PRIMARY STRUCTURE OF CARBOXYPEPTIDASE III FROM MALTED BARLEY

by

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The primary structure of malt carboxypeptidase III has been determined. The enzyme is a single N-terminally blocked polypeptide chain containing 411 amino acid residues. The sequence of these amino acid residues was deduced from analysis of fragments of the polypeptide chain obtained by chemical cleavages with either cyanogen bromide or hydroxylamine and by enzymatic cleavages with either trypsin, *S. aureus* V8 protease or proteinase A from yeast. A glycosylated asparagine was found in position 71.

The determined sequence was 97% homologous with the amino acid sequence derived from the nucleotide sequence of a gene coding for a wheat protein postulated to be a carboxypeptidase. The malt carboxypeptidase III sequence showed 34% homology with the amino acid sequence of the single-chain carboxypeptidase Y, and about 25% homology with the combined A- and B-chains of malt carboxypeptidase I and II as well as wheat carboxypeptidase II.

1. INTRODUCTION

Carboxypeptidases play an important role in hydrolysis of reserve proteins in temperate zone cereals (21). In germinating barley L. MIKOLA (16) has suggested the presence of five carboxypeptidases with different substrate preferences and three of these, carboxypeptidase I, II and III have been purified and characterized in this laboratory (6, 7, 8). The amino acid sequences of malt carboxypeptidases I (19) and II (20) and wheat carboxypeptidase II (9) are known and all three enzymes consist, like a number of other serine carboxypeptidases from higher plants (4), of two subunits, each composed of two polypeptide chains, cross-linked by disulphide bridges (8). Recently, DOAN & FINCHER (10) reported the isolation of a cDNA

clone encoding both peptide chains of malt carboxypeptidase I, indicating that these chains are formed by excision of a 55-residue long linker peptide from a single precursor polypeptide. The amino acid sequence of the two peptide chains are homologous with the N-terminal and C-terminal portion, respectively, of the single-chain carboxypeptidase Y from yeast (5, 15, 18). In the present report, the primary structure of malt carboxypeptidase III has been determined, and it is demonstrated that the sequence of this single peptide chain is almost identical to the amino acid sequence derived from the nucleotide sequence of a wheat gene (2) and homologous with the two peptide chains of malt carboxypeptidases I and II and the single peptide chain of carboxypeptidase Y.

Abbreviations: DPCC = diphenylcarbamyl chloride; EDTA = ethylenediaminetetraacetic acid, disodium salt; GYBS-Sepharose = [(N-glycyl-L-tyrosine)-p-azo-benzyl] succinyl-Sepharose 4B; HPLC = high performance liquid chromatography; PTH = phenylthiohydantoin; TFA = trifluoroacetic acid.

2. MATERIALS AND METHODS

2.1. Materials

Malt carboxypeptidase III was prepared as previously described (6) from malted Gula barley (obtained from the Carlsberg Breweries, Denmark) by affinity chromatography on GYBS-Sepharose.

Bio-Gels P-60 and P-6 were from Bio-Rad, USA. DPCC-treated trypsin and dithiothreitol were from Sigma, USA. *S. aureus* V8 protease was from ICN ImmunoBiologicals, USA. Proteinase A from yeast was a gift from dr. THOMAS DREYER, Carlsberg Laboratory. 2-Vinylpyridine was from Aldrich, W. Germany. Reagents and solvents used in sequencing were from Applied Biosystems, USA. All other chemicals were analytical or HPLC-grade from Merck, W. Germany.

2.2. Methods

2.2.1. Cleavage of peptide bonds

Malt carboxypeptidase III was reduced and pyridylethylated and subsequently cleaved with cyanogen bromide, hydroxylamine or trypsin as previously described (7, 19). Digestion of the modified enzyme with *S. aureus* V8 protease (EC 3.4.21.19) (13) was performed at 37 °C in 0.1 M ammonium bicarbonate and 2 M urea at pH 7.8. The protein was dissolved in 6 M urea and diluted with 2 volumes 0.15 M ammonium bicarbonate pH 7.8, enzyme was added (1:30 w/w) and the reaction stopped after 6 or 24 hours digestion by addition of acetic acid to a final concentration of 15%.

