PRIMARY STRUCTURE OF CARBOXYPEPTIDASE I FROM MALTED BARLEY

by

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The primary structure of malt carboxypeptidase I has been determined. The enzyme is composed of two peptide chains, an A- and a B-chain, linked by disulphide bridges. Fragments were obtained by chemical cleavages with either cyanogen bromide or hydroxylamine and by enzymatic cleavages with either trypsin, *S. aureus* V8 protease or proline specific endopeptidase (E.C. 3.4.21.26), sequenced and aligned to give the total sequence of the two chains. The A- and B-chains contain 266 and 148 amino acid residues, respectively. Glycosylated asparagines are found in positions 118 and 232 of the A-chain and position 56 of the B-chain. Alignment of the sequence of the A-chain with the N-terminal part of carboxypeptidase Y revealed 30% homology. Similarly, the B-chain showed 27% homology with the C-terminal part of carboxypeptidase Y. No homology was observed with other proteins by a computer search of a sequence data base provided by the National Biomedical Research Foundation. A region of the A-chain was found to be identical to the region around the essential seryl residue in position 146 of carboxypeptidase Y.

1. INTRODUCTION

All known carboxypeptidases may be classified either as metallo carboxypeptidases or as serine carboxypeptidases (6). For a number of metallo carboxypeptidases from different organisms the amino acid sequences have been determined and the three-dimensional structure of bovine carboxypeptidase A is also known (16). Among the serine carboxypeptidases the most extensively characterized is yeast carboxypeptidase Y, of which both the amino acid sequence and the corresponding nucleotide sequence are known (7, 15, 20). While this en-

zyme, like most other such enzymes from fungi, consists of a single peptide chain, a number of serine carboxypeptidases from higher plants and mammalian tissues are composed of two peptide chains of thus far unknown structure (6). In the present paper the amino acid sequence of such an enzyme, previously isolated from malted barley (8), i.e. carboxypeptidase I, is reported. The longest of its constituent peptide chains is homologous with the N-terminal portion of yeast carboxypeptidase, whereas the shortest peptide chain exhibits homology with the C-terminal part of yeast carboxypeptidase.

Abbreviations: CABS-Sepharose = [N-(ϵ -aminocaproyl)-p-amino-benzyl]succinyl-Sepharose 4B; DPCC = diphenylcarbonyl chloride; EDTA = ethylenediaminetetraacetic acid, disodium salt; HPLC = high pressure liquid chromatography; PTH = phenylthiohydantoin; TFA = trifluoroacetic acid. The binding site notations for the enzyme is that of SCHECHTER and BERGER (17). Accordingly, the binding site for the C-terminal amino acid residue of the substrate is denoted S; and those for the amino acid residues in the amino-terminal direction away from the scissile bond are denoted S₁, S₂, ..., S_n. Amino acid residues in the substrate are referred to as P₁, P₂, ..., P_n and P_i in correspondence with the binding site.

2. MATERIALS AND METHODS

2.1. Materials

Malt carboxypeptidase I was prepared as previously described (8) from malted Gula barley (obtained from the Carlsberg Breweries, Denmark) by affinity chromatography on CABS-Sepharose.

Bio-Gels P-200, P-60, P-30 and P-6 were from Bio-Rad, USA. DPCC-treated trypsin and dithiothreitol were from Sigma, USA. *S. aureus* V8 protease was from Miles, USA. Proline specific endopeptidase (EC 3.4.21.26) from *Flavobacterium meningosepticum* was from Seikagaku Kogyo Co., Japan. Carboxypeptidase Y was a product of Carlsberg Biotechnology, Denmark and malt carboxypeptidase II was prepared in this laboratory as earlier described (9). 2-vinylpyridine was from Janssen Chimica, Belgium. All other chemicals were analytical or HPLC-grade.

2.2. Methods

2.2.1. Chemical modifications

Malt carboxypeptidase I (100 mg) dissolved in 5 ml 7 M-guanidine hydrochloride, 0.2 M-Tris, 5 mM-EDTA, pH 7.6, was flushed with nitrogen and reduced for 2 hours by addition of 20 mg dithiothreitol. Alkylation was then performed with 3×50 µl 2-vinylpyridine added over a period of 30 min. After 2 hours, the pH of the reaction mixture was adjusted to 4.0 with glacial acetic acid and excess of reagents removed by dialysis against 2% acetic acid with 5% ethanol (21).

