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The Complete Amino Acid Sequence of the Zn^{2+}-Containing D-Alanyl-D-Alanine-Cleaving Carboxypeptidase of *Streptomyces albus* G

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The 22076-M_r Zn^{2+}-containing D-alanyl-D-alanine-cleaving carboxypeptidase of *Streptomyces abuls* G effectively catalyses the transfer of the N^{α},N^{ϵ}-diacetyl-L-lysyl-D-alanyl electrophilic group of the standard tripeptide substrate N^{α},N^{ϵ}-diacetyl-L-lysyl-D-alanyl-D-alanine to water. It also performs a weak β-lactamase activity, hydrolysing penicillin into penicilloate at a very low rate. This protein consists of 212 amino acid residues in a single polypeptide chain. The N terminus is partially blocked as a result of the cyclization of the dipeptide Asn-Gly into anhydroaspartylglycine imide. The protein has been fragmented by cyanogen bromide into five fragments whose sequences have been determined via appropriate subcleavages with various proteases. The ordering of the cyanogen bromide peptide fragments has been carried out (a) by submitting the *S*-carboxymethylated protein to complete tryptic digestion and labelling the methionine-containing peptides thus obtained with iodo[^{14}C]-acetamide, and (b) by submitting to limited tryptic digestion the *S*-[2-(4′-pyridyl)ethyl]-cysteine protein whose amino groups have been blocked by reaction with exo-*cis*-3,6-endoxo-Δ^4-tetrahydrophthalic anhydride prior to digestion. The protein contains six cysteine residues in the form of three disulfide bridges. No homology is found by comparing this peptidase with other Zn^{2+}-containing enzymes (carboxypeptidase A, thermolysin, carbonic anhydrase B and alcohol dehydrogenase) and several completely or partially sequenced, serine-containing D-alanyl-D-alanine-cleaving peptidases and Zn^{2+}/serine-containing β-lactamases.

The D-alanyl-D-alanine-cleaving peptidases (in short DD-peptidases) are enzymes involved in the bacterial wall peptidoglycan metabolism. They catalyse transfer of LAla-DGlu-(LRaa-DAla) (where DGlu is a γ-glutamyl residue and Raa is a diamino acid or an amino acid grouping possessing a free ω-amino group) from LAla-DGlu(LRaa-DAla-DAla) pentapeptide units either to water (carboxypeptidation) or to the ω-amino group at the L-Raa position of another peptide (transpeptidation). On the basis that the sequence LRaa-DAla-DAla is that part of the carbonyl donor mainly involved in substrate activity, the tripeptide N^{α},N^{ϵ}-diacetyl-L-lysyl-D-alanyl-D-alanine (Ac_2LLys-DAla-DAla) has been used as a substrate analogue to isolate several DD-peptidases exhibiting, with varying efficiencies, carboxypeptidase activity (Ac_2-LLys-DAla-DAla + H_2O → DAla + Ac_2LLys-DAla) and/or transpeptidase activity (Ac_2LLys-DAla-DAla + a suitable amino compound NH_2-X → DAla + Ac_2LLys-DAla-CONH-X) (for a recent review, see [1]). The R61 (from *Streptomyces* R61) and R39 (from *Actinomadura* R39) DD-peptidases are highly penicillin-sensitive, bifunctional carboxypeptidases/transpeptidases. They operate by covalent catalysis via an active serine residue. In contrast, the G (from *Streptomyces albus* G) DD-peptidase is a highly penicillin-resistant, monofunctional carboxypeptidase. It operates by liganding catalysis via a Zn^{2+} cofactor which is firmly bound to the apoprotein (K_A: 2×10^{14} M^{-1}) [2]. On the basis of X-ray crystallographic studies [3], this G Zn^{2+} DD-carboxypeptidase is a two-domain protein. A cleft, with the Zn^{2+} cofactor located in it, extends throughout the large domain and serves as binding site for the two enzyme competitive inhibitors, the dipeptide AcDAla-DGlu and the β-lactam compound *p*-iodo-7-β-phenylacetylaminocephalosporanic acid. The interpretation of the 2.5-Å (0.25-nm) electron density map of the G Zn^{2+} DD-carboxypeptidase has been carried out simultaneously with the establishment of its primary structure. This paper is the first report of the complete amino acid sequence of a member of this special class of bacterial DD-peptidases that characterize themselves by their ability specifically to attack peptide bonds extending between two D centres.

Abbreviations. Cya, cysteic acid; CmCys, *S*-carboxymethyl-cysteine; PeCys, *S*-[2-(4′-pyridyl)ethyl]cysteine; SCM-protein, protein treated with iodoacetic acid giving rise to CmCys residues; SPE-protein, protein treated with 4-vinyl-pyridine giving rise to PeCys residues; DABITC, 4-*N*,*N*-dimethylaminoazobenzene-4′-isothiocyanate; PITC, phenylisothiocyanate; dansyl and Dns, 5-dimethylaminonaphthalene-1-sulfonyl; Nbs_2, 5,5′-dithio-bis(2-nitrobenzoic acid); ETPA, exo-*cis*-3,6-endoxo-Δ^4-tetrahydrophthalic anhydride; EtSH, 2-mercaptoethanol; Hse, homoserine; $MetO_2$, methionine sulfone; HPLC, high-performance liquid chromatography; SDS, sodium dodecyl sulfate.

Enzymes. D-Alanyl-D-alanine-cleaving carboxypeptidase or DD-peptidase (EC 3.4.17.8); *Armillaria mellea* protease (EC 3.4.99.32); carboxypeptidase Y (EC 3.4.16.1); chymotrypsin (EC 3.4.21.1); pepsin (EC 3.4.23.1); *Staphylococcus aureus* V8 protease (EC 3.4.21.19); thermolysin (EC 3.4.24.4); trypsin (EC 3.4.21.4).

MATERIALS AND METHODS

Preparation of the DD-Peptidase (Native Protein)

The DD-peptidase was isolated and purified to protein homogeneity as described in [4].

Reduced and Alkylated Protein

After unfolding the protein in 6 M guanidinium chloride and cleavage of the S-S bridges with dithiothreitol, alkylation

of the SH groups in the reduced protein was carried out by treatment with iodoacetic acid, giving rise to *S*-carboxymethyl cysteine (CmCys) residues (SCM-protein), [5] or 4-vinyl-pyridine, giving rise to *S*-[2-(4′-pyridyl)ethyl]cysteine (PeCys) residues (SPE-protein) [6]. The efficacy of each of the treatments was checked by amino acid analysis to detect cystic acid (Cya) after further performic acid oxidation [7] of the reduced and alkylated protein.

Proteolytic Enzymes

Trypsin (treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone) and chymotrypsin were from Worthington (Freehold, NJ, USA). Carboxypeptidase Y and pepsin were from Boehringer (Mannheim, FRG). Thermolysin was from Calbiochem (San Diego, CA, USA) and *Staphylococcus aureus* protease V8 from Miles (Slough, England). *Armillaria mellea* protease was a gift from Dr V. Barkholt Pedersen (Copenhagen, Denmark).

Chemicals

Analytical grade reagents were used. Cyanogen bromide and 4-vinyl-pyridine were from Aldrich-Europe (Beerse, Belgium). Mercaptoethanesulfonic acid and anhydrous hydrazine were from Pierce Chemicals Co. (Rockford, IL, USA). Fluorescamine was from Fluka (Buchs, Switzerland), 5,5′-dithio-bis(2-nitrobenzoic acid) (Nbs_2) from Sigma (St Louis, MO, USA), and iodo[^{14}C]acetamide (50 Ci/mol) from New England Nuclear (Dreieich, FRG). Exo-*cis*-3,6-endoxo-Δ^4-tetrahydrophtalic anhydride (ETPA) was synthesized as described by Riley et al. [8]. All the other chemicals were from Merck (Darmstadt, FRG).

Thin-Layer Sheets, Chromatography and Electrophoresis Papers

Polyamide-coated thin-layer sheets (F1700) were either from Pierce Chemicals Co. or from Schleicher & Schüll (Dassel, FRG). Chromatography and electrophoresis papers were from Whatman (Maidstone, Kent, UK).

Solvents and Reagents for Manual Amino Acid Sequencing

1-Dimethylaminonaphtalene-5-sulphonyl (dansyl) chloride was from BDH (Poole, England). Phenylisothiocyanate (PITC), 4-*N*,*N*-dimethylaminoazobenzene-4′-isothiocyanate (DABITC) and trifluoroacetic acid were from Pierce Chemicals Co. Pyridine (analytical grade from Merck) was purified by two successive distillations, first over ninhydrin (20 g/l) and then over KOH pellets under nitrogen. Butyl acetate was distilled over ninhydrin (10 g/l). Heptane and ethyl acetate were spectroscopic grade reagents from Merck.

Solvents and Reagents for Automatic Liquid Amino Acid Sequencing and High-Performance Liquid Chromatography (HPLC)

The solvents and reagents used in the sequenator were 'sequenal grade' and were not purified further. The compounds 1 M quadrol, *n*-propanol (used to dilute the quadrol to a 0.3 M concentration), benzene, ethyl acetate and chlorobutane were from Merck. Hexafluorobutyric acid was from Pierce and trifluoroacetic acid was from Rathburn (Scotland). The dithiothreitol added to the chlorobutane to a final concentration of 0.001% was from Calbiochem. The acetonitrile used in the HPLC analysis of the phenylhydantoins was Lichrosolv grade from Merck. The HPLC water was from Alltech Assoc. (IL, USA).