Digestion of the reduced and alkylated enzyme with proteinase A from yeast (EC 3.4.23.6) was performed in 0.1 M Na-formate and 2 M urea at pH 3.0 and 25 °C. The protein was dissolved in 6 M urea and two volumes 0.15 M Na-formate pH 3.0 were added. Enzyme (1:100 w/w) was added and the solution stirred for 6 hours at 25 °C. Proteinase A was inactivated by heating for 5 min in a boiling waterbath (11).

2.2.2. C-terminal sequence determination

The C-terminal amino acid sequence of the native enzyme was determined by digestion with wheat carboxypeptidase II. The following condi-

tions were used: 0.1 mM malt carboxypeptidase III, 50 mM sodium acetate, 1 mM EDTA, pH 4.5, 0.2 µM wheat carboxypeptidase II (mol. weight 55,000). At suitable time intervals aliquots were withdrawn, pH adjusted to 2.0 by addition of 0.5 M HCl and the aliquots applied directly to a Durrum D500 amino acid analyzer.

2.2.3. Separation of peptide fragments

Separation and purification of peptide fragments were performed by chromatography on Bio-Gel P-60 in 30% (v/v) acetic acid or by reverse phase HPLC using equipment from Waters and columns i.d. 4.6 mm and length 25 cm. The HPLC columns employed were Vydac 214TP54 Protein C₄, preferentially applied for isolation of large peptides and Vydac 218TPb or J.T. Baker RP7104-0 wide pore C₁₈ columns applied for smaller fragments. Gradient elution was accomplished with linear gradients of acetonitrile in 0.1% (w/w) trifluoroacetic acid at a flow rate of 1.0 ml · min⁻¹. The eluate was monitored at 215 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, or another column. Appropriate peaks in the chromatograms were lyophilized or taken to dryness on a Savant Speed Vac Concentrator before sequence analysis and amino acid analysis.

2.2.4. 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 program provided by the company. The phenylthiohydantoin amino acid derivatives from the gas-phase sequencer were identified on-line by reverse phase HPLC using an Applied Biosystems Model 120A PTH-analyser, while those from the liquid phase sequencer were identified with a Hewlett-Packard HPLC system using a linear gradient from 10 to 45% methanol (17). Amino acid compositions were determined as previously described (7).

Figure 1. Separation of soluble cyanogen bromide fragments of malt carboxypeptidase III (1 mg) by reverse phase HPLC using a wide pore C_{18} column (Bakerbond). Elution was accomplished over 60 min with a linear gradient from 0 to 54% acetonitrile in 0.1% TFA at a flow rate of $1.0 \text{ ml} \cdot \text{min}^{-1}$. The eluate was monitored at 215 nm.

2.2.5. Peptide nomenclature

Peptide fragments obtained by cleavage with cyanogen bromide, hydroxylamine, trypsin, *S. aureus* V8 protease or proteinase A from yeast are designated CB, NG, T, E and A, 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 solu-

tion during cleavage with cyanogen bromide are named DP followed by their position in and the name of the corresponding cyanogen bromide fragment.

3. RESULTS

3.1. N-terminal sequence

N-terminal sequence determination of the reduced and alkylated enzyme showed the polypeptide chain to be N-terminally blocked. The blocking group was both acid and base labile. Heterogeneity at the N-terminus was observed as the cyanogen bromide peptide CB1 was obtained in two forms, one form missing the first three amino acid residues.

3.2. Cyanogen bromide fragments

According to the amino acid analysis, malt carboxypeptidase III contains 12 methionyl residues (6) corresponding to 13 cyanogen bromide fragments. Cyanogen bromide cleavage of reduced and pyridylethylated malt carboxypeptidase III resulted in a supernatant and a precipitate of fragments insoluble at pH 3.5.

The supernatant was subjected to HPLC-chromatography which revealed 6 major peaks

Figure 2. Separation of dissolved precipitate (pH 3.5) of cyanogen bromide fragments of malt carboxypeptidase III (1 mg) on Bio-Gel P-60 ($1.5 \times 90 \text{ cm}$). The eluant was 30% acetic acid at a flow rate of $1.3 \text{ ml} \cdot \text{h}^{-1}$. The eluate was monitored at 280 nm and fractions of 1.3 ml collected.