2.2.2. Separation of A- and B-chain

After reduction and alkylation of malt carboxypeptidase I, the peptide chains were separated by gel filtration on Bio-Gel P-200 (5.0×88 cm) equilibrated with 5% (v/v) acetic acid. This provided an improved separation compared to the previously employed method using Sephadex G-100 (8).

2.2.3. Chemical cleavages of peptide bonds

Cyanogen bromide cleavages of reduced and alkylated peptide chains (50 mg · ml⁻¹) were

performed in 70% (v/v) formic acid or trifluoroacetic acid at room temperature for 20 hours in the dark with 50 mg · ml⁻¹ cyanogen bromide. After 20 hours, 20 volumes of water were added and the mixture lyophilized.

Cleavage at Asn-Gly bonds in peptides (2-5 mg · ml⁻¹) was effectuated with freshly prepared 2 M-hydroxylamine in 6 M-guanidine hydrochloride, adjusted to and kept at pH 9.0 with 4.5 M-LiOH. After 4 hours at 45 °C the reaction was stopped by adding glacial acetic acid at 10% (v/v) and the mixture was desalted on Bio-Gel P-6 eluted with 10% acetic acid (3).

2.2.4. Enzymatic cleavages of peptide bonds

Tryptic digestion of peptide chains was performed in a pH-stat in 2 M-urea at 30 °C and pH 8.0. In a typical experiment 5 mg peptide was dissolved in 670 µl 6 M-urea, 1000 µl distilled water and 5 µl 0.1 M-calcium chloride was added. pH was adjusted to 8.0 with 50 mM-NaOH and 50 µl DPCC-treated trypsin (1 µg/µl) in 0.1 mM-HCl was added (1:100 w/w). pH was kept at 8.0 by titration with 50 mM-NaOH and after 30 min, 50 µl trypsin solution was again added and the digestion continued for 30 min at pH 8.0. The reaction was stopped by addition of trifluoroacetic acid at 0.5% and the peptide mixture injected directly into a Waters HPLC system equipped with a reverse-phase column (see section 2.2.7).

Digestion with *S. aureus* V8 protease (1:40 w/w) (13) was performed at 40 °C in 0.1 M-ammonium bicarbonate pH 7.8 (cleavage after glutamic acid). After 4 hours of digestion, the reaction mixture was lyophilized and the peptide fragments separated by HPLC.

Cleavage with proline specific endopeptidase at the carboxyl side of proline residues (23) was performed in 0.1 M-sodium phosphate buffer pH 7.0 at 35 °C for 4 hours (1:40 w/w). The peptides generated were separated by HPLC.

2.2.5. C-terminal sequence determination

C-terminal sequences of the NG2 fragment of the B-chain and the entire A-chain were determined by digestion with malt carboxypeptidase II. The following conditions were used: 0.17

mM-NG2, 0.2 μ M-carboxypeptidase, 0.05 M-sodium acetate, pH 4.0; 0.075 mM A-chain, 10 μ M-carboxypeptidase, 0.05 M-Mes, 1 mM-ED-TA, 0.1% SDS, pH 6.5. Aliquots were withdrawn during the reaction, pH was lowered to 2.0 by addition of 0.5 M-HCl and the aliquots were applied directly to a Durrum D500 amino acid analyzer.

2.2.6. Separation of large peptides

Separation and purification of large peptide fragments were performed by gel filtration through Bio-Gel P-60 or P-30 in 10-30 % (v/v) acetic acid or on a Pharmacia FPLC system equipped with a Mono S column eluted with a linear gradient from 0 to 0.2 M-NaCl in 10 mM-sodium acetate, 7 M-urea, pH 5.0, over a period of 20 min (22).

2.2.7. Separation of small peptides

A Waters chromatograph was used for purification of small peptides by reverse phase HPLC using wide pore C_{18} columns i.d. 4.6 mm and length 25 cm (J.T. Baker no. RP 7104-0 or Vydac no. 218TPb). Gradient elution was performed with linearly increasing concentrations of acetonitrile in 0.1% (w/w) trifluoroacetic acid at a flow rate of 1.0 ml $\cdot$ min⁻¹. The eluent was monitored at 220 nm supplemented with fluorescence detection at 375 nm after excitation at 280 nm, to detect tryptophan containing peptides. Inhomogeneous fractions were rechromatographed in the same system but employing a more flat and narrow gradient of acetonitrile.

