond: Ala^{34}-Glu^{35}-Asn^{36}, Ala^{39}-Gly^{40}-Ala^{41}, Ala^{53}-Leu^{54}-Ala^{55}.

Bovine proinsulin, with the following alterations of the amino acid sequence: $Ala^{34} \rightarrow$ Val, $Ala^{39} \rightarrow$ Val, $Ala^{53} \rightarrow$ Leu, and $Ala^{55} \rightarrow$ Gly, is cleaved only in the two remaining positions as with the porcine peptide.

Position P_3. Substrates of the general formula W-Ala_3-pNA are split at approximately the same rate (table 3). In contrast to pancreatic elastase, in which the electrostatic interaction at an analogous position has been presumed, the anionic residue is not indispensable for the endopeptidase. This is also confirmed by our previous finding that Ac-Ala_3-pNA was hydrolyzed 1.7 times faster than Sc-Ala_3-pNA. Similarly, other residues at P_3 do not affect the endopeptidase activity to any considerable extent.

The alanine peptides are likewise suitable for the post-proline cleaving enzyme[14], which, however, prefers the bond with C-terminal proline. Intestinal alanine endopeptidase does not hydrolyze Z-Gly-Pro-pNA at all.

1 Abbreviations: Ac-, acetyl; Sc-, 3-carboxypropionyl; MBS-, 4-methoxybenzenesulphonyl; -pNA, 4-nitroanilide; all amino acids had the L-configuration.
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