Figure 3. Separation of hydroxylamine fragments of malt carboxypeptidase III (1.2 mg) by reverse phase HPLC using a protein C_4 column from Vydac. A linear gradient over 60 min from 27 to 63% acetonitrile in 0.1% TFA at a flow rate of $1.0 \text{ ml} \cdot \text{min}^{-1}$ was applied for elution. The eluate was monitored at 215 nm.

and 4 minor peaks. Sequence analysis allowed partial or complete determination of the primary structures of the following cyanogen bromide fragments: CB1, CB4, CB5, CB6, CB8, CB10, CB12 and CB13 (Figures 1 and 6). Several of the cyanogen bromide fragments were found in two peaks (e.g. CB8) presumably due to different retention times for peptides containing homoserine and homoserine lactone as the C-terminal residue.

Cyanogen bromide fragments in the precipitate were dissolved in 30% acetic acid and separated on Bio-Gel P-60 (Figure 2). In this way the partial amino acid sequences of cyanogen bromide fragments DP1-CB2, DP2-CB2, CB3, CB7 and CB9 were obtained (Figure 6).

3.3. Hydroxylamine fragments

The fragments formed by cleavage with hydroxylamine could be purified by HPLC-chromatography on a Vydac C_4 column (Figure 3). Cleavage or separation was apparently incomplete as the same N-terminal sequence was found in several peaks. The NG4-fragment could be sequenced 49 cycles and gave the overlaps between CB4, CB5, CB6 and CB7. The NG3 fragment provided overlap between two tryptic peptides (T17 and T18+19) (Figure 6).

3.4. Tryptic peptides

The amino acid composition of malt carboxypeptidase III indicated 24 Lys and 17 Arg residues (6), corresponding to 42 tryptic peptides. HPLC-chromatography of the digestion

Figure 4. Fractionation of a tryptic digest of malt carboxypeptidase III (0.8 mg) by reverse phase HPLC using a wide pore C_{18} column (Bakerbond). Elution was accomplished over 60 min with a gradient from 0 to 54% acetonitrile in 0.1% TFA at a flow rate of $1.0 \text{ ml} \cdot \text{min}^{-1}$. The eluate was monitored at 215 nm.

Figure 5. Separation of *S. aureus* V8 protease fragments from malt carboxypeptidase III (0.7 mg) by reverse phase HPLC using a wide pore C_{18} column (Bakerbond). A linear gradient over 60 min from 0 to 54% acetonitrile in 0.1% TFA at a flow rate of 1.0 ml $\cdot$ min $^{-1}$ was applied for elution. The eluate was monitored at 215 nm.

mixture revealed a complex chromatogram with fairly well resolved peaks (Figure 4). Isolated fragments were sequenced as far as possible. Incomplete tryptic cleavages were observed at repeated basic residues and at a basic residue followed by an acidic residue (Figure 6).

3.5. *S. aureus* V8 protease fragments

The reduced and alkylated malt carboxypeptidase III was digested with *S. aureus* V8 protease. The resulting fragments were separated by reverse phase HPLC (Figure 5). Sequence analysis of the separated fragments contributed valuable sequence information, e.g. missing

overlaps, especially between tryptic fragments. Two unexpected cleavages occurred at Gly⁹⁵-Thr⁹⁶ and Ala¹⁵³-Ser¹⁵⁴ (Figure 6) and the latter provided, after isolation of both E9a, E9b and E9, a missing overlap. E9 was obtained after digestion of fragment NG2 and E16 was isolated by HPLC after cleavage of CB7 with *S. aureus* V8 protease.

3.6. Proteinase A fragments

Throughout the sequence determination comparisons with the amino acid sequence derived from the nucleotide sequence of the corresponding carboxypeptidase from wheat (2) facilitated the alignment of the isolated fragments. An overlap was missing between E5 and E6 and a stretch connecting E20 with T33+34 (Figure 6). This information was obtained by digestion of reduced and alkylated malt carboxypeptidase III with proteinase A from yeast and isolation of the fragments A1 and A2 by HPLC.

3.7. Assignment of glycosylated asparagines and C-terminus

Malt carboxypeptidase III contains 2 glucosamine residues (6). These were assigned to the glycosylated asparagine Asn⁷¹ due to the absence of a PTH-amino acid in this position after Edman degradation of fragment E6a and NG2 and the presence of a hydroxyamino acid two positions from the vacant position towards the C-terminal end. Furthermore, amino acid analysis of NG2 indicated the presence of glucosamine.