2.2.8. Amino acid sequence determinations

Peptides were sequenced either on a Beckman model 890C liquid phase sequencer as previously described (14) or on an Applied Biosystems model 470A gas-phase sequencer, using the programme provided by the company. The phenylthiohydantoin amino acid derivatives were identified by reverse-phase HPLC using a linear gradient from 10 to 45% methanol, (19). Amino acid compositions were determined as previously described (8).

2.2.9. Peptide nomenclature

Peptide fragments obtained by cleavage with cyanogen bromide, hydroxylamine or trypsin, are designated CB, NG, and T, respectively, followed by a number indicating their positions in the polypeptide chain from the N-terminus of the protein. Similarly, fragments originating from cleavage of Asp-Pro bonds in acidic solution during cleavage with cyanogen bromide are named DP followed by their position in and the name of the corresponding cyanogen bromide fragment. Secondary fragments generated by digestion of tryptic peptides or cyanogen bromide fragments with *S. aureus* V8 protease or proline specific endopeptidase are designated E and PSE, respectively, followed by a number according to their positions in the corresponding primary fragments and terminated by the designation of the original primary fragment.

3. RESULTS

3.1. N-terminal sequences of A- and B-chain

The A- and B-chain of malt carboxypeptidase I were separated by chromatography on Bio-Gel P-200, the A-chain (mol.wt. 32,000) eluting ahead of the B-chain (mol.wt. 19,000). The fractions were pooled and the two pools subjected to N-terminal sequence determination. The A-chain was sequenced 42 cycles and the B-chain 58 cycles. The results agreed with the N-terminal sequence already published (8) and the unknown residue in position four of the B-chain was identified as a cysteinyl residue.

3.2. Cyanogen bromide fragments

According to the amino acid analysis, both the A- and B-chain of malt carboxypeptidase I contain three methionyl residues (8) and cleavage of each chain with cyanogen bromide should thus give rise to four fragments.

Chromatography of the cyanogen bromide fragments from the A-chain on Bio-Gel P-60 (Figure 1) produced four peaks. Pool 1 presumably contained uncleaved A-chain. Pool 2 was subjected to 30 cycles of Edman degradation. It contained two peptides, the N-terminal cyanogen bromide fragment (CB1, 102 res.) and Ile¹⁰³-Hse¹⁸⁹ (CB2, 87 res.). Pool 3 was rechromatographed.

Figure 1. Separation of cyanogen bromide fragments of the A-chain (13 mg) on Bio-Gel P-60. The column (1.5×89 cm) was eluted with 10% acetic acid at a flow rate of 7 ml · h⁻¹. Fractions (1.4 ml) were pooled as indicated. Pool 3 was further purified by rechromatography using Bio-Gel P-30.

matographed on Bio-Gel P-30. It contained three peptides, one peptide being the C-terminal cyanogen bromide fragment (CB4, Gly²¹²-Ser²⁶⁶) which was sequenced 25 cycles. The remaining two peptides originated from CB1 by an Asp-Pro split. One of these, DP2-CB1 (Pro⁵¹-Hse¹⁰²), was sequenced 21 cycles. Pool 4 contained a single peptide species (CB3, Val¹⁹⁰-Hse²¹¹), which was sequenced 19 cycles.

Chromatography of the cyanogen bromide fragments of the B-chain on Bio-Gel P-60 yielded one broad peak and several minor peaks. One of the minor peaks contained the N-terminal pentapeptide already known from the N-terminal sequence data while the broad peak by rechromatography on Mono S was resolved into several peaks (figure not shown) allowing CB3 and CB4 to be sequenced 17 and 19 cycles, respectively. The main part of CB2 was already known from the N-terminal sequence determination.

3.3. Hydroxylamine fragments

Treatment of the intact A-chain with hydroxylamine resulted in three peptide fragments consistent with the two Asn-Gly sequences seen

Figure 2. Separation of hydroxylamine fragments of the A-chain (5.0 mg) on Bio-Gel P-60. The column (1.5×90 cm) was eluted with 30% acetic acid at a flow rate of 4 ml · h⁻¹. Fractions (1.0 ml) were pooled as indicated.

in DP2-CB1 and CB3, respectively. They could be purified by gel filtration on Bio-Gel P-60 (Figure 2). Pool 1 contained Gly⁵⁸-Asn¹⁹² while pool 2 contained Gly¹⁹³-Ser²⁶⁶. Sequence analysis of this fragment provided the overlap between CB3 and CB4. Pool 3 contained the already known N-terminal peptide Ala¹-Asn⁵⁷.