Amino Acid Analysis

Protein samples (40 nmol) were hydrolyzed under vacuum in 150 µl of 6 M HCl at 110 °C for 24 h, 48 h and 72 h and analysed with a Beckman 120B analyser. Peptide samples (5 – 30 nmol) were hydrolyzed for 24 h under the same conditions and analysed with a Beckman Multichrom 4255 or a Dionex D300. Cysteine and methionine were determined as Cya and methionine sulfone ($MetO_2$) after performic oxidation of the protein [7]. Tryptophan was estimated after hydrolysis with 3 M mercaptoethane sulfonic acid for 96 h at 110 °C [9].

Determination of the N-Terminal Residues

Dansyl chloride was used according to the procedure recommended for the proteins by Gray [10]. For peptide fragments, the determination of the N-terminal residues was carried out by the method of Hartley [11].

Determination of the C-Terminal Residues

Samples (1 mg) of SCM-protein and of peptide CB_3 (obtained by cyanogen bromide cleavage of the protein) were treated with 0.5 ml anhydrous hydrazine for 24 h at 80 °C. Each hydrazinolysate was dried under vacuum over concentrated sulphuric acid, and the residue was dissolved in 0.5 ml H_2O. The solution was adjusted to pH 3 with 6 M HCl and filtered in water on a column (0.9 × 11 cm) of Amberlite IRC50 (H^+ form). The first 15 ml of the eluent were collected and, after freeze-drying, the residue was submitted to an additional chromatography under the same conditions as above (in order to remove all the hydrazides). The free amino acid residue released by hydrazinolysis was then identified with the amino acid autoanalyser.

Sequence Determination

The SCM-protein (100 nmol) and large-size peptides (100 nmol) were submitted to automatic liquid-phase Edman degradation [12] in a Socosi 110 sequenator (Saint-Maure, France), using 0.3 M quadrol as coupling buffer and 2 mg of polybrene [13] as carrier. The thiazolinones were converted manually using 20% trifluoroacetic acid [14] for 25 min at 55 °C. The phenylthiohydantoins were analysed by 'reversed-phase' HPLC on a column (0.46 × 30 cm) of C18 HL RSil (RSL, Eke, Belgium) using either an isochratic [15] or a gradient [16] elution method with acetonitrile as the organic solvent.

Small-size peptides (5 – 50 nmol) were sequenced manually, following the micro dansyl-Edman [17] or the DABITC/PITC [18] method. In some cases where the presence of amides or tryptophan residues was expected and when the initial degradation with the DABITC/PITC method resulted in a severe wash-out of the residual peptides, a combination of the two methods was used. In those cases, the first N-terminal residues were cleaved by the dansyl-Edman method without removing samples for dansylation and the following degradation steps were carried out by the DABITC/PITC

method. All the peptides were sequenced in 1-ml conic vials closed with a teflon septum.

Estimation of the SH Groups

Quantitative estimation was carried out spectrophotometrically as described in [19], using NBs_2 as reagent. Either sodium dodecylsulfate (SDS) (4%; w/v) or a mixture of 6 M guanidinium chloride and 2 mM EDTA were used as denaturing agents. Glutathione served as control.

Estimation of Sugars and S-Glyceryl-cysteine Thioether in the Native Protein

The possible occurrence of a carbohydrate moiety in the protein was tested by using the method of Dubois [20] and that of *S*-glyceryl-cysteine was tested as described in [21], except that the quantities and volumes were divided by 10.

Cyanogen Bromide Cleavage of the SCM-Protein

A solution of the SCM-protein (10 mg/ml) made in 70% formic acid was supplemented with cyanogen bromide (40 mg/ml, final concentration), left in the dark for 24 h at room temperature under nitrogen and finally freeze-dried.

Purification of the Cyanogen Bromide Peptide Fragments CB2, CB3, CB4, CB5 and CB6

Two procedures were used (see flow sheet in Fig. 1). In a first experiment and as shown in Fig. 1 M (M refers to the miniprint section), the soluble peptides originating from 5 µmol of SCM-protein (and separated from an insoluble core) were fractionated by filtration on a column (2.5 × 150 cm) of Sephadex G-50, fine, in 0.2 M NH_4HCO_3 buffer pH 8.6 (flow rate 35 ml/h; volume of the fractions 5.3 ml; manual detection at 280 and 214 nm). Fraction CB1 was the uncleaved protein. Peptide CB2 (115 residues) and peptide CB3 (58 residues) were obtained in pure form. Peptides CB5 and CB6 (5 and 4 residues, respectively) were recovered in the salt volume and further isolated from each other by paper electrophoresis at pH 3.5. In a second experiment and as shown in Fig. 2 M, all the peptide fragments originating from 0.8 µmol of SCM-protein were solubilized in 50 mM ammonium formate buffer pH 3.5 supplemented with 2 M guanidinium chloride. Filtration of the solution on a column (1.25 × 150 cm) of Sephadex G-50 fine in 50 mM ammonium formate buffer pH 3.5 (flow rate 15 ml/h; volume of the fractions 1.2 ml; detection at 230 nm) resulted in the isolation of an additional peptide CB4 (29 residues) which coeluted with peptide CB3. Complete separation of CB3 and CB4 from each other was achieved by paper electrophoresis at pH 6.5 (pyridine/glacial acetic acid/water; 100/4/900; v/v/v).

Enzymatic Cleavage of the Cyanogen Bromide Peptide Fragments CB2, CB3 and CB4, and Isolation of the Digested Peptides

CB2, CB3 and CB4 were digested as indicated in the flow sheet (Fig. 1) and under the conditions given in Table 1. The digested samples were freeze-dried. The large peptides (≥ 30 residues) were first filtered on Sephadex using, depending on the samples, columns of varying sizes (1 × 100 cm, or 1 × 150 cm), Sephadex G-25 fine or Sephadex G-50 fine, and 5% formic acid or 50 mM ammonium formate buffer, pH 3.5,

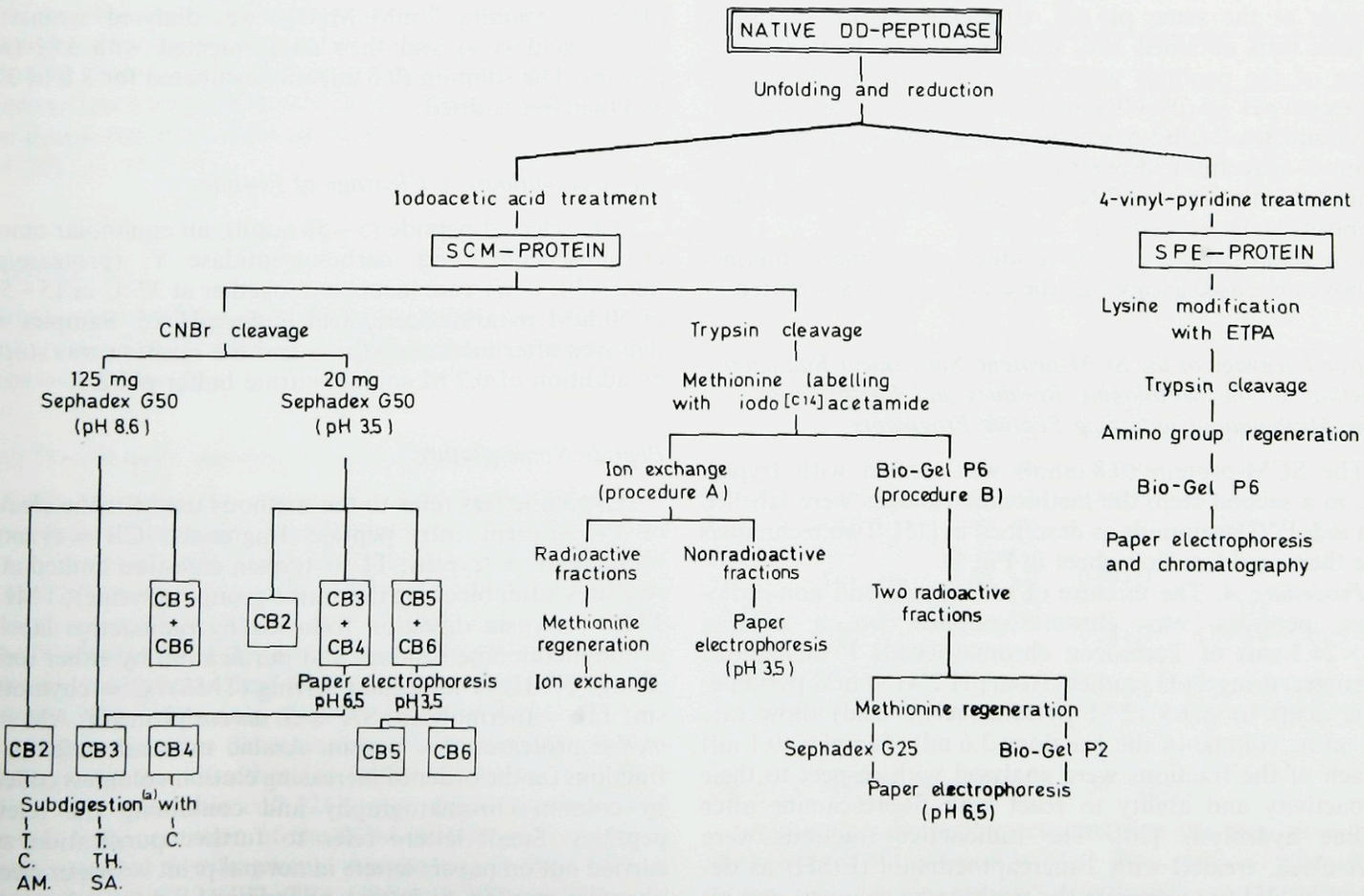


Fig. 1. *Fragmentation of the G Zn^{2+} DD-peptidase.* Flow sheet. (a) T = trypsin; C = chymotrypsin; AM = *Armillaria mellea* protease; TH = thermolysin; SA = *Staphylococcus aureus* protease