Digestion of native malt carboxypeptidase III with wheat carboxypeptidase II identified the three amino acid residues at the C-terminus to be -Lys-Leu-Lys.

4. DISCUSSION

The amino acid sequence of malt carboxypeptidase III is consistent with this enzyme being composed of a single N-terminally blocked polypeptide chain, 411 amino acid residues long (Figure 6). The polypeptide chain is polymor-

Figure 6. Amino acid sequence of malt carboxypeptidase III. Peptides were sequenced by automated Edman degradation (full line) and digestion with wheat 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; A, proteinase A fragments; DP, Asp-Pro split during cleavage with cyanogen bromide in acidic solution. N is a glycosylated asparagine and X the unknown N-terminal blocking group.

Figure 7. Alignment of the amino acid sequence derived from the nucleotide sequence of a putative wheat carboxypeptidase (2) with that of malt carboxypeptidase III. Residues which are not identical are boxed. N is a glycosylated asparagine and X the unknown N-terminal blocking group. CPD-W III = putative wheat carboxypeptidase, CPD-M III = malt carboxypeptidase III.

phous at position 185, one form containing a glutamoyl, the other a valyl residue indicating the presence in barley of at least two genes each coding for one form. No evidence of polymorphism at other positions was found. The enzyme is a glycoprotein with a glycosylated asparagine at position 71. No unused potential N-glycosylation sites were found and O-glycosylation was absent. The amino acid composition, as calculated from the sequence, is in fairly good agreement with the composition determined by amino acid analysis (6).

A comparison of the sequence of malt carboxypeptidase III with the nucleotide sequence of a putative wheat carboxypeptidase (2) revealed almost identity (97% homology) indicating that the malt and the wheat enzyme are functionally equivalent (Figure 7). The malt carboxypeptidase III sequence deviates in 10 positions from the putative wheat carboxypeptidase and all deviations except the polymorphic spot are the result of single base mutations. The distribution of the substitutions seems not

to be random as they are concentrated in the central region of the sequences. This could be explained by the lack of important structures in this segment. A similar close homology was observed between carboxypeptidase II from malt and wheat (9, 20).

The amino acid sequence derived from the nucleotide sequence of the homologous wheat carboxypeptidase is extended both N-terminally and C-terminally compared to the determined amino acid sequence of malt carboxypeptidase III (Figure 7). The N-terminal extension comprises apparently a signal peptide (2) and additionally about 50 amino acid residues before the homology with malt carboxypeptidases begins. This region could be a pro-peptide, indicating that the wheat enzyme and presumably the corresponding malt carboxypeptidase III are synthesized as inactive zymogens. The C-terminal extension which is 16 amino acid residues, suggests that the enzyme has been subject to proteolysis after its synthesis.

A comparison of the amino acid sequence of

S.B. SØRENSEN et al.: Sequence of malt carboxypeptidase III

CPD-M I, A-chain: 1 10 20 30 40
 APQGA E V T G L P G F D G A L P S K - H Y A G Y V T V D E G H G R N L F Y Y V V E S E R D

CPD-M II, A-chain: A G G H A A D R I V R L P G Q P E V D F D M - - Y S G Y I T V D E A A G R S L F Y L L Q E A P E E

CPD-M III: X
 P G L P E G V A D L G H H A G Y Y R L P N T H D A R M F Y F F F E S R G K

CPD-Y: K I K D P K I L G I D P N V T Q Y T G Y L D V - E D E D K H F F F W T F E S R N D

CPD-M I, A-chain: 50 60 70 80
 P G K D P V V L W L N G G P G C S S F D - G F V Y E H G P F N F E S G G S V K S L P K L H L N P Y A W

CPD-M II, A-chain: A Q P A P L V L W L N G G P G C S S V A Y G A S E E L G A F R V M P R G A G - - - - L V L N E Y R W

CPD-M III: K E - D P V V I W L T G G P G C S S E L - A V F Y E N G P F T I A N N M S - - - - L V W H K F G W

CPD-Y: P A K D P V I L W L N G G P G C S S L T - G L F F E L G P S S I G P D L - - - - K P I G N P Y S W

CPD-M I, A-chain: 90 100 110 120 130
 S K V S T M I Y L D S P A G V G L S Y S K N V S D Y E T - G D L K T A T D S H T F L L K W F Q L Y P E

