AMINO ACID SEQUENCE STUDIES OF A NOVEL SUBTILISIN - ALKALINE MESENTERICOPEPTIDASE

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The amino acid sequence of mesentericopeptidase, originating from a strain of *B. mesentericus*, has been determined in part (95% of total) by: a) direct sequencing of intact protein and peptides obtained by cleavage with cyanogen bromide, trypsin and *S. aureus* protease, b) indirectly by comparison of partially characterized chymotryptic peptides with the sequence of subtilisin amylosacchariticus. Mesentericopeptidase belongs to the family of subtilisins related to subtilisin BPN'/Novo and it is highly homologous with subtilisin amylosacchariticus. A minor error in the sequences of subtilisin Novo and subtilisin amylosacchariticus has been corrected.

1. INTRODUCTION

Alkaline mesentericopeptidase (peptidyl peptide hydrolase, EC 3.4.21) is an extracellular protease originating from a strain of *Bacillus mesentericus*. The enzyme has been isolated in crystalline state (12) and studies on the structure in solution and mechanism of action have been performed. Mesentericopeptidase is DFP and PMSF-sensitive (8, 12); kinetic and photooxidation studies showed that a histidyl residue is also involved in the active site (6, 18). The reactivities, environment and role in the catalytic

mechanism of tyrosyl, tryptophyl, methionyl, lysyl and arginyl residues were investigated by chemical, photochemical and spectroscopic methods (4, 5, 7). All these studies showed a structural similarity between alkaline mesentericopeptidase and the members of the subtilisin family. However, some quantitative differences in the specificity toward ester substrates have been observed (6). On the basis of amino acid composition, kinetic and fluorescence properties it could be concluded that the protease from *B. mesentericus* is nearer to the subtilisins

Abbreviations: DPC-trypsin = trypsin treated with diphenylcarbonyl chloride; PMSF = phenylmethanesulfonylfluoride; Polybrene = 1,5-dimethyl-1,5-diazuundecamethylene polymethobromide; TCA = trichloroacetic acid; THEED = N,N,N',N'-tetrakis (2-hydroxyethyl)ethylene diamine.

Novo and Amylosacchariticus than to subtilisin Carlsberg, i.e. this enzyme belongs to the subtilisin BPN⁻-group of alkaline bacterial proteases according to the classification of KEAY and MOSER (13). It should be noticed also that the amino acid composition of mesentericopeptidase and its poor solubility closely resemble those of subtilisin Amylosacchariticus (6).

Knowledge of the covalent structure is very important for the exact classification of alkaline mesentericopeptidase and for the future investigations of the structure-function relationship. This enzyme easily crystallizes during the last step of purification which makes it suitable object for X-ray analysis. Amino acid sequence studies described in the present paper yielded 95% of the total sequence assuming that the polypeptide chain of the examined protease contains 273 residues (4). The results show close similarities in the primary structures of mesentericopeptidase and subtilisin Amylosacchariticus.

2. MATERIALS AND METHODS

2.1. Materials

Alkaline mesentericopeptidase was prepared in crystalline form as reported in reference (12). Phenylmethanesulphonylfluoride (PMSF), 1-dimethylaminonaphthalene-5-sulphonylchloride (DNS-Cl) and acetonitril (Uvasol) for spectroscopic purposes were obtained from Merck (Darmstadt, West Germany). Sephadex G-75, Sephadex G-25 and Sephadex G-10 were purchased from Pharmacia (Uppsala, Sweden). Dowex 50×2, 200 to 400 mesh and α -chymotrypsin (four times crystallized) were from Fluka AG (Basel, Switzerland). All solvents and reagents in the sequencer were from Rathburn, Walkerburn, U.K., except THEED (ICN-KK Laboratories, Inc., N.Y., USA) and Polybrene (Pierce, Europe, Rotterdam, Holland). All other reagents used were of analytical purity.