Table 1. *Enzymatic digestion of the peptides obtained after CNBr cleavage of the SCM-protein*
Buffers: (a) 0.2 M ammonium acetate pH 8.5; (b) 0.1 M *N*-ethylmorpholine/acetate pH 8.0; (c) 0.1 M ammonium bicarbonate pH 7.8 containing 2 mM EDTA

Protease	Peptide concentration	Protease/peptide (w/w)	Buffer	Duration of the incubation at 37 °C (unless otherwise stated)
	mg/ml			h
Trypsin	10	1/40	a	2
Chymotrypsin	10	1/40	a	1 or 2
Thermolysin	10	1/100	b	1 (50 °C)
S. aureus protease	1.3 to 2.5	1/50	c	4 or 16
A. mellea protease	7	1/3000	b	6.5

as eluants (flow rate 20 ml/h; size of the fractions 1.5 ml). Detection was made with a Uvicord II at 280 nm (in the presence of formic acid) or manually at 230 nm (in the presence of ammonium formate). In all cases, samples of the collected fractions were spotted along the same base line (0.5 cm for each fraction) on a Whatman 3MM paper and submitted to electrophoresis at pH 6.5. Detection of the peptides was made by successive staining with fluorescamine (0.001% in dry acetone) [22], ninhydrin/acetic acid/acetone (0.25/1/99; w/v/v) [23] and Pauly's reagent as described in [24]. On the basis of the maps thus obtained, the relevant fractions were pooled and then purified by preparative paper electrophoresis at the same pH 6.5. Checking the purity of the peptides thus obtained and, when necessary, further purification of the peptides were carried out by paper electrophoresis at pH 3.5 (pyridine/glacial acetic acid/water; 1/10/89; v/v/v) and finally, by descending paper chromatography in butane-1-ol/acetic acid/pyridine/water (15/3/10/12; v/v/v/v). Purity tests also involved N-terminal group and amino acid composition determinations.

For small peptides ($\leq$ 30 residues), the same techniques as above were used except that the gel filtration was omitted.

Trypsin Cleavage of the SCM-protein, Subsequent Radioactive Labelling of the Methionine Residues and Identification of the Methionine-Containing Peptide Fragments

The SCM-protein (0.8 µmol) was treated with trypsin and, in a second step, the methionine residues were labelled with iodo[^{14}C]acetamide as described in [25]. Two techniques were then used (see flow sheet in Fig. 1).

Procedure A. The mixture of radioactive and non-radioactive peptides was chromatographed on a column (0.8 × 24.5 cm) of Technicon chromatobeads P (a cationic exchanger) using a pH gradient from pH 2.4 (50 mM pyridine/acetic acid) to pH 5 (2 M pyridine/acetic acid) (flow rate 16.3 ml/h; volume of the fractions 3.6 ml). Samples (0.1 ml) of each of the fractions were analysed with respect to their radioactivity and ability to react with fluorescamine after alkaline hydrolysis [26]. The radioactive fractions were freeze-dried, treated with 2-mercaptoethanol (EtSH) as described in [25] to regenerate the methionine residues, and rechromatographed under the same conditions as above.

Procedure B. Filtration of the mixture of radioactive and nonradioactive peptides on a column (1.15 × 140 cm) of Bio-Gel P6 (200 mesh) in 50 mM ammonium formate buffer, pH 3.5 (flow rate 9.8 ml/h; volume of the fractions 1.1 ml) yielded two radioactive fractions. Each of these fractions was freeze-dried, treated with EtSH and, depending on the size of the peptides to be purified, filtered in 50 mM ammonium bicarbonate buffer pH 8 either on Sephadex G-25 fine (1 × 140 cm; 10 ml/h; volume of the fractions 0.8 ml) or on Bio-Gel P2 (100–200 mesh; 1 × 110 cm; 10 ml/h; volume of the fractions 0.2 ml). In all cases, the peptides were purified by paper electrophoresis and/or paper chromatography as described above.

Limited Tryptic Digestion of the SPE-Protein after Blocking the Lysine Residues

The following procedure was used (see flow sheet in Fig. 1). The SPE-protein (36 mg in 3.6 ml 0.15 M sodium tetraborate buffer pH 8.5) was treated with three successive additions of 6.5 mg of ETPA as described in [8]. The solution was dialysed at 4 °C against 50 mM NH_4HCO_3 buffer pH 8.0 and after freeze-drying, the residue was submitted to tryptic digestion as described in [4]. Subsequently, the amino groups were generated by dissolving the digested material in 2 ml 10% acetic acid and gently stirring the solution at room temperature for 16 h. The solution was then filtered on Bio-Gel P6 in 50 mM ammonium formate buffer pH 3.5 and the peptides were purified by paper electrophoresis and paper chromatography as described above.

Pepsin Cleavage of the Native Protein

The native protein (5.7 mg in 0.3 ml 10 mM Tris/HCl pH 8.3 containing 2 mM $MgCl_2$) was dialysed against 5% formic acid (v/v) and then supplemented with 1% (w/w) pepsin). The solution (0.5 ml) was incubated for 8 h at 37 °C and then freeze-dried.

Carboxypeptidase Y Cleavage of Peptides

The selected peptide (5–50 nmol), an equimolar amount of norleucine, and carboxypeptidase Y (protease/peptide: 1/30; w/w) were incubated together at 37 °C in 15–50 µl of 50 mM pyridine/acetic acid buffer pH 5.5. Samples were removed after increasing times and the reaction was stopped by addition of 0.2 M sodium citrate buffer pH 2.2.

Peptide Nomenclature

Capital letters refer to the methods used for the cleavage of the protein into peptide fragments. CB = cyanogen bromide; T = trypsin; TL = trypsin digestion limited at the Arg sites (after blocking the amino groups of lysine); TMI and TMS = trypsin digestion followed by radioactive labelling of the methionine residues and purification by either ion exchange (TMI) or molecular seiving (TMS); C = chymotrypsin; TH = thermolysin; SA = *S. aureus* protease; AM = *A. mellea* protease; P = pepsin. Arabic numerals refer to the fractions (in the order of increasing elution volumes) collected by column chromatography and containing the relevant peptides. Small letters refer to further purification steps carried out on paper: letters in normal print = paper electrophoresis at pH 6.5; letters underlined = paper electrophoresis at pH 3.5; letters followed by an asterisk = descend-

ing paper chromatography. In each case, letters a, b, c, etc. indicate the relative position (from anode to cathode during electrophoresis or on the basis of increasing R_F values during chromatography) of the relevant peptide when compared to the other peptides present in the same preparation. Thus for example, peptide CB2T1eeb* was a peptide generated by cyanogen bromide cleavage of the SCM-protein and recovered in fraction 2 after column chromatography. Following further cleavage with trypsin, the peptide was recovered in fraction 1 after column chromatography. Finally, it was purified by paper electrophoresis at pH 6.5, followed by paper electrophoresis at pH 3.5, and paper chromatography. The peptide was in position e during the first and the second preparation methods, and in position b during the third one.

Allocation of the Disulfide Bridges

The diagonal technique of Brown and Hartley [27] was applied to the pepsin digest of the native protein. After the first electrophoresis at pH 6.5 and subsequent treatment with performic acid, the second electrophoresis, carried out in a direction perpendicular to the first one, gave rise to three groups of peptides located outside the diagonal.

Amide Assignment

The amide groups were assigned both by identifying the phenylthiohydantoin and 4-*N*,*N*-dimethylaminoazobenzene-thiohydantoin derivatives and on the basis of the electrophoretic mobilities of the peptides at pH 6.5 [28].

Search for Sequence Homology and Repetitive Fragments

The search for homology between different sequences or repetitive fragments in the same sequence was carried out by comparing all possible spans of n (3 to 25) contiguous amino acids [29]. The amino acids were compared on the basis of a structural test which made use of the relative frequencies of substitution found in different families of homologous proteins [30].

RESULTS

The amino acid sequence of the DD-peptidase is shown in Fig. 2. It was established as described in the ensuing and miniprint sections.

Intact Protein and Cyanogen Bromide Fragments

Table 2 presents the amino acid composition of the protein as found after acid hydrolysis and as deduced from the primary structure. The value of 22076 thus obtained for the M_r was 20% larger than that determined by polyacrylamide gel electrophoresis in the presence of SDS. Asp (or Asn) was at the N terminus and Ile at the C terminus of the protein, as shown by dansylation and hydrazinolysis experiments, respectively. Nbs_2 (Ellman's reagent) failed to detect any free SH group (even after denaturation of the protein). Neither S-glyceryl-cysteine thioether (as found in the Braun's lipoprotein of *Escherichia coli* [21]), nor sugar residues were detected. Fragmentation of the SCM-protein with cyanogen bromide yielded five fragments designated, in the order of decreasing sizes, CB2, CB3, CB4, CB5 and CB6 (see Materials and Methods, and Table 3).

