Complete amino acid sequence of alkaline mesentericopeptidase

A subtilisin isolated from a strain of *Bacillus mesentericus*

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Received 22 November 1985; revised version received 3 December 1985

This paper reports the complete amino acid sequence of a novel subtilisin: alkaline mesentericopeptidase. The protein contains 275 amino acid residues in a single polypeptide chain. The sequence data presented allow the linear arrangement of the whole molecule. Comparison with the subtilisins amylosacchariticus, novo, carlsberg, DY, and a subtilisin isolated from *B. subtilis* strain I 168, respectively classifies the enzyme as a serine protease of the subtilisin novo type closely related to subtilisin amylosacchariticus. Special features of mensentericopeptidase with respect to enzymatic and physico-chemical properties are discussed.

Amino acid sequence *Sequence homology* *Mesentericopeptidase* *Subtilisin*

1. INTRODUCTION

Alkaline mesentericopeptidase (peptidyl peptide hydrolase, EC 3.4.21) is a serine protease isolated in crystalline state from a strain of *Bacillus mesentericus* [1]. The enzyme belongs to the subtilisin type family of bacterial proteases [2,3]. In a previous work on the primary structure of mesentericopeptidase [3] the partial sequence of this enzyme was published. The sequence data showed a high homology with subtilisin amylosacchariticus. Additional information was obtained from peptides generated by chymotryptic and tryptic hydrolyses and led to the determination of the complete amino acid sequence of mesentericopeptidase which is reported in this paper.

2. MATERIALS AND METHODS

Crystallized and lyophilized alkaline mesentericopeptidase was prepared by the method of Karadjova et al. [1]. Trypsin, 2 × crystallized, was purchased from Koch-Light (England). The methods and other chemicals used for the cleavage of phenylmethanesulfonyl-mesentericopeptidase and for the purification and characterization of the obtained peptides were described [3]. Tryptic cleavage of PMS-enzyme in which the lysyl residues were not modified yielded a digest from which peptides were isolated in pure form by gel filtration on Sephadex G-25 or Sephadex G-50 or by HPLC technique. Automated sequence analysis was performed using an 890 Beckman sequencer with modifications according to Svendsen et al. [3].

3. RESULTS AND DISCUSSION

The amino acid sequence of mesentericopeptidase has in part been determined by direct sequencing from the N-terminal and by sequencing of fragments obtained by cleavage with cyanogen