2.2. Methods

2.2.1. Preparation of the PMS-derivative

1.5 g of the native mesentericopeptidase were dissolved in 150 ml of 1 M-NaCl, pH = 7.0. The reaction vessel was put into an ice bath and stirred. 3.75 ml of the solution of PMSF in dioxan

(320 mg PMSF dissolved in 10 ml of dioxan) were added to the enzyme solution. After 30 min of stirring an additional portion of 3.75 ml of the inhibitor was added and the mixture was stirred for 1 hour. The fine sediment was removed by centrifugation for 20 min at 6,500 rpm in tubes which in advance had been cooled in the chamber of a refrigerator. The clear solution was gel filtered through a Sephadex G-75 column equilibrated and eluted with a 0.3 M-NaCl, pH = 7.0. The protein fraction was force-dialyzed and lyophilized.

2.2.2. Hydrolysis of PMS-mesentericopeptidase with chymotrypsin

The PMSF-inhibited mesentericopeptidase was dialyzed 60 hours against 0.01 M-CaCl₂, pH = 6.0. An equal volume of 20% trichloroacetic acid was added and the mixture was stirred for 1 hour. The precipitate was filtered and washed with 10% trichloroacetic acid, ethanol, acetone and ether as described in (15). 1.042 g of the denatured protein was suspended in 200 ml of 0.005 M-CaCl₂ and the pH was raised to 10.1 with NaOH. After 16 hours of stirring the pH of the mixture was lowered to 9.3 and it was adjusted to 8.1 with 1 N-acetic acid. The enzymic hydrolysis was performed in a jacketed vessel at 40 °C with 40 mg of α -chymotrypsin added in three portions. The pH was maintained constant at 8.1 by addition of 0.1 N-NaOH and using a digital pH-meter Präcitronic model MV 87. The reaction was terminated after 8 hours and 50 min.

2.2.3. Separation of chymotryptic peptides on Dowex 50×2

The reaction mixture containing chymotryptic peptides of mesentericopeptidase was centrifuged and the clear supernatant applied to a column (2.8×142 cm) of Dowex 50×2 at a flow rate of 50 ml · h⁻¹. After washing with 4.5 litres of 0.2 M-pyridine-acetic acid buffer at pH 3.1 the peptides were eluted with a pyridine-acetic acid gradient as described in (14). Aliquots of 0.25 ml were withdrawn from the fractions and analyzed by the ninhydrin method.

2.2.4. HPLC separation of chymotryptic peptides

Peptide fractions obtained after the elution of

the Dowex 50×2 column were further purified by means of gel filtration on Sephadex G-25, Sephadex G-10 or HPLC-technique. Perkin-Elmer Liquid Chromatograph Series 2, with an LC-75 spectrophotometric detector was used. Chymotryptic fragments of PMS-mesentericopeptidase were separated on a preparative reversed phase HPLC column with a linear gradient of 0.05% CF₃COOH/H₂O (solvent A) and 0.05% CF₃COOH/CH₃CN (solvent B, 0-50%) at a flow rate of 8 ml · min⁻¹.

2.2.5. Terminal and sequence analysis of the chymotryptic peptides

The NH₂-terminal amino acids were determined by the method of GRAY and HARTLEY (10) using the solvent systems described in (17). COOH-terminal analysis was performed according to the procedures given in (3) or with carboxypeptidase A (1). The amino acid sequence of chymotryptic peptides was studied by using a combination of the dansyl and phenylthiohydantoin techniques (9).

2.2.6. Amino acid analyses

The peptides were hydrolysed in 5.7 N-HCl for 20 hours at 110 °C in evacuated and sealed tubes. The amino acid analyses were performed using a Michrotechna AAA 881 amino acid analyzer.

2.2.7. Amino acid sequence determination

Automated sequence analysis was performed using a Beckman 890C sequencer as originally described by EDMAN and BEGG (2). The use of THEED and other modifications have been described (11). In several cases Polybrene was added to the cup together with the peptide to be sequenced. Identification of the resulting PTH-amino acids was made by HPLC as described by SVENDSEN et al. (20). Additional information was obtained by back hydrolysis to the parent amino acids (16).

2.2.8. Cyanogen bromide cleavage

15 mg of enzyme was dissolved in one ml of 70% formic acid and 150 mg of cyanogen bromide added. The reaction mixture was left overnight at room temperature in the dark, lyophilized and redissolved in 30% acetic acid. After centrifugation the supernatant was applied to a column of

Sephadex 50 (fine) (1.6×92 cm) equilibrated with 30% acetic acid and eluted with the same solvent. Peak identification was made from measurement of the O.D. at 280 nm and appropriate samples were pooled and lyophilized.