Table 2. *Amino acid composition of the performic-acid-oxidized protein*
The first three columns give the amounts of amino acid residues as obtained after increasing periods of hydrolysis with 6 M HCl at 110 °C. Trp was determined on a separate sample of the native protein after hydrolysis for 96 h at 110 °C with 3 M mercaptoethane sulfonic acid

Amino acid	Amount after				Number of residues as calculated from	
	24 h	48 h	72 h	mean	hydrolysis	sequence
	nmol/mg					
Lys	0.233	0.242	0.256	0.244	5.07	5
His	0.328	0.362	0.341	0.344	7.14	7
Arg	0.459	0.462	0.471	0.464	9.64	10
Cya	0.261	0.265	0.233	0.253	5.25	6
Asp	1.074	1.100	1.063	1.079	22.41	22
Thr	0.618	0.619	0.619	0.619	12.85	13
Ser	0.682	0.683	0.677	0.681	14.14	15
Glu	0.728	0.743	0.713	0.728	15.12	15
Pro	0.360	0.392	0.417	0.389	8.08	8
Gly	1.487	1.485	1.520	1.497	31.09	31
Ala	1.427	1.462	1.417	1.435	29.80	29
Val	0.459	0.466	0.462	0.462	9.59	10
$MetO_2$	0.166	0.175	0.152	0.164	3.41	4
Ile	0.336	0.336	0.336	0.336	6.99	7
Leu	0.519	0.530	0.543	0.531	11.03	11
Tyr	0.237	0.239	0.278	0.251	5.21	7
Phe	0.385	0.392	0.408	0.395	8.20	8
Trp	4.82				4.82	4

Table 3. *Amino acid composition of the peptides produced by CNBr fragmentation of the SCM-protein*
Number of residues as obtained after acid hydrolysis with 6 M HCl for 24 h at 110 °C. The figures in parentheses give the relevant numbers as obtained by sequencing

Residue	Amount in fragment				
	CB2	CB3	CB4	CB5	CB6
Lys	3.25 (3)		0.94 (1)	0.87 (1)	
His		4.49 (5)	0.68 (1)		1.10 (1)
Arg	5.31 (5)	1.95 (2)	1.69 (2)		0.91 (1)
CmCys	2.29 (3)	2.31 (2)	0.19 (1)		
Asp	12.98 (12)	5.90 (5)	5.10 (5)		
Thr	8.41 (9)	2.11 (2)	1.74 (2)		
Ser	6.86 (8)	2.93 (3)	3.60 (4)		
Glu	10.92 (11)	2.69 (3)		0.97 (1)	
Pro	3.98 (4)	3.20 (3)	0.93 (1)		
Gly	14.16 (14)	10.98 (12)	4.90 (5)		
Ala	16.53 (17)	7.56 (9)	1.23 (1)	0.99 (1)	1.32 (1)
Val	5.31 (6)	1.30 (1)	2.83 (3)		
Ile	3.39 (4)	1.68 (2)	0.84 (1)		
Leu	6.42 (7)	2.93 (3)		1.17 (1)	
Tyr	4.28 (5)	1.71 (2)			
Phe	3.91 (4)	3.05 (3)	0.90 (1)		
Trp	? (2)	? (1)	?	? (1)	
Hse	0.99 (1)		0.54 (1)	0.56 (1)	0.95 (1)
Total	115	58	29	6	4

The CB2 Peptide Fragment (115 Residues)

Automatic sequencing revealed that the first six residues were Asn-Gly-Xaa-Tyr-Thr-Trp. The yield of phenylthiohydantoins was very low (2.5%) although, as controlled with whale sperm myoglobin, the sequenator was in perfect ope-

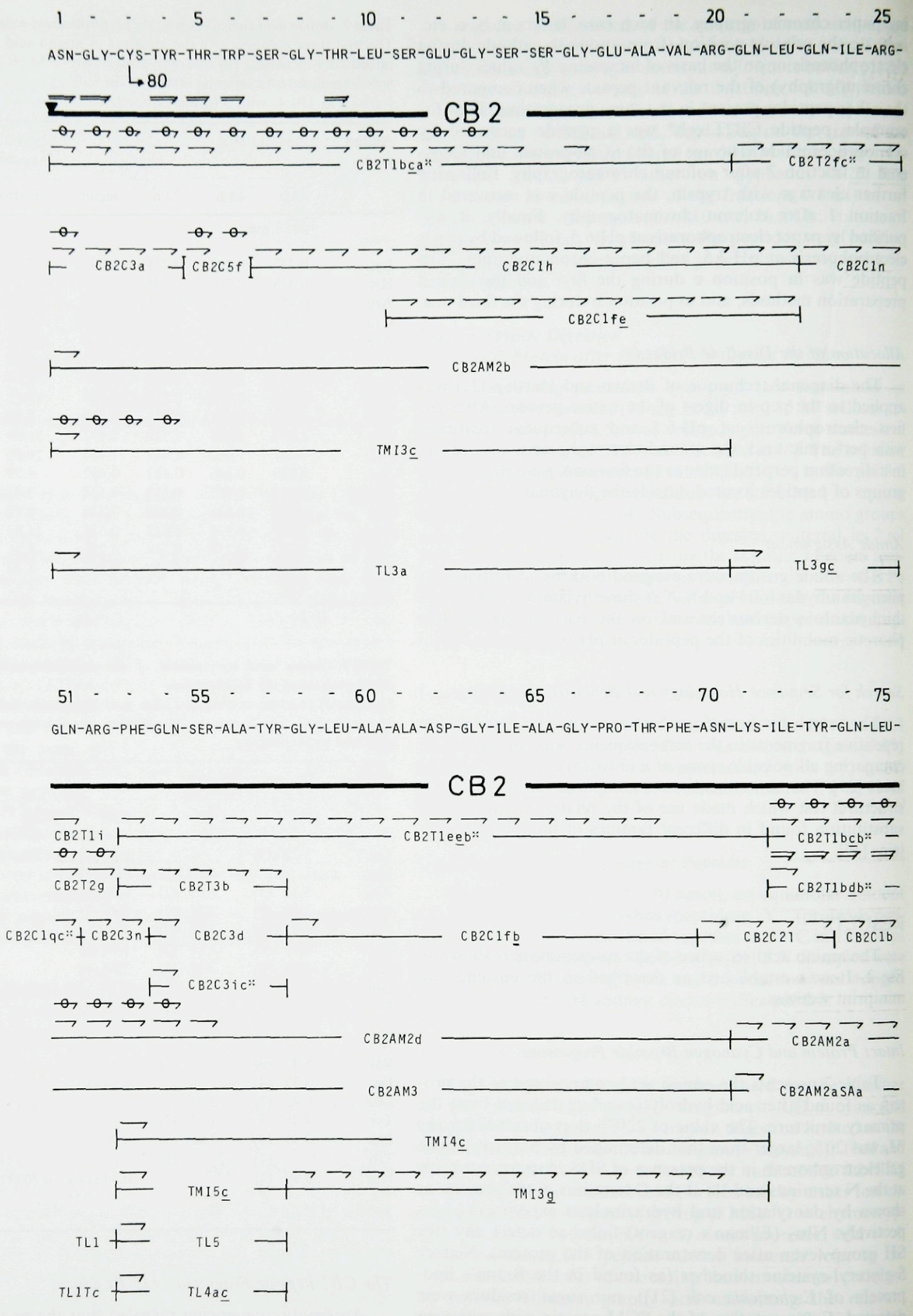
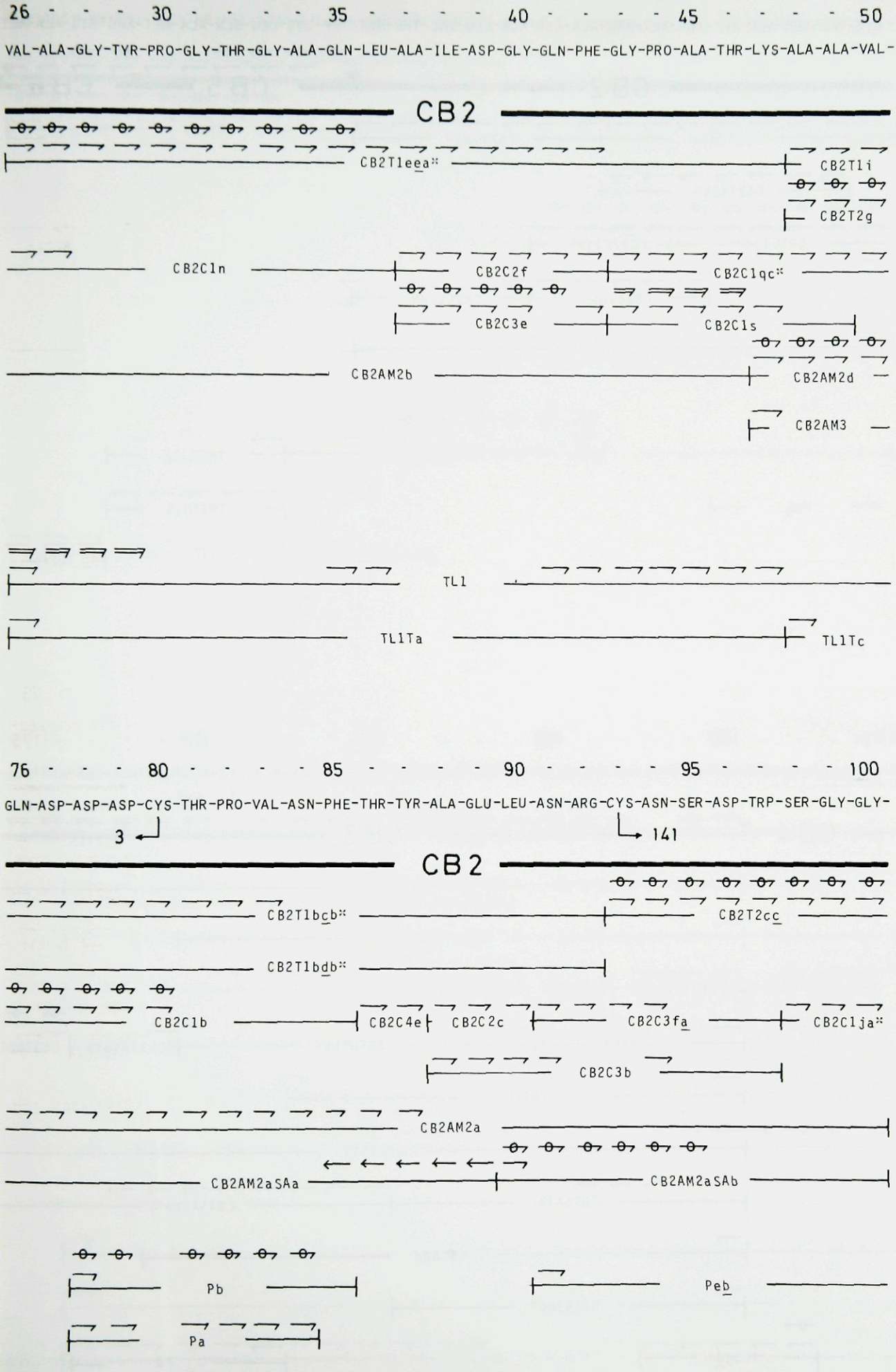
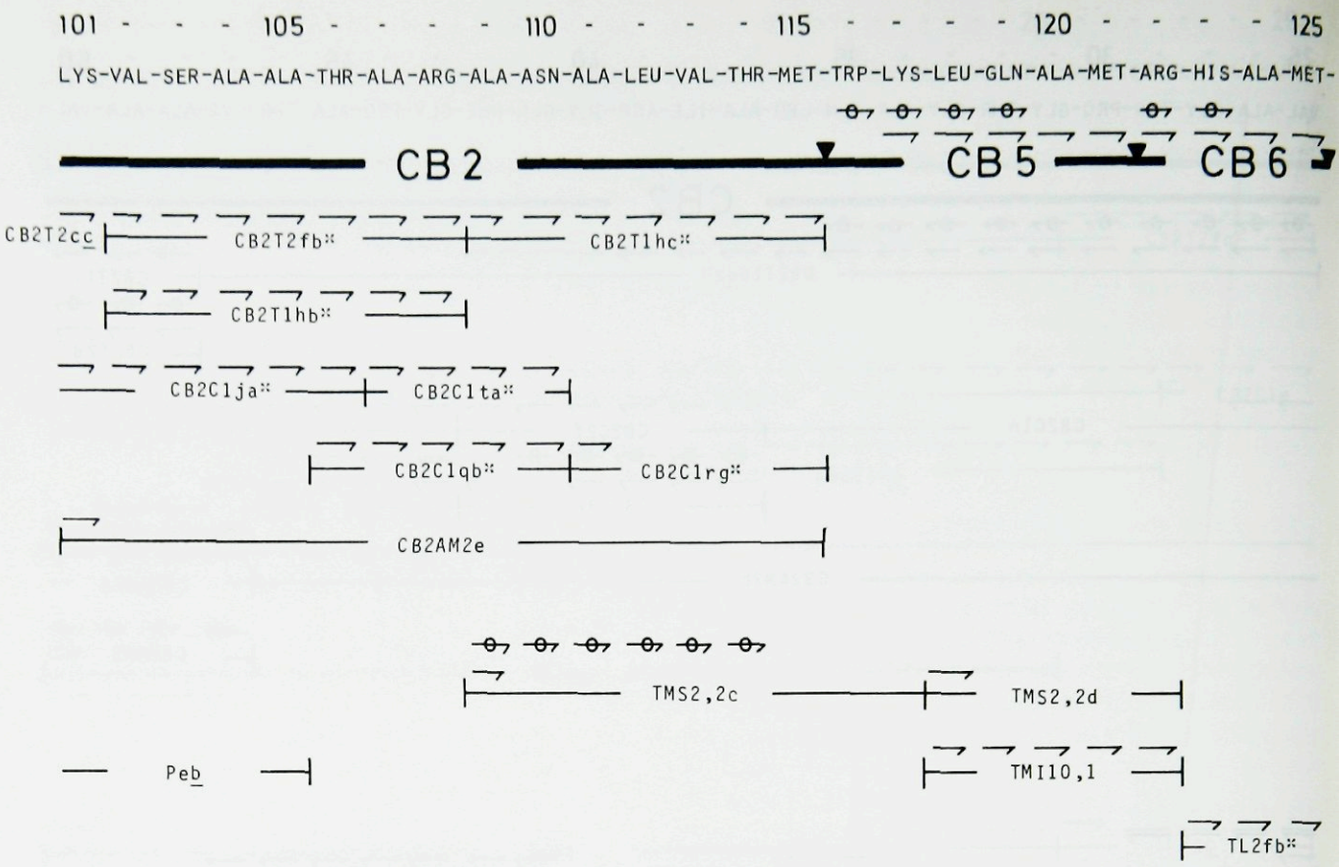