Cleavage of the B-chain with hydroxylamine and subsequent chromatography on Bio-Gel P-60 indicated the existence of a single Asn-Gly sequence (Figure 3). Pool 1 is uncleaved B-chain, pool 2 the N-terminal fragment Gly¹-Asn¹⁰¹ and pool 3 the fragment Gly¹⁰²-Leu¹⁴⁸ which was sequenced 40 steps.

3.4. Sequence studies on tryptic peptides

The amino acid composition of the A-chain of malt carboxypeptidase I (8) indicated 12-13 Lys

Figure 3. Separation of hydroxylamine fragments of the B-chain (3.5 mg). Conditions as in Figure 2. Fractions (1.5 ml) were pooled as indicated.

and 3 Arg residues, corresponding to 16-17 tryptic peptides. HPLC-chromatography of the digestion mixture revealed a complex chromatogram with fairly well resolved peaks (Figure 4). The amino acid analyses combined with the available sequence data lead to identification of several of the peptides. The remaining peptides were sequenced as far as possible, in most cases by means of the gas-phase sequencer.

Figure 4. Fractionation of tryptic digest of the A-chain by reverse phase HPLC using a wide pore C_{18} column (Bakerbond). The column was eluted over 80 min with a gradient from 5 to 40% acetonitrile in 0.1% TFA at a flow rate of $1.0 \text{ ml} \cdot \text{min}^{-1}$. The elution was monitored at 220 nm.

Figure 5. Fractionation of a tryptic digest of the B-chain by reverse phase HPLC using a wide pore C_{18} column (Bakerbond). The column was eluted over 60 min with a gradient from 18 to 36% acetonitrile in 0.1% TFA at a flow rate of $1.0 \text{ ml} \cdot \text{min}^{-1}$. The elution was monitored at 220 nm.

Incomplete tryptic cleavages were observed between Arg⁴⁵-Asp⁴⁶, Lys⁴⁹-Asp⁵⁰ and Lys¹⁸⁰-Pro¹⁸¹. Sequencing of T3+4+5 provided the overlap between the N-terminal sequence and the Asp-Pro fragment DP2-CB1 obtained after cleavage with cyanogen bromide.

Amino acid analysis of the B-chain indicated 6 Lys and 4 Arg residues (8) consistent with 11 tryptic peptides. However, the HPLC-chromatogram of the digestion mixture showed only 7 peaks (Figure 5) and sequence analysis revealed that Lys⁴⁰-Leu⁴¹, Arg⁹⁶-Pro⁹⁷ Lys¹³¹-Pro¹³² and Lys¹⁴⁷-Leu¹⁴⁸ had not been cleaved. Sequencing of T5 provided the overlap between CB3 and CB4 while sequencing of T6+7 revealed the position of CB4 relative to NG2.

3.5. Sequence studies on *S. aureus* V8 protease fragments

The mixture of CB1 and CB2 from the A-chain (section 3.2) was digested with *S. aureus* V8 protease. The resulting fragments were separated by reverse phase HPLC (Figure 6). Sequence analysis of E7-CB1 provided, together with the known sequence of T8, the position of

Figure 6. Separation of *S. aureus* V8 protease fragments from CB1 and CB2 of the A-chain by reverse phase HPLC using a wide pore C_{18} column (Vydac). The column was eluted over 60 min with a gradient from 18 to 36% acetonitrile in 0.1% TFA at a flow rate of $1.0 \text{ ml} \cdot \text{min}^{-1}$. The elution was monitored at 220 nm.

CB2 in the chain. Similarly, the sequence determined for E2-CB4 and E(3+4)-CB4 after digestion of CB4 with V8 protease provided the C-terminal sequence together with a tryptic fragment T16 and C-terminal digestion with malt carboxypeptidase II. Sequence analysis of E2-CB2 and E5-CB2 provided overlap between tryptic fragments.

The C-terminal hydroxylamine fragment NG2 from the B-chain was digested with V8 protease and the fragments separated by reverse phase HPLC. Sequence analysis gave the missing overlaps between tryptic fragments in the C-terminal part of the B-chain.