2.2.9. Cleavage with DPC-trypsin

15 mg of enzyme was dissolved in 4 ml of 0.1 M-Tris buffer, pH 8.0 and 25 mg of succinic anhydride was added. pH was kept at 8 by dropwise addition of 4 N-NaOH. Three more portions of reagent were added with intervals of 20 min. After the addition the reaction mixture was left for one hour to ensure complete hydrolysis of the anhydride. After precipitation with TCA (10%) the protein was dissolved in ammonia, titrated to pH 9.0 and digested with DPC-trypsin overnight (1:100 w/w). The supernatant was gel filtered as described in 2.2.8, but with 0.5 % ammonia as the eluant.

2.2.10. Cleavage with *S.aureus* protease

Ten ml of enzyme was dissolved in 1.5 ml 0.1 M-ammonium bicarbonate/3 M-urea. 0.4 mg *S.aureus* protease was added and the mixture left at 40 °C for 5 hours. Separation of peptides was made on Sephadex G50 as in 2.2.8 but using 0.5% ammonia instead of acetic acid.

3. RESULTS

3.1. Amino-terminal sequence analysis of native mesentericopeptidase

Three mg of native enzyme was sequenced 58 steps with a repetitive yield of 98%. A second sequence amounting to 15% of the main sequence was determined for 24 steps. This sequence turned out to be one starting at the active Ser₂₂₁ residue and is probably the consequence of an internal autolytic cleavage step (Figure 1).

3.2. Sequence studies on cyanogen bromide peptides

Fractionation of the peptides resulting from cleavage with cyanogen bromide on Sephadex G50 resulted in six peaks (see Figure 2) indicating incomplete cleavage or aggregation since only five peptides were expected. Two of the peaks contained only one component each. Peak IV had the N-terminal sequence: Ala₂₂₃-Thr₂₂₄-Pro₂₂₅- and was sequenced 41 steps with a repeti-

Figure 1. Partial amino acid sequence of mesentericopeptidase. → denotes start and direction of sequencing. C1-C12 denotes chymotryptic peptides.

Figure 2. Gel filtration of peptides resulting from cleavage of mesentericopeptidase by cyanogen bromide on Sephadex G50 in 30% acetic acid. Column dimension 1.6×92 cm. Flowrate 10 ml · hr⁻¹.

tive yield of 95%. It was shown to be the C-terminal CNBr-peptide (Figure 1) which was confirmed by the absence of homoserine and homoserine lactone in the amino acid analysis. Peak VI was sequenced 18 steps with a repetitive yield of 90%. The sequence was compatible with Ala₂₀₀-Met₂₂₂ (Figure 1).

Peak III contained two peptides in the ratio 3 to 1. The minor peptide was that of peak VI while the major peptide started with Ser-Leu-Gly compatible with the CNBr-peptide Ser₁₂₅-Met₁₉₉ (Figure 1). It was sequenced 36 steps with a repetitive yield of 98%.

The N-terminal CNBr-peptide was found in peaks I and II in both cases contaminated with the major peptide of peak III. No further analysis were performed with these peaks.

3.3. Sequence studies on tryptic peptides

Gelfiltration of the peptides from tryptic cleavage of succinylated mesentericopeptidase resulted in three peaks (see Figure 3) of which peak I was sequenced 34 steps and shown to be a peptide starting at Gly₄₆ (Figure 1). It is noteworthy (see discussion) that the sequence Asn₅₆-Pro₅₇ was confirmed.

A second sequence, 15% of the major one, was sequenced only 10 steps and was compatible

with the peptide segment Val₉₅-Tyr₁₀₄ which probably indicates incomplete succinylation of Lys₉₄. The amino acid composition was in fair agreement with the peptide Val₉₅-Arg₁₈₆.

Peak II was sequenced 31 steps and started Ala₁₈₇-Ser₁₈₈-Phe₁₈₉. Sequencing stopped abruptly after Tyr₂₁₇ indicating an Asn-Gly combination as found in the sequence of *B.amylosaccariticus* protease.

Finally, peak III contained three peptides, the major one (50%) of which was the C-terminal fragment of the protease. It was sequenced 14 steps through Tyr₂₆₃ confirming the sequence already obtained from the C-terminal CNBr-peptide.