Fig. 2. *Amino acid sequence of the G Zn^{2+} DD-carboxypeptidase.* The CNBr peptide fragments of the SCM-protein are represented by heavy lines between hydrolysis and sequencing gave rise to identical or at least consistent data. The methods used for sequencing are represented by the following symbols: ⟵ : carboxypeptidase Y



triangles (▼——▼). The other full lines (|——|) refer to all the other peptides analysed. In all cases, the amino acid composition as determined by acid
——: manual dansyl-Edman degradation; —○→: DABITC/PITC method; ⟹: automatic method (phenylthiohydantoins); ⇜⇝: hydrazinolysis;


101 - - - 105 - - - - - 110 - - - - - 115 - - - - - 120 - - - - - 125
LYS-VAL-SER-ALA-ALA-THR-ALA-ARG-ALA-ASN-ALA-LEU-VAL-THR-MET-TRP-LYS-LEU-GLN-ALA-MET-ARG-HIS-ALA-MET-
CB2
CB5
CB6
CB2T2cc
CB2T2fb*
CB2T1hc*
CB2T1hb*
CB2C1ja*
CB2C1ta*
CB2C1qb*
CB2C1rg*
CB2AM2e
TMS2,2c
TMS2,2d
Peb
TMI10,1
TL2fb*