CPD-M II, A-chain: N K V A N V L F L D S P A G V G F S Y T N T S S D I Y T S G D N R T A H D S Y A F L A A W F E R F P H

CPD-M III: D K I S N I I F V D Q P T G T G F S Y S S D D R D T R H - D E T G V S N D L Y D F L O V F F K K H P E

CPD-Y: N S N A T V I F L D Q P V N V G F S Y S G S S G V S N T - - - V A A G K D V Y N F L E L F E D Q F P E

CPD-M I, A-chain: 140 150 160 170
 F L S N P - - F Y I A G E S Y A G V Y V P T L S H E V V K G I Q G G A K P T I N F K G Y M V G N G V C

CPD-M II, A-chain: Y K R R E - - F Y V A G E S Y A G H Y V P E L S Q L V - - - - H R S G N P V I N L K G F M V G N G L I

CPD-M III: F I K N D - - F F I T G E S Y A G H Y I P A F A S R V H Q G N K K N E G T H I N L K G F A I G N G L T

CPD-Y: Y V N K G Q D F H I A G E S Y A G H Y I P V F A S E I - - - - L S H K D R N F N L T S V L I G N G L T

CPD-M I, A-chain: 180 190 200 210 220 230
 D T I F D G N A L V P F A H G M G L I S D E I Y Q Q A S T S C H G N Y W N A T D G K C D T A I S K I E

CPD-M II, A-chain: D D Y H D Y V G T F E F W W N H G I V S D D T Y R R L K D A C L H D S F I H P S P A C D A A T D V A T

CPD-M III: D P A I V Y K A Y T D Y A L E M N L I Q K A D Y E R - - - - I N K F I P P C E F A I K L C G T N - G K

CPD-Y: D P L T Q Y N Y Y E P M A C G E G G E P S V L P S E E C S A M E D S L E R C L G L I E S C Y D S Q S V

CPD-M I, A-chain: 240 250 260 270 280
 S L I S G L N I Y D I L E P - C Y H S R S S - S S G V P C M - S D E V A T A W L D

CPD-M II, A-chain: A E Q G N I D M Y S L Y T P V C N I S S CPD-M II, B-chain: T G S Y D P C T - - E R Y S T A Y Y N

CPD-M III: A S C M A A Y M V C N T I F N S I M K L V G T K N Y Y D V R K E C E G - K L C Y D F S N L E K F F G D

CPD-Y: W S C V P A T I Y C N N A Q L - A P Y Q R T G R N V Y D I R K D C E G G N L C Y P T L Q D I D D Y L N

CPD-M I, B-chain: 290 300 310 320
 N A A V R S A I H A Q S V S A I G P - W L L C T D K L Y F - V H D A G - S M I A Y H K - - - N L T S Q

CPD-M II, B-chain: R R D V Q T A L H A N Y T G A M N Y T W T N T C S D T I N T H W H D A P R S M L P I Y R - - - E L I A A

CPD-M III: K - A V R Q A I - - - - G V G D I E F V S C S T S V Y Q A M L T - - D W M R N L E V G I P A L L E D

CPD-Y: Q D Y V K E A V - - - - G A E V D H Y E S C N F D I N R N F L F A G D W M K P Y H T A V T D L L N Q

CPD-M I, B-chain: 330 340 350 360 370
 G Y R A I I F S G D H D M C V P F T G S E A W T K S L - - - - - G Y G V V D S W R P W I T N G Q

CPD-M II, B-chain: G L R I W V F S G D T D A V V P L T A T R Y S I G A L - - - - - G L A T T T S W Y P W Y D D L Q

CPD-M III: G I N V L I Y A G E Y D L I C N W L G N S R W V H S M E W S G Q K D F A - - K T A E S S F L V D D A Q

CPD-Y: D L P I L V Y A G D K D F I C N W L G N K A W T D V L P W K Y D E E F A S Q K Y R N W T A S I T D - -

CPD-M I, B-chain: 380 390 400 410 420
 V S G Y T E G Y E H G L T F A T I K G A G H T V P E Y K P Q E A F A F Y S R W L - A G S K L