3.4. Sequence studies on peptides from cleavage with *S.aureus* protease

Separation of the peptides obtained from cleavage with *S.aureus* protease gave rise to a number of peaks which upon sequencing turned out to be mixtures of peptides, except one. This was the C-terminal peptide of the protease starting at Ser₂₅₂ which was sequenced 14 steps.

Figure 3. Gel filtration of peptides resulting from tryptic cleavage of succinylated mesentericopeptidase on Sephadex G50 (1.6×100 cm) in 0.5% ammonia. Flowrate 18 ml · hr⁻¹.

Table I.

Compositions of peptides from chymotryptic digests of PMS-mesentericopeptidase.

Amino acid	C1 (51-58)	C2 (59-67)	C3 (76-82)	C4 (83-91)	C5 (127-135)	C6 (172-185)	C7 (197-206)	C8 (207-214)	C9 (227-233)	C10 (246-256)	C11 (268-271)	C12 (268-275)
Histidine		2.0(2)										
Arginine										1.5(2)		
Aspartic acid	1.0(1)	0.9(1)	2.1(2)			1.7(2)	1.2(1)			1.2(1)	1.1(1)	1.2(1)
Threonine	1.2(1)	0.8(1)			1.6(2)	1.0(1)		1.7(2)		2.1(2)		
Serine	1.3(1)	1.8(2)	1.0(1)	1.9(2)	1.0(1)	1.7(2)	1.2(1)	1.1(1)		1.2(1)		
Glutamic acid	1.1(1)	1.1(1)				0.7(1)	1.0(1)			1.2(1)	1.0(1)	1.7(2)
Proline	1.8(2)			1.0(1)	0.8(1)	0.9(1)	1.1(1)	1.2(1)				
Glycine		1.8(2)	1.1(1)	1.2(1)	2.6(3)	1.3(1)	1.1(1)	2.0(2)	1.0(1)			
Alanine				2.1(2)	1.2(1)	2.7(3)	1.0(1)		4.1(4)	1.2(1)		3.0(3)
Valine	0.8(1)		0.7(1)	0.8(1)		1.7(2)	1.5(2)		0.8(1)	0.3?(1)	0.8(1)	0.9(1)
Methionine							0.7(1)					
Isoleucine			0.8(1)			0.8(1)	0.7(1)				0.9(1)	0.9(1)
Leucine			0.7(1)	1.0(1)	1.3(1)			0.8(1)	0.9(1)	1.1(1)		
Tyrosine	1.1(1)			0.8(1)				0.9(1)		1.0(1)		
Total residues	8	9	7	9	9	14	10	8	7	11	4	8
Purification procedures	Dowex 50x2											
	HPLC	HPLC	HPLC	HPLC	Seph G25,G10	Seph G25	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC

Although position 19 in the intact protein was shown to be a glutamine a peptide Gly₂₀-Tyr₂₁-Thr₂₂ etc. appeared in several of the peaks obtained by gel filtration. Deamidation at Gln₁₉ was

characteristic protease permitted the incorporation of all the peptides in the sequence of the enzyme under study. The sequence positions are given in brackets at the top of each column in Table I

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4. DISCUSSION

Previous studies (4, 5, 6, 7, 8, 12, 18) including amino acid analyses and kinetic analysis have strongly indicated that mesentericopeptidase belongs to the group of alkaline proteases related to subtilisin BPN'/Novo (13). The present investigation was undertaken to prove this relationship directly through amino acid sequence studies. Although the sequence determination has not been completed the partial amino acid sequence obtained directly in the sequencer or suggested from amino acid composition of chymotryptic peptides covers 95 % of the total molecule and demonstrates that mesentericopeptidase indeed is related to subtilisin BPN'/Novo. More striking is the finding that a very close relationship exists between mesentericopeptidase and the subtilisin isolated from *B.amylosacchariticus* (14). Only six differences have been demonstrated during these studies as seen from the comparison between the two proteases made in Figure 4. Asn₅₆-Pro₅₇ in mesentericopeptidase was published as Pro₅₆-Asn₅₇ in subtilisin amylosacchariticus (14). Thr₁₃₀ and Thr₁₆₂ in our enzyme are serines in the other, Ser₂₅₉ in the present enzyme is an Asp in the other, and finally either Ser₁₈₂ or Ser₁₈₃ is an Ala in the other enzyme. Of these differences the first two are probably not true differences, but due to errors in the original papers on the amino acid sequences of subtilisin amylosacchariticus (14) and subtilisin BPN'/Novo (15). Inspection of these papers shows that direct sequencing through residues 56 and 57 has not been possible with the manual method available at that time. The positioning of the Pro and Asn was made from conclusions made about the specificity of carboxypeptidase A (20).