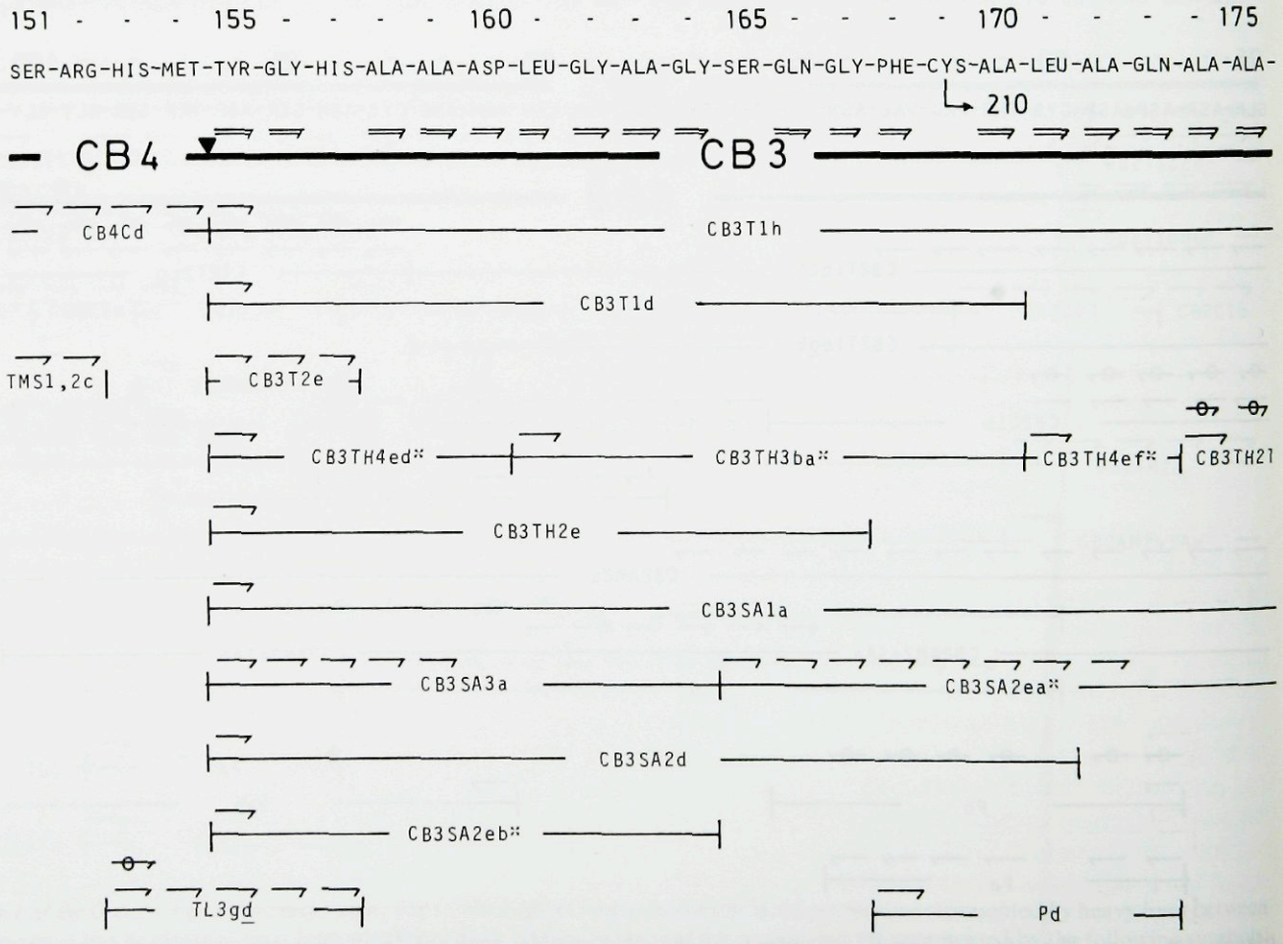
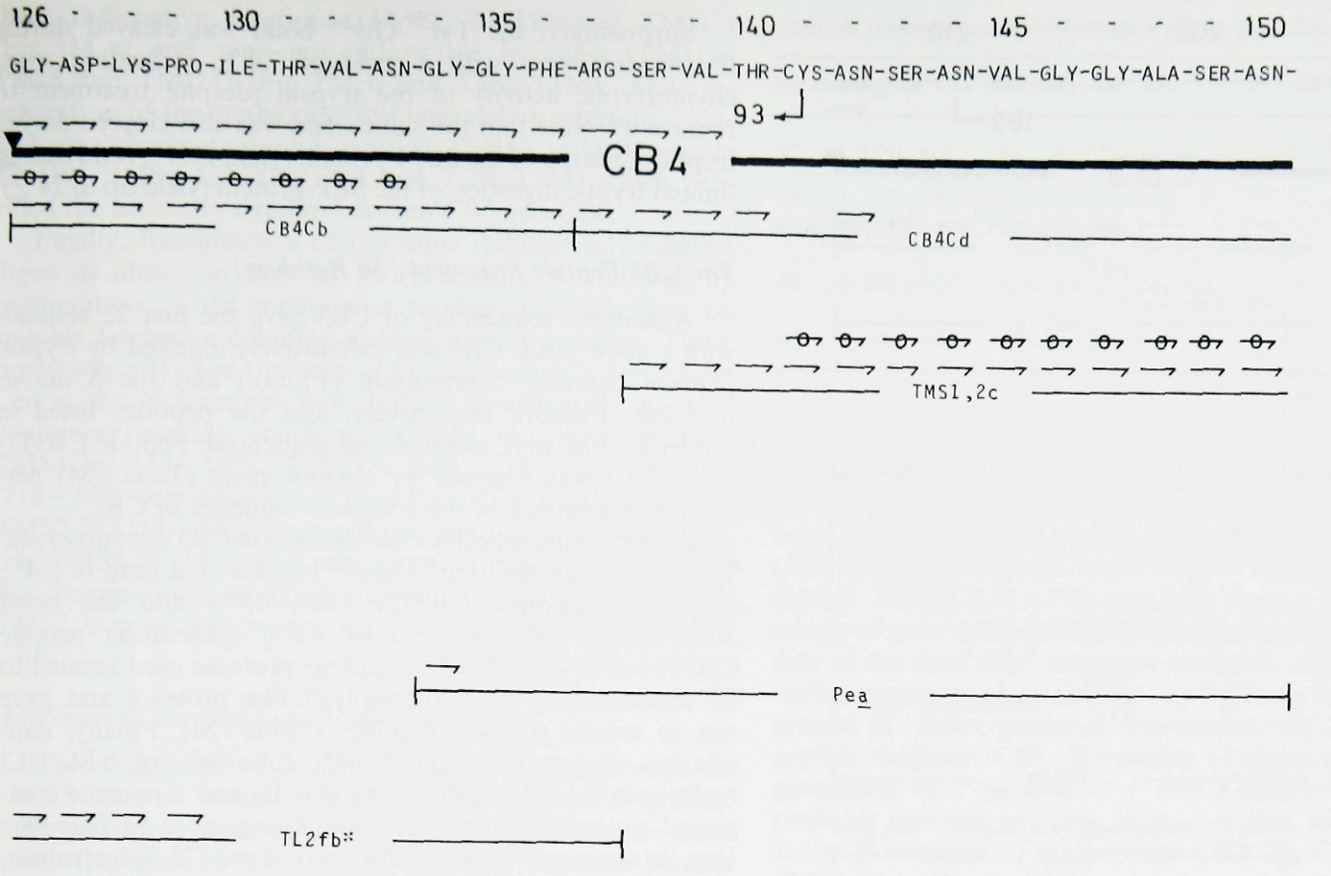
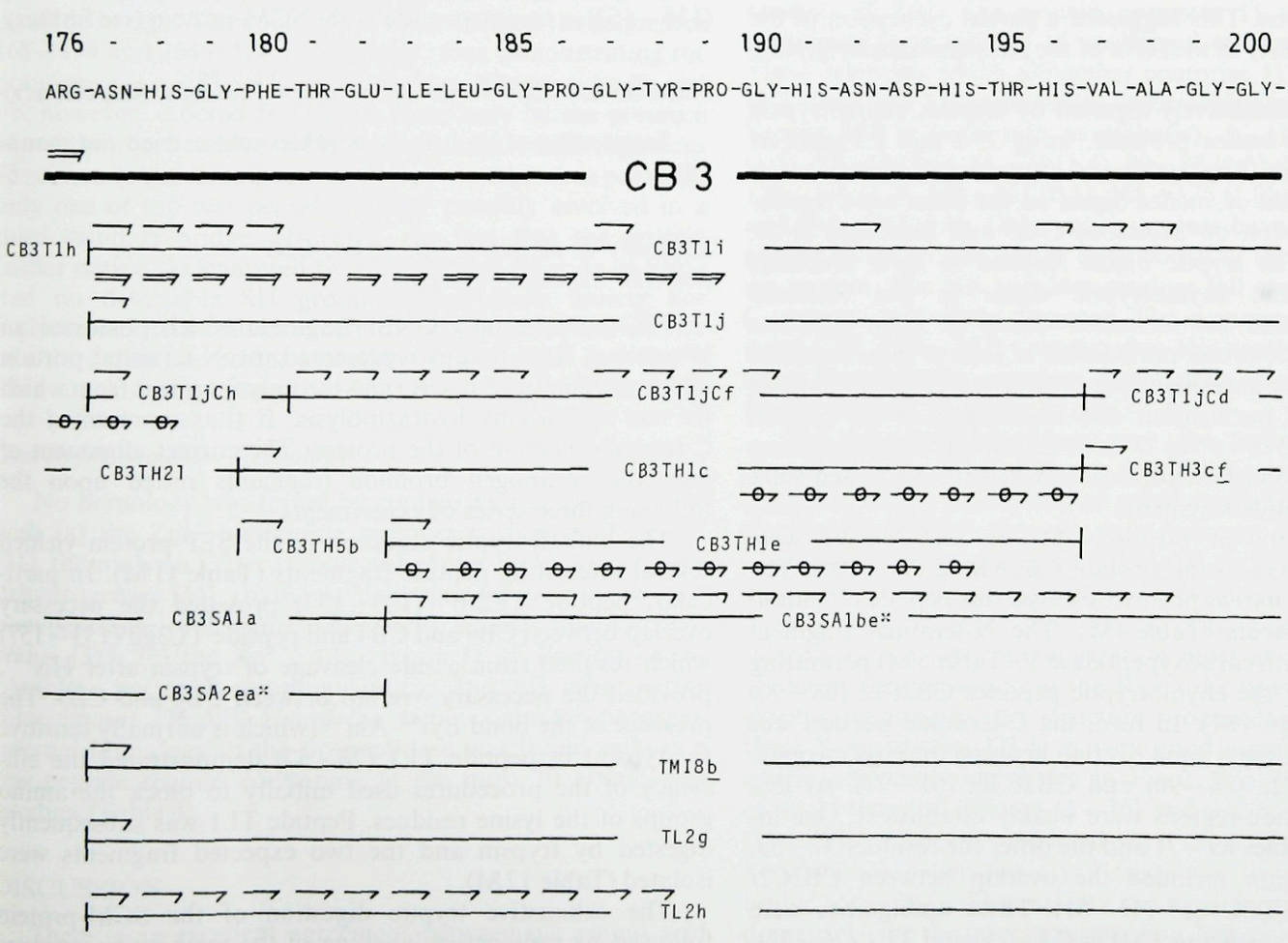

151 - - - 155 - - - - - 160 - - - - - 165 - - - - - 170 - - - - - 175
SER-ARG-HIS-MET-TYR-GLY-HIS-ALA-ALA-ASP-LEU-GLY-ALA-GLY-SER-GLN-GLY-PHE-CYS-ALA-LEU-ALA-GLN-ALA-ALA-
210
CB4
CB3
CB4Cd
CB3T1h
CB3T1d
TMS1,2c
CB3T2e
CB3TH4ed*
CB3TH3ba*
CB3TH4ef*
CB3TH2e
CB3SA1a
CB3SA3a
CB3SA2ea*
CB3SA2d
CB3SA2eb*
TL3gd
Pd