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00145793/86/$3.50

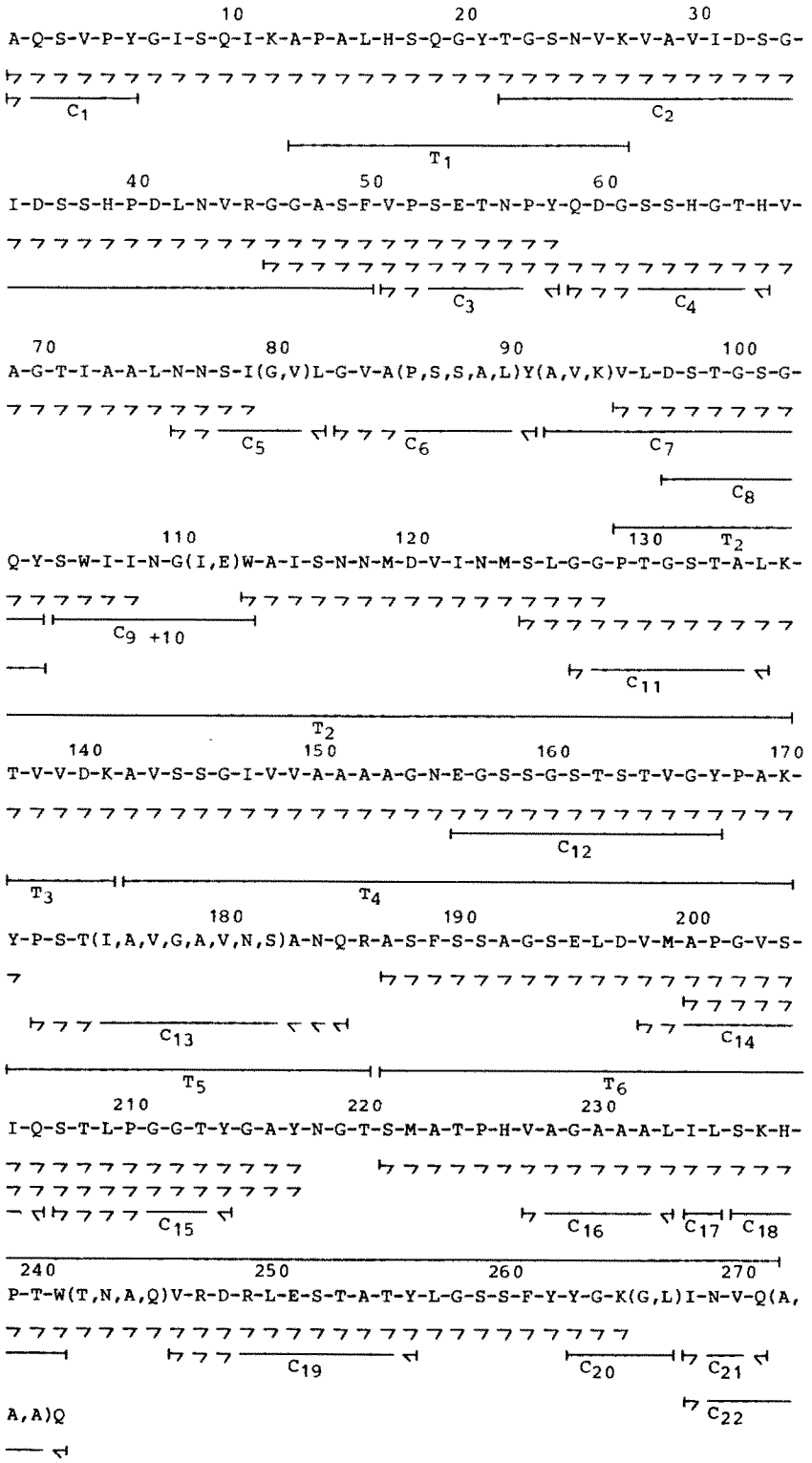

Fig.1. The complete amino acid sequence of mesentericopeptidase. ⊢ and ⊣ : start and direction of sequencing. C_1–C_{22}, chymotryptic peptides; T_1–T_6, tryptic peptides. For further detail see text.

bromide, trypsin and *Staphylococcus aureus* V8 protease [3]. In addition a peptide preexisting in the batch used in this study and probably generated by autolysis of the bond Thr_{220}-Ser_{221} was sequenced [3]. In fig.1 these sequence data are included together with the results of the present study necessary for the construction of the complete sequence. Twenty-two chymotryptic peptides covering 180 of the 275 residues, and 6 tryptic peptides covering 192 residues were isolated as indicated in fig.1. In those cases where direct sequencing did not give the necessary overlaps, amino acid composition of purified peptides in combination with the close similarity between the sequence of this enzyme and subtilisin amylosaccharíticus permitted the construction of the complete sequence (peptides C_5, C_6, C_7, C_{9+10}, C_{13}, C_{20}, C_{21}, T_5 and T_6 in fig.1).

The 4 Asp/Asn residues in positions 109, 181, 218, and 269 determined by amino acid analysis only were identified as Asn as follows: the total number of all free carboxyl groups was determined by using *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride and glycineamide hydrochloride [4]. The total number of free carboxyl groups was 14.4 accounting for the 15 Glu, Asp and the C-terminal carboxyl group determined by sequencing, leaving the 4 positions occupied by Asn. The presence of Asn in positions 109 and 218 was further verified by the observation that sequencing was abruptly terminated after positions 108 and 217 indicating the presence of an Asn-Gly bond.

In fig.2 a comparison is made of the sequence of mesentericopeptidase with that of subtilisins I 168 (isolated from a strain of *B. subtilis* [5]), amylosaccharíticus [6], novo [7], carlsberg [8] and DY [9]. Great homology is shown especially in the case of the first 3 proteases. The differences between mesentericopeptidase and subtilisin amylosaccharíticus are limited to 6 positions. The substitution in positions 130, 162, and 183 are conservative in nature. In subtilisin amylosaccharíticus there are seryl residues in these places which change to a threonine or alanine in the second enzyme, respectively. It is noteworthy that in all other subtilisins, except mesentericopeptidase and subtilisin I 168, there is a seryl residue in position 130 which is close to the important Tyr_{105} of the substrate binding region [10]. A more radical change was observed in position 259 where the noncharged serine in mesentericopeptidase becomes an aspartic acid in subtilisin amylosaccharíticus. The sequence Asn_{56}-Pro_{57} is in inverse order in comparison with the respective sequence in subtilisin amylosaccharíticus and novo. We and others [3,11] have shown that in the case of the novo enzyme there is an error in the original determination of the sequence in these positions. The same is probably valid for the enzyme from *B. amylosaccharíticus*, because the positions of the respective Pro_{56}-Asn_{57} had not been determined by direct sequencing.

In view of the close similarity between the amino acid sequences of mesentericopeptidase and subtilisin amylosaccharíticus in the substrate binding area it was surprising to find that they differ in the hydrolysis of tosyl-L-arginine methyl ester. V_{max} for the hydrolysis of this substrate by subtilisin amylosaccharíticus is independent of pH from 6 to 8 [12] while pH dependence of V_{max} for the hydrolysis of the same substrate by mesentericopeptidase follows a sigmoidal curve [2]. Quantitative differences in their specificity toward ester substrates have also been demonstrated [2].