Since subtilisin BPN'/Novo has a Met in position 50 and since no other CNBr-peptide than the one of interest has Pro or Asn in positions 6 and 7 the following experiment was carried out: 500 nanomoles of peptide-free subtilisin Novo was treated with cyanogen bromide under the same conditions as for mesentericopeptidase, lyophilized and applied directly to the sequencer. All of the amino acids expected in each step were released except in step 6 and 7 where first Asn₆ and then Pro₇ was identified. The correct sequence in subtilisin BPN'/Novo is therefore

Asn₅₆-Pro₅₇. Since the amino acid sequence in subtilisin amylosacchariticus around these residues was deduced from comparison with chymotryptic peptides obtained from subtilisin BPN'/Novo it is almost certain that the correct sequence in the amylosacchariticus protease is also Asn₅₆-Pro₅₇ and therefore an even closer relationship with mesentericopeptidase exists.

The peptide starting at Val₉₅, when sequenced, probably originated from a tryptic digest of a succinylated derivative in which Lys₉₄ was incompletely modified. It has been shown in a previous study (5) that seven of the eight lysine residues can be modified by maleylation. The single residue which is difficult to modify therefore most likely is Lys₉₄. This suggestion is compatible with the finding from the study of the three-dimensional model of subtilisin Novo that Lys₉₄ is located in a cavity and hence partially inaccessible to reagents. In subtilisin Novo 10 of the 11 lysine residues could be modified by carbamylation or succinylation (19) while all 11 residues were accessible to maleic anhydride (5).

When PMS-mesentericopeptidase was sequenced two peptides were obtained: the expected N-terminal and, as a minor component, Ser₂₂₁-Met₂₂₂-Ala₂₂₃ etc. Ser₂₂₁ is the active amino acid of the catalytic center and should according to the procedure described in section 2.2.1 be blocked with PMSF. Apparently, approximately 15% of the molecules had not reacted with PMSF. One reason for this may be that the molecule was in part unfolded destroying the extraordinary reactivity of Ser₂₂₁ towards PMSF, but making the peptide segment in which it is located vulnerable to hydrolytic attack by protease still active. Further studies are needed to show if this case of "limited proteolysis" is the initial step in the autolytic degradation of mesentericopeptidase.