3.6. Sequence studies on fragments obtained after cleavage with proline specific endopeptidase

The tryptic fragment T11 from the A-chain

was digested with proline specific endopeptidase as described in the literature (23) and the fragments separated by reverse phase HPLC. Sequence analysis provided the missing overlap between E4-CB2 and E5-CB2.

3.7. Assignment of glycosylated asparagines

The A-chain of malt carboxypeptidase I was previously found to contain 4 glucosamine residues (8). These were assigned to two glycosylated asparagines (Asn^{118} and Asn^{232}) from the absence of detectable PTH-amino acid after Edman degradation, a hydroxylamine acid two positions from the vacant position towards the C-terminal end, and finally by the amino acid analysis of T9 and E2-CB4 indicating the presence of glucosamine and the excess of a single Asx as compared with the composition obtained from the sequence determined. Similarly, the B-chain was found to contain a glycosylated asparagine at position 56.

4. DISCUSSION

The determination of the amino acid sequence of the serine carboxypeptidase I from barley malt confirms that this enzyme is composed of two chains, linked by disulfide bridges, the A-chain containing 266 amino acid residues (Figure 7) and the B-chain 148 amino acid residues (Figure 8). The amino acid composition of the A- and B-chain (Table I) determined by sequence analysis is in fair agreement with the composition found by amino acid analysis (8). The glucosamine and neutral sugar (8%) appears to be attached to the asparagine residues at positions 118 and 232 of the A-chain and position 56 of the B-chain. No unused N-glycosylation sites are found and O-glycosylation is absent. The amino acid residue at position 74 of the B-chain has not yet been identified since it has escaped detection as either an ordinary or as a carbohydrate carrying amino acid residue. The enzyme contains seven cysteinyl residues consistent with the presence of a single unblocked sulfhydryl group (8) and three disulfide bridges.

Comparison of the amino acid sequence of malt carboxypeptidase I with those of other proteins has only demonstrated homology with

Figure 7. Amino acid sequence of the A-chain of malt carboxypeptidase I. Peptides were sequenced by either automated Edman degradation (-) or by digestion with malt carboxypeptidase II (—). Each peptide fragment is indicated by a full line for sequenced residues followed by a broken line for residues not sequenced in that particular fragment. Peptide fragments are designated by the following nomenclature: CB, cyanogen bromide fragments; NG, hydroxylamine fragments; T, tryptic fragments; E, *S. aureus* V8 protease fragments; PSE, proline specific endopeptidase fragments; DP, Asp-Pro split during cleavage with cyanogen bromide in acidic solution. N are glycosylated asparagines.

Figure 8. Amino acid sequence of the B-chain of malt carboxypeptidase I. See legend for Figure 7.

the serine carboxypeptidase from yeast, i.e. carboxypeptidase Y. This enzyme consists of a single peptide chain, 421 amino acid residues long. However, when the A- and B-chains of malt carboxypeptidase I are aligned with the N-terminal and C-terminal portions of carboxypeptidase Y, respectively, and due allowance is made for gaps, 30% and 27% identity is demonstrated (Figure 9). The regions 44-58 and 142-149 in the carboxypeptidase Y numbering are highly conserved. The latter region contains the essential Ser¹⁴⁶ of carboxypeptidase Y (12) and the fact that this residue is conserved in malt carboxypeptidase I strongly suggests that it is the active site serine in this enzyme as well. The sequence around the reactive seryl residue has been determined for three serine carboxypeptidases from citrus fruits (10, 18) (see Figure 10). These regions exhibit high homology with the corresponding regions of mammalian serine endopeptidases, but only slight homology with those of carboxypeptidase Y and malt carboxypeptidase I, suggesting that two groups of serine carboxypeptidases exist.

In carboxypeptidase Y, Cys³⁴¹ and Met³⁹⁸ are located in the S_i and S'_i binding sites, respectively (7). These amino acid residues are not conserved in malt carboxypeptidase I which is consistent with this enzyme being insensitive to para-hydroxymercuribenzoate and phenacylbromide which modify Cys³⁴¹ and Met³⁹⁸ in carboxypeptidase Y, respectively (2, 4, 5). Malt carboxypeptidase I does contain a cysteinyl residue at another position, but it is only accessible to chemical modification after denaturation of the enzyme (8).