Fig. 2e–h


126 130 135 140 145 150
GLY-ASP-LYS-PRO-ILE-THR-VAL-ASN-GLY-GLY-PHE-ARG-SER-VAL-THR-CYS-ASN-SER-ASN-VAL-GLY-GLY-ALA-SER-ASN-
93
CB4
CB4Cb
CB4Cd
TMS1,2c
Pea
TL2fb*


176 180 185 190 195 200
ARG-ASN-HIS-GLY-PHE-THR-GLU-ILE-LEU-GLY-PRO-GLY-TYR-PRO-GLY-HIS-ASN-ASP-HIS-THR-HIS-VAL-ALA-GLY-GLY-
CB 3
CB3T1h
CB3T1i
CB3T1j
CB3T1jCh
CB3T1jCf
CB3T1jCd
CB3TH2l
CB3TH1c
CB3TH3cf
CB3TH5b
CB3TH1e
CB3SA1a
CB3SA1be*
CB3SA2ea*
TMI8b
TL2g
TL2h

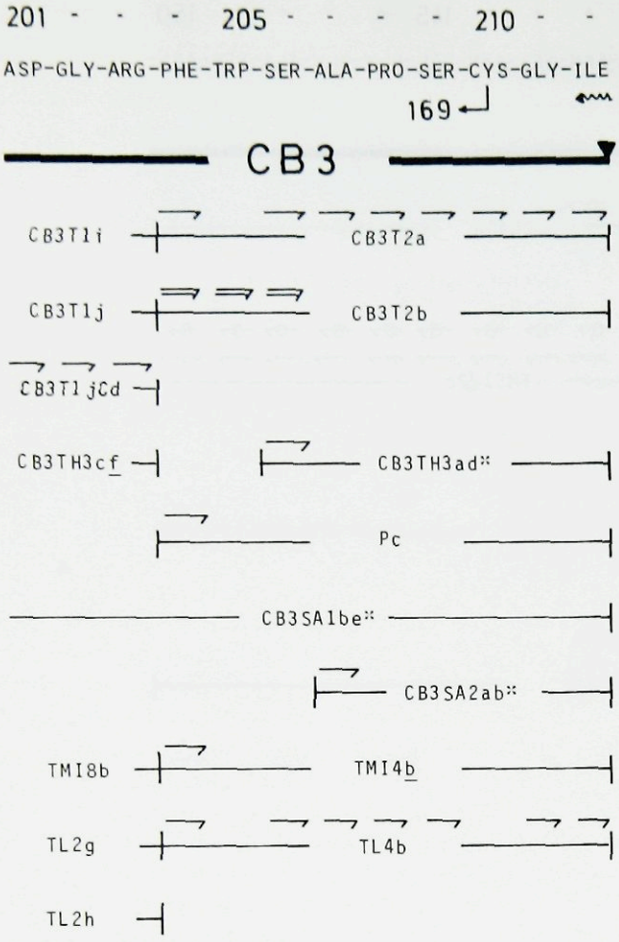


Fig. 2i

ration conditions. This suggested a partial cyclization of the dipeptide Asn-Gly in the form of the anhydro-aspartylglycine imide [31].

CB2 was exhaustively digested by trypsin, chymotrypsin and *Armillaria mellea* protease, using 2, 2 and 1.2 µmol of CB2, respectively. The tryptic and chymotryptic digests on the one hand and the *A. mellea* digest on the other were fractionated by filtration on Sephadex G-25 and Sephadex G-50, respectively. The tryptic digest resulted in three fractions (CB2T1–3), the chymotryptic digest in five fractions (CB2C1–5), and the *A. mellea* digest in three fractions (CB2AM1–3). Further purification of each of these fractions by paper electrophoresis and paper chromatography permitted isolation, final purification and sequencing of the peptide fragments listed in Tables 1–3M. On the basis of their amino acid composition, these peptides covered the whole sequence of the CB2 peptide fragment.

Two overlaps at positions 85–86 and 90–91 were elucidated thanks to the peptide CB2AM2a (71–100). The *Staphylococcus aureus* protease cleaved this peptide (75 nmol) into two fragments (Table 4M). The N-terminal fragment was digested with carboxypeptidase Y (Table 5M) permitting the ordering of the chymotryptic peptides CB2C2c (88–90) and CB2C4e (86–87). In turn, the C-terminal portion was sequenced, yielding a good overlap between the chymotryptic peptides CB2C2c (88–90) and CB2C3fa (91–97). At this stage, two peptide regions were weakly established. One involved the residues 69–71 and the other the residues 37–53. This latter region included the overlap between CB2C2f (37–42) and CB2C1qc* (43–51). These ambiguities were elucidated by sequencing TMI3g (58–71) and TL1 (26–52), respectively.

Surprisingly, the Tyr^{57}-Gly^{58} bond was cleaved during trypsin treatment with a yield of 7.4%, indicating some chymotryptic activity of the trypsin (despite treatment to remove it). Note that the same bond was also cleaved during tryptic digestion of the SCM-protein (yield 11.4%) and during limited tryptic digestion of the SEP-protein (yield up to 24%).

The CB3 Peptide Fragment (58 Residues)

Automatic sequencing of CB3 gave the first 22 residues with a good yield. CB3 was exhaustively digested by trypsin (2 µmol peptide), thermolysin (1 µmol) and the *S. aureus* protease (1 µmol), respectively, and the peptides listed in Tables 6–8M were isolated and sequenced. Peptide CB3T1j (177–203) was digested by chymotrypsin (Table 9M) permitting elucidation of the complete sequence of CB2.

The following aspecific cleavage sites in CB3 were observed. Trypsin cleaved the His^{157}-Ala^{158} bond with a yield of 2.4% [generating peptide CB3T2e (155–157)] and the bond Ala^{170}-Leu^{171} with a yield of 6.2% [generating peptide CB3T1d (155–170)]. The *S. aureus* protease used seemed to be contaminated by a thermolysin-like protease and gave rise to several parasite peptides (Table 8M). Finally, dansylation of peptide CB3SA1be*, followed by 6 M HCl hydrolysis for 8 h, yielded both Dns-Ile and a parasite compound probably Dns-Ile-Leu, migrating close to Dns-Phe. Dns-Ile alone was obtained after prolonged (72-h) hydrolysis.

The CB4 Peptide Fragment (29 Residues)

Manual sequencing of CB4 gave the first 14 residues. The complete sequence was elucidated after subdigestion of CB4 with chymotrypsin (Table 10M) and by purifying TMS1,2c (138–152), a tryptic peptide of the SCM-protein (see further).

The CB5 and CB6 Fragments (6 and 4 Residues, Respectively)

Sequencing of both these peptides was carried out manually.

Assembly of the CB2, CB3, CB4, CB5 and CB6 Peptide Fragments

CB2 was the only CNBr fragment which possessed an N-terminal Asn. It thus represented the N-terminal portion of the protein. In turn, CB3 was the only fragment from which Ile was released hy hydrazinolysis. It thus represented the C-terminal portion of the protein. The correct alignment of the other cyanogen bromide fragments rested upon the following three series of experiments.

The limited tryptic digestion of the SEP-protein yielded several interesting peptide fragments (Table 11M). In particular, peptide TL2fb* (123–137) provided the necessary overlap between CB6 and CB4 and peptide TL3gd (153–157) which resulted from a side cleavage of trypsin after His^{157}, provided the necessary overlap between CB4 and CB3. The presence of the bond Lys^{47}-Ala^{48} (which is normally sensitive to trypsin) in peptide TL1 (26–52) demonstrated the efficiency of the procedures used initially to block the amino groups of the lysine residues. Peptide TL1 was subsequently digested by trypsin and the two expected fragments were isolated (Table 12M).

The exhaustive tryptic digestion of the SCM-protein followed by radioactive labelling of the methionine residues yielded, after ion-exchange chromatography (procedure A),

three radioactive fractions (TMI9, TMI10 and TMI12) (Fig. 3M, a) and nine non-radioactive fractions (TMI1–TMI8 and TMI11) (Fig. 3M, b and Table 13M). After regeneration of the methionine residues and an additional ion-exchange chromatography, peptide TMI10 yielded peptide TMI10,1 (118–122) (Fig. 4M and Table 13M) which provided the necessary overlap between CB5 and CB6.

Finally, fractionation of the same radioactively labelled digest as above on Bio-Gel P6 (procedure B) followed by regeneration of the methionine residues and filtration on suitable Sephadex columns yielded several peptides. Those possessing a His (on the basis of Pauly's test) or a Ser, Ala or Leu at the N terminus were further investigated. No His-containing peptide could be purified but three peptides TMS1,2c (138–152), TMS2,2c (109–117) and TMS2,2d (118–122) were obtained in pure form (Table 14M). Peptide TMS2,2d (118–122) contained one methionine residue. Peptide TMS1,2c (138–152) was a tryptic peptide of CB4 and was helpful in completing the sequence of CB4. Peptide TMS2,2c provided the necessary overlap between CB2 and CB5.