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	1	10	20
BA	Ala-Gln-Ser-Val-Pro-Tyr-Gly-Ile-Ser-Gln-Ile-Lys-Ala-Pro-Ala-Leu-His-Ser-Gln-Gly-		
BM	Ala-Gln-Ser-Val-Pro-Tyr-Gly-Ile-Ser-Gln-Ile-Lys-Ala-Pro-Ala-Leu-His-Ser-Gln-Gly-		
	30	40	
BA	Tyr-Thr-Gly-Ser-Asn-Val-Lys-Val-Ala-Val-Ile-Asp-Ser-Gly-Ile-Asp-Ser-Ser-His-Pro-		
BM	Tyr-Thr-Gly-Ser-Asn-Val-Lys-Val-Ala-Val-Ile-Asp-Ser-Gly-Ile-Asp-Ser-Ser-His-Pro-		
	50	60	
BA	Asp-Leu-Asn-Val-Arg-Gly-Gly-Ala-Ser-Phe-Val-Pro-Ser-Glu-Thr-Pro-Asn-Tyr-Gln-Asp-		
BM	Asp-Leu-Asn-Val-Arg-Gly-Gly-Ala-Ser-Phe-Val-Pro-Ser-Glu-Thr-Asn-Pro-Tyr-Gln-Asp-		
	70	80	
BA	Gly-Ser-Ser-His-Gly-Thr-His-Val-Ala-Gly-Thr-Ile-Ala-Ala-Leu-Asn-Asn-Ser-Ile-Gly-		
BM	Gly-Ser-Ser-His-Gly-Thr-His-Val-Ala-Gly-Thr-Ile-Ala-Ala-Leu-Asn-Asn-Ser-Ile,Gly,		
	90	100	
BA	Val-Leu-Gly-Val-Ala-Pro-Ser-Ser-Ala-Leu-Tyr-Ala-Val-Lys-Val-Leu-Asp-Ser-Thr-Gly-		
BM	Val,Leu-Gly-Val-Ala,Pro,Ser,Ser,Ala,Leu,Tyr	Lys-Val-Leu-Asp-Ser-Thr-Gly-	
	110	120	
BA	Ser-Gly-Gln-Tyr-Ser-Trp-Ile-Ile-Asn-Gly-Ile-Glu-Trp-Ala-Ile-Ser-Asn-Asn-Met-Asp-		
BM	Ser-Gly-Gln-Tyr	Glu-Trp-Ala-Ile-Ser-Asn-Asn-Met-Asp-	
	130	140	
BA	Val-Ile-Asn-Met-Ser-Leu-Gly-Gly-Pro-Ser-Gly-Ser-Thr-Ala-Leu-Lys-Thr-Val-Val-Asp-		
BM	Val-Ile-Asn-Met-Ser-Leu-Gly-Gly-Pro-Thr-Gly-Ser-Thr-Ala-Leu-Lys-Thr-Val-Val-Asp-		
	150	160	
BA	Lys-Ala-Val-Ser-Ser-Gly-Ile-Val-Val-Ala-Ala-Ala-Ala-Gly-Asn-Glu-Gly-Ser-Ser-Gly-		
BM	Lys-Ala-Val-Ser-Ser-Gly-Ile-Val-Val-Ala-Ala-Ala-Ala-Gly-Asn-Glu-Gly-Ser-Ser-Gly-		
	170	180	
BA	Ser-Ser-Ser-Thr-Val-Gly-Tyr-Pro-Ala-Lys-Tyr-Pro-Ser-Thr-Ile-Ala-Val-Gly-Ala-Val-		
BM	Ser-Thr-Ser-Thr-Val-Gly-Tyr-Pro-Ala-Lys-Tyr-Pro-Ser-Thr,Ile,Ala,Val,Gly,Ala,Val,		
	190	200	
BA	Asn-Ser-Ser-Asn-Gln-Arg-Ala-Ser-Phe-Ser-Ser-Ala-Gly-Ser-Glu-Leu-Asp-Val-Met-Ala-		
BM	Asx,Ser,Ala,Asn-Gln-Arg-Ala-Ser-Phe-Ser-Ser-Ala-Gly-Ser-Glu-Leu-Asp-Val-Met-Ala-		
	210	220	
BA	Pro-Gly-Val-Ser-Ile-Gln-Ser-Thr-Leu-Pro-Gly-Gly-Thr-Tyr-Gly-Ala-Tyr-Asn-Gly-Thr-		
BM	Pro-Gly-Val-Ser-Ile-Gln-Ser-Thr-Leu-Pro-Gly-Gly-Thr-Tyr-Gly-Ala-Tyr-		-
	230	240	
BA	Ser-Met-Ala-Thr-Pro-His-Val-Ala-Gly-Ala-Ala-Ala-Leu-Ile-Leu-Ser-Lys-His-Pro-Thr-		
BM	Ser-Met-Ala-Thr-Pro-His-Val-Ala-Gly-Ala-Ala-Ala-Leu-Ile-Leu-Ser-Lys-His-Pro-Thr-		
	250	260	
BA	Trp-Thr-Asn-Ala-Gln-Val-Arg-Asp-Arg-Leu-Glu-Ser-Thr-Ala-Thr-Tyr-Leu-Gly-Asp-Ser-		
BM	Trp-Thr-Asn-Ala-Gln-Val-Arg-Asp-Arg-Leu-Glu-Ser-Thr-Ala-Thr-Tyr-Leu-Gly-Ser-Ser-		
	270		
BA	Phe-Tyr-Tyr-Gly-Lys-Gly-Leu-Ile-Asn-Val-Gln-Ala-Ala-Ala-Gln		
BM	Phe-Tyr-Tyr-Gly-Lys	Ile,Asx,Val,Gln,Ala,Ala,Ala,Gln	

- ◀ Figure 4. Partial amino acid sequence of mesentericopeptidase (BM) and comparison with that of subtilisin amylosacchariticus (BA) (14). Boxed amino acids show sequence differences.