It is well established that a histidyl residue functions as an acid/base catalyst in the active site of the serine endopeptidases (1) and it is probable that this is also the case for the serine carboxypeptidases (6, 11). It is reasonable to assume that such an amino acid residue would be preserved in the two homologous enzymes carboxypeptidase Y and malt carboxypeptidase I. If so, the histidyl residues at positions 317, 386 and 397 (see Figure 9) are the only possibilities, and of these the one at position 397 is situated in a fairly conserved region and hence, the most

Figure 9. Alignment of the sequences of the A- and B-chain of malt carboxypeptidase I with the nucleotide derived amino acid sequence of carboxypeptidase Y (nucleotide sequence differs from the amino acid sequence at four positions (10)). The numbering is that of carboxypeptidase Y. Gaps (-) were introduced to obtain maximum homology and positions with identical residues are boxed. In the sequence of carboxypeptidase Y the unblocked cysteinyl residue is indicated by SH and the reactive seryl residue by an asterisk. N are glycosylated asparagines. CPD-MI = malt carboxypeptidase I; CPD-Y = carboxypeptidase Y.

probable candidate.

The homology found by the alignment of the two chains of malt carboxypeptidase I with the single chain of carboxypeptidase Y might indicate that malt carboxypeptidase I is synthesized as a single chain which subsequently is cleaved at two positions, i.e. after Ser²⁵¹ and before Gly²⁶⁵, thus releasing an approximately 13 amino acid residue long peptide. However, it is unknown whether these cleavages are required for activity, or, although it seems unlikely, take place during purification of the enzyme. It is of interest to note that the two enzymes are terminated at the same positions in the C-terminus but at different positions in the N-terminus.

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Table I. Amino acid composition of A- and B-chain of malt carboxypeptidase I

Amino acid	Residues per molecule				
	A-chain		B-chain		
	Sequence analysis	Amino acid Analysis (per 28,800) ^{a)}	Sequence analysis	Amino acid analysis (per 16,300) ^{a)}	
Aspartic acid	15 } 27	27.2	7 } 10	11.0	
Asparagine	12 }		3 }		
Threonine	13	12.9	10	10.1	
Serine	25	24.6	13	13.7	
Glutamic acid	13 } 18	17.3	6 } 10	10.0	
Glutamine	5 }		4 }		
Proline	17	15.1	6	6.6	
Glycine	32	31.6	15	15.7	
Alanine	15	15.6	16	16.0	
Cysteine	5	3.2	2	1.8	
Valine	19	20.0	10	11.2	
Methionine	3	2.7	3	3.0	
Isoleucine	12	12.9	7	6.9	
Leucine	21	22.3	9	10.5	
Tyrosine	18	17.7	8	9.2	
Phenylalanine	13	14.2	6	6.6	
Histidine	8	9.2	6	6.1	
Lysine	13	12.2	6	6.1	
Arginine	3	2.8	4	3.5	
Tryptophan	4	4	6	3	
Unknown	0	—	1	—	
Total	266	265.5	148	151	

^{a)}Based on the calculated mol.wt. of the protein part.

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Enzyme	Amino acid sequence					
CPD-M I, A-chain (Barley malt):	-A	G	E	S	Y	A G V-
CPD-Y (Yeast):	-A	G	E	S	Y	A G H-
CPD-C _{Ua} (Mandarin orange):	-E	G	D	S	G	G E L-
CPD-C _{Ub} (Mandarin orange):	-E	G	D	S	G	G E L-
CPD-C _N (Citrus natsudaikai):	-Z	G	B	S	G	G E L-
Chymotrypsin (Bovine):	-M	G	D	S	G	G P L-
Elastase (Porcine):	-Q	G	D	S	G	G P L-
Plasmin (Bovine):	-Q	G	D	S	G	G P L-
Trypsin (Bovine):	-Q	G	D	S	G	G P V-
Thrombin (Bovine):	-E	G	D	S	G	G P F-
Subtilisin BPN':	-N	G	T	S	M	A S P-

Figure 10. Amino acid sequence around the reactive seryl residue of serine proteases. The reactive seryl residue is marked with an asterisk. Sequence data from ref. 10. CPD-MI = malt carboxypeptidase I; CPD-Y = carboxypeptidase Y, CPD-C_{Ua}, CPD-C_{Ub} and CPD-C_N carboxypeptidases from citrus fruits (10, 18).

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