CPD-M II, B-chain: E V G G W S Q V Y K G L T L V S V R G A G H E V P L H R P R Q A L I L F Q O F L Q G K P M P G R T T N

CPD-M III: - - A G V L K S H G A L S F L K V H N A G H M V P M D O P K A A L E M L R R F T Q G K L K

CPD-Y: E V A G E V K S Y K H F T Y L R V F N G G H M V P F D V P E N A L S M V N E W I H G G F S L

malt carboxypeptidase III with those of other proteins by a computer search of a sequence data base provided by the National Biomedical Research Foundation, demonstrated homology only with malt carboxypeptidase I, wheat and malt carboxypeptidase II, a putative wheat carboxypeptidase and carboxypeptidase Y. Alignment of the three malt carboxypeptidases with carboxypeptidase Y is shown in Figure 8 with due allowance for gaps. The number of identical amino acid residues in malt carboxypeptidase III and carboxypeptidase Y is 141 (34%), whereas only 108 (26%) and 104 (25%) residues are common in malt carboxypeptidase I or malt carboxypeptidase II, respectively. These results are unexpected, because they indicate that malt carboxypeptidase III is more closely related to carboxypeptidase Y from yeast than to the two other malt carboxypeptidases. This conclusion is supported by the positions of the cysteinyl residues: All the nine cysteinyl residues of malt carboxypeptidase III are found in homologous positions in carboxypeptidase Y. This implies that the disulfide bridges are conserved as well and hence a similar folding of the polypeptide backbone in the two enzymes. Consequently, the free sulfhydryl group of malt carboxypeptidase III (6) is most likely located in position 341 (Figure 8) where the free sulfhydryl group of carboxypeptidase Y is known to be situated (5). Carboxypeptidase Y contains two additional cysteinyl residues (Cys¹⁹³ and Cys²⁰⁷) which are known to be connected by a disulfide bridge (18). A comparison of malt carboxypeptidase I and II reveals that they have six cysteinyl residues in homologous positions, and three of these (residue 56, 268 and 298 in Figure 8) are conserved in malt carboxypeptidase III and carboxypeptidase Y as well.

The number of identical amino acid residues present in all four carboxypeptidases (Figure 8) is 57 (14%). 12 of these residues, corresponding to 21%, are glycyl residues and 8 residues, corresponding to 14%, are prolyl residues. As glycine and proline are known to be situated at bends or twists of the polypeptide chain, these high figures further underline that the spatial conformations of these polypeptide chains might be similar.

Two regions in the four enzymes are highly conserved, i.e. the regions 49-58 and 144-149 in the carboxypeptidase Y numbering. The essential Ser¹⁴⁶ of carboxypeptidase Y (12) is located in the latter region and the conservation of this residue in all four carboxypeptidases suggests that it is the active site serine in these enzymes as well. The conserved region, residue 49-58, is probably important for the three-dimensional structure of the enzymes, due to the presence of three glycines and one proline in this segment.

Recently, it has been shown that His³⁹⁷ is the essential histidyl residue functioning as an acid/base catalyst in the active site of carboxypeptidase Y (3). As this histidine is the only conserved histidine in all the presently characterized serine carboxypeptidases, it is probably the essential histidine in these enzymes as well. It is still not clear which of the aspartic acid residues participate in the active site (1), since four aspartic acid residues are conserved in all four enzymes.

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◀ Figure 8. Alignment of the sequences of malt carboxypeptidases I, II and III with the nucleotide derived amino acid sequence of carboxypeptidase Y (nucleotide sequence differs from the amino acid sequence at four positions (5)). His⁶⁶ in malt carboxypeptidase I originates from nucleotide sequencing (10) while Pro was found by amino acid sequencing (19). The numbering is that of carboxypeptidase Y. Gaps (—) were introduced to obtain maximum homology. In the sequence of carboxypeptidase Y the unblocked cysteinyl residue is indicated by SH, the reactive seryl residue by an asterisk (*) and the essential His³⁹⁷ by ♦. Substrate binding sites in carboxypeptidase Y are marked by ●. Residues identical in all four enzymes are in boxes. N are glycosylated asparagines and X the unknown N-terminal blocking groups. CPD-M I = malt carboxypeptidase I; CPD-M II = malt carboxypeptidase II; CPD-M III = malt carboxypeptidase III; CPD-Y = carboxypeptidase Y.

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