REFERENCES

1. BAILEY, J.L.: In "Techniques in Protein Chemistry", J.L. Bailey, ed., Elsevier Publishing Company, 1967, Amsterdam-London-New York, p. 222
2. EDMAN, P. & G. BEGG: A protein sequenator. *Eur. J. Biochem.* 1, 80-91 (1967)
3. FRAENKEL-CONRAT, H. & C.M. TSUNG: Hydrazinolysis. *Methods in Enzymology* 11, 151-155 (1967)
4. GENOV, N. & G. JORI: Conformational studies on alkaline protease from *Bacillus mesentericus*. *Int. J. Peptide Protein Res.* 5, 127-133 (1973)
5. GENOV, N. & M. SHOPOVA: Maleylation of alkaline mesentericopeptidase. *Int. J. Peptide Protein Res.* 6, 149-154 (1974)
6. GENOV, N. & M. SHOPOVA: Hydrolysis of amino acid esters by alkaline mesentericopeptidase. *Int. J. Peptide Protein Res.* 6, 347-351 (1974)
7. GENOV, N.: Spectrophotometric titration of tyrosyl residues in alkaline mesentericopeptidase. *Int. J. Peptide Protein Res.* 7, 325-332 (1975)
8. GENOV, N. & M. SHOPOVA: Circular dichroism studies on native and phenylmethanesulfonyl-mesentericopeptidase. *Int. J. Peptide Protein Res.* 11, 185-189 (1978)
9. GRAY, W.R. & B.S. HARTLEY: The structure of a chymotryptic peptide from *Pseudomonas* cytochrome C.-551. *Biochem. J.* 89, 379-380 (1963)
10. GRAY, W.R. & B.S. HARTLEY: A fluorescent end-group reagent for proteins and peptides. *Biochem. J.* 89, 59_p (1963)
11. JOHANSEN, J.T., C. OVERBALLE-PETERSEN, B. MARTIN, V. HASEMANN & I. SVENDSEN: The complete amino acid sequence of copper, zink superoxide dismutase from *Saccharomyces cerevisiae*. *Carlsberg Res. Commun.* 44, 201-217 (1979)
12. KARADJOVA, M., A. BAKURDJIEVA & P. VELCHEVA: On the composition of the enzyme preparation E-30 - characteristics of the proteolytic enzymes in it. *Compt. Rend. Acad. Bulg. Sci.* 23, 431-434 (1970)
13. KEAY, L. & P.W. MOSER: Differentiation of alkaline proteases from bacillus species. *Biochem. Biophys. Res. Commun.* 34, 600-604 (1969)
14. KURIHARA, M., F.S. MARKLAND & E.L. SMITH: Subtilisin Amylosacchariticus. III. Isolation and sequence of the chymotryptic peptides and the complete amino acid sequence. *J. Biol. Chem.* 247, 5619-5631 (1972)
15. MARKLAND, F.S., G. KREIL, B. RABADEAU-DUMAS & E.L. SMITH: Subtilisin BPN'. V. Isolation and sequence of chymotryptic peptides. *J. Biol. Chem.* 241, 4642-4664 (1966)
16. MENDEZ, E. & C.Y. LAI: Regeneration of amino acids from thiazolinones formed in the Edman degradation. *Anal. Biochem.* 68, 47-53 (1975)
17. NEDKOV, P. & N. GENOV: Extending the range of application of the Edman method. Application to short peptides in small amounts. *Biochim. Biophys. Acta* 127, 541-543 (1966)
18. SHOPOVA, M. & N. GENOV: Photochemical evidence for the presence of histidyl residue in the active site of alkaline mesentericopeptidase. *Int. J. Peptide Protein Res.* 10, 369-374 (1977)
19. SVENDSEN, I.: Chemical modification of the subtilisins with special reference to the binding of large substrates. *Carlsberg Res. Commun.* 41, 237-291 (1977)
20. SVENDSEN, I., B. MARTIN & I. JONASSEN: Characteristics of hiproly barley III. Amino acid sequences of two lysine rich proteins. *Carlsberg Res. Commun.* 45, 79-85 (1980)