In regard to their physico-chemical properties it should be noted that both enzymes have very low solubility in water in contrast to subtilisin novo which is very soluble in water (>50 mg/ml). This feature is not readily explained from a comparison with the three-dimensional structure of subtilisin novo, since most of the differences in amino acid side-chains on the enzyme surface (as determined in subtilisin novo) are very conservative and no major change in net-charge is observed.

Another difference between mesentericopeptidase and subtilisin novo (and carlsberg) is found in the stability of the enzymes in urea solutions. While the novo and carlsberg enzymes are stable in 6 M urea, mesentericopeptidase unfolds after short incubation in 3 M urea [13]. Again, this difference in behaviour cannot be explained from the differences in the primary structures of the enzymes. The answer may come from an inspection of the three-dimensional structure of mesentericopeptidase when this becomes available.

The results obtained support the classification of mesentericopeptidase as a serine protease of the subtilisin novo/BPN' type.

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    1                   10                  20                  30
BM: A-Q-S-V-P-Y-G-I-S-Q-I-K-A-P-A-L-H-S-Q-G-Y-T-G-S-N-V-K-V-A-V-I-D-S-G-I-
BA:
NO:                 V
DY:     T             P-L       D-K-V-Q-A         K   A          G-I     T
CA:     T             P-L       D-K-V-Q-A       F-K   A            L   T

           40                  50                  60                  70
BM: D-S-S-H-P-D-L-N-V-R-G-G-A-S-F-V-P-S-E-T-N-P-Y-Q-D-G-S-S-H-G-T-H-V-A-G-
BA:                                               P-N
NO:                 K   A         M                 F     D-N
DY: A-A     T       K   V             S-G   S ● Y-N-T     N-G
CA: Q-A             V             A-G   A ● Y-N-T     N-G

                    80                  90                  100
BM: T-I-A-A-L-N-N-S-I-G-V-L-G-V-A-P-S-S-A-L-Y-A-V-K-V-L-D-S-T-G-S-G-Q-Y-S-
BA:
NO:   V                                                 G-D-A
DY:   V       D   T-T               N-V-S       I       N   S       T
CA:   V       D   T-T                 V-S               N   S       S

          110                 120                 130                 140
BM: W-I-I-N-G-I-E-W-A-I-S-N-N-M-D-V-I-N-M-S-L-G-G-P-T-G-S-T-A-L-K-T-V-V-D-
BA:                                                 S
NO:                     A                           S     A       A-A
DY: A   V-S           T-Q   G-L                     S             Q-A
CA: G   V-S           T-T   G                     A-S         M   Q-A

                   150                 160                 170
BM: K-A-V-S-S-G-I-V-V-A-A-A-A-G-N-E-G-S-S-G-S-T-S-T-V-G-Y-P-A-K-Y-P-S-T-I-
BA:                                             S
NO:       A     V     V                 T     S             G         V
DY:     Y-A           V           S           Q-N   I             D   V
CA: N   Y-A-R   V     V           S   N         N   I             D   V

          180                 190                 200                 210
BM: A-V-G-A-V-N-S-A-N-Q-R-A-S-F-S-S-A-G-S-E-L-D-V-M-A-P-G-V-S-I-Q-S-T-L-P-
BA:               S
NO:           D   S                 V   P
DY:           D   N-K-N             V   A     E               V-Y     Y
CA:           D   N-S-N             V   A     E           A-G-V-Y     Y

                   220                 230                 240
BM: G-G-T-Y-G-A-Y-N-G-T-S-M-A-T-P-H-V-A-G-A-A-A-L-I-L-S-K-H-P-T-W-T-N-A-Q-
BA:
NO:   N-K                       S                           N       T
DY: S-N     T-S-L               S                       Y     L-S-A-S
CA: T-N     A-T-L               S                           N-L-S-A-S

          250                 260                 270
BM: V-R-D-R-L-E-S-T-A-T-Y-L-G-S-S-F-Y-Y-G-K-G-L-I-N-V-Q-A-A-A-Q
BA:                           D
NO:     S-S   Q-N   T   K     D
DY:     N     S         N     D                       E
CA:     N     S                                       E
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Fig.2. Comparison of amino acid sequences of mesentericopeptidase (BM), subtilisin I 168 (isolated from strain I 168 of *B. subtilis*, BS), subtilisin amylosachariticus (BA), subtilisin novo/BPN′ (NO), subtilisin DY (DY) and subtilisin carlsberg (CA). Only the deviations from the sequence of BM are shown for the other enzymes. (●) Gap.

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