Allocation of the Disulfide Bridges

The technique of diagonal paper electrophoresis at pH 6.5 allowed several peptides to be detected (Fig. 5M). After performic oxidation, two acidic peptides became more acidic (peptides Pa and Pb), two neutral peptides became acidic (peptides Pc and Pd) and two basic peptides became neutral. These latter peptides, Pea and Peb, were further separated by paper electrophoresis at pH 3.5. The analyses of all the peptides are shown in Table 15M. The couple Pea and Peb represented the sequences 134–150 and 91–106, respectively, thus demonstrating the occurrence of a S^{93}-S^{141} disulfide bridge. The couple Pd-Pc represented the sequences 168–174 and 204–211, respectively, thus demonstrating the occurrence of a S^{169}-S^{210} disulfide bridge. The peptides Pa and Pb, however, differed from each other only by the presence of an additional residue in Pb; they represented the sequences 78–84 and 78–85, respectively. The procedure thus provided only one of the two peptide partner possibly involved in a third disulfide bridge. However, the fact that the protein (either native or denatured by guanidinium chloride or SDS) had no detectable SH group, carbohydrate moiety nor *S*-glycerol-cysteine thioether left little doubt on the occurrence of a third disulfide bridge extending between Cys^3 and Cys^{80}. This conclusion was confirmed by crystallographic data [3].

Search for Sequence Homology

No homology was found by comparing the DD-peptidase with (a) the Zn^{2+}-containing enzymes carboxypeptidase A [31], thermolysin [32], carbonic anhydrase B [33] and alcohol dehydrogenase [34]; (b) the partially sequenced serine DD-carboxypeptidase of *Bacillus subtilis* and *Bacillus stearothermophilus* [35]; (c) the Zn^{2+} β-lactamase II of *Bacillus cereus* (Ambler; personal communication); and (d) other serine β-lactamases [36, 37]. Using the same computer program, internal homology in the DD-peptidase was found only in a few peptide regions containing at the most 10 amino acid residues (Table 4).

Table 4. *Internal sequence identities in the DD-peptidase*

Peptide region	Sequence
57–66, 155–164	Tyr-Gly-$\frac{\text{Leu}}{\text{His}}$-Ala-Ala-Asp-$\frac{\text{Gly}}{\text{Leu}}$-$\frac{\text{Ile}}{\text{Gly}}$-Ala-Gly
28–31, 187–190	Gly-Tyr-Pro-Gly
6–8, 96–98	Trp-Ser-Gly
52–56, 203–207	Arg-Phe-$\frac{\text{Gln}}{\text{Trp}}$-Ser-Ala
93–95, 141–143	Cys-Asn-Ser

DISCUSSION

There is an excellent agreement between the amino acid composition of the DD-peptidase shown in Table 2 and the amino acid sequence proposed in Fig. 2. However, a few peptide bonds were only proved weakly. The Arg^{203}-Phe^{204} bond was established on the basis of peptide CB3SA1be* (182–212) only and the Met^{154}-Tyr^{155} bond on the basis of peptide TL3gd (153–157) resulting from a tryptic side reaction of poor yield (3.5%). Deamidation and cyclization of Asn in the Asn^1-Gly^2 sequence probably explains the difficulties encountered in attempts to sequence both the SCM-protein (C. Duez, personal communication) and the CNBr peptide fragment CB2. Reopening of the cyclic imide with generation of a mixture of α and β-aspartyl peptides [38] probably explains why Asp, instead of Asn, has been located at the N terminus of the peptides CB2C3a, CB2T1bca* and TMI3c. Usually, α and β-aspartyl peptides can be separated by electrophoresis at pH 3.5 [39] and peptide CB2C3a was indeed found to be heterogenous under these conditions.

Among the 212 residues which constitute the DD-peptidase, 14 are acidic (4 Glu and 10 Asp) and 15 are basic (5 Lys and 10 Arg), providing a ratio of uncharged residues to charged residues of 7.31. The protein possesses 11 segments, each containing from 8 up to 15 uncharged residues in sequence. These segments which altogether comprise 113 residues, are as follows (the hydrophobicity index calculated according to Segrest [40] is given into parenthesis): 1–11 (1.2), 26–38 (1.5), 53–61 (1.6), 63–70 (1.4), 80–88 (1.6), 109–116 (2.1), 129–136 (1.7), 138–151 (0.1), 161–175 (1.0), 183–192 (1.6) and 204–212 (2.0). Only two fragments have a high hydrophobic character and one of them occurs at the C terminus of the protein. The His and Met residues fall exclusively in the C-terminal half of the sequence. The cyanogen peptide fragment CB3 (155–212) contains five His residues out of the seven found in the complete polypeptide chain. Out of the 15 Glx present, 11 occur as Gln.

The DD-peptidase shows very few internal repetitive sequences and no homology is found by comparing the sequence of Fig. 2 with those of other mechanistically and/or functionally related enzymes. In agreement with this latter conclusion, the interpretation of the 2.5-Å (0.25-nm) electron density map of the DD-peptidase has revealed a secondary and tertiary structure not found in any other protein [3]. Considering that a full characterization must await final fitting of the protein molecule in the Richard's box and crystallographic refinements, the structure proposed on the basis of these X-ray studies [3] is in very good agreement with the amino acid sequence shown in Fig. 2. The protein consists of an N-terminal domain (1–76) and a C-terminal domain (81–212) which are connected via a single peptide sequence Leu^{75}-Gln-Asp-Asp-Asp-Cys^{80} (itself involved in a disulfide bridge with Cys^3). The catalytic cavity is located in the large domain. Using the present numbering, important residues are His^{153}, His^{194} and His^{196} (Zn^{2+} ligands) and, probably,

His^{191} (proton donor?) and Arg^{137} (charge pairing with the carboxylate substrate?).

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Miniprint section to

The complete amino acid sequence of the Zn^{++}-containing D-alanyl-D-alanine-cleaving carboxypeptidase of *Streptomyces albus* G

by

B. Joris, J. Van Beeumen, F. Casagrande, Ch. Gerday, J.M. Frère and J.M. Ghuysen

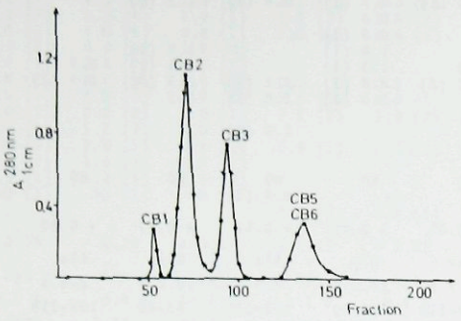

Fig. 1M. Separation of the peptide fragments produced by CNBr cleavage of the SCM-protein (5 µmol).
The peptides soluble in 0.2 M NH_4HCO_3 buffer pH 8.6 were filtered on a 2.5 × 150 cm column of Sephadex G-50 fine in the same bicarbonate buffer. Flow rate : 35 ml/h . Volume of the fractions : 5.3 ml .

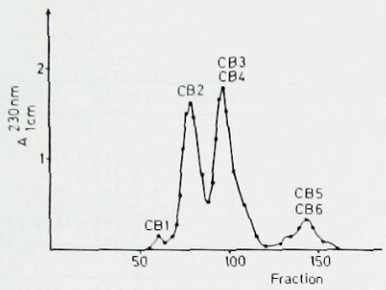

Fig. 2M. Separation of the peptide fragments produced by CNBr cleavage of the SCM-protein (0.8 µmol).
All the peptides were solubilized in 50 mM ammonium formate buffer pH 3.5 supplemented with 2 M guanidinium chloride and the resulting solution was filtered on a 1.25 × 150 cm column of Sephadex G-50 fine in the same ammonium formate buffer (without guanidinium chloride). Flow rate : 15 ml/h . Volume of the fractions : 1.2 ml .

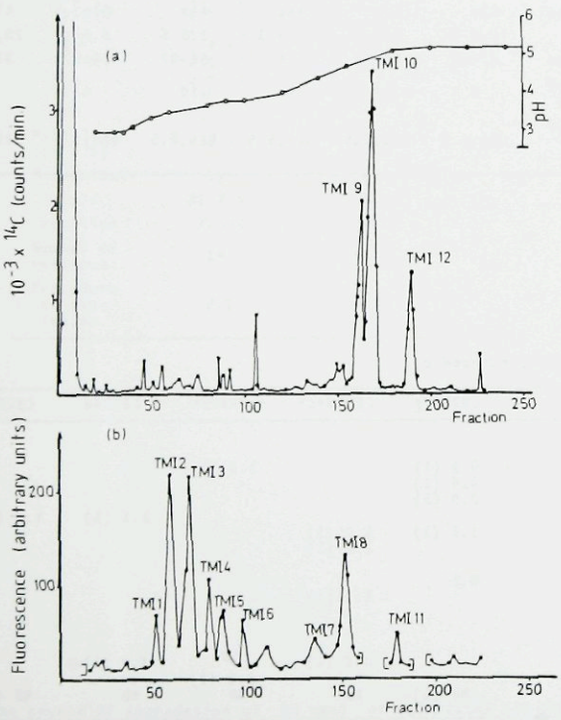

Fig. 3M. Fractionation of the tryptic digest of the SCM-protein by chromatography on chromatobeads P.
After tryptic digestion, the methionine residues were labelled with iodo[^{14}C]acetamide. The sample, dissolved in 30 % acetic acid, was applied to a 0.8 × 24.5 cm column preequilibrated against buffer A (50 mM pyridinium acetate pH 2.4). Elution was carried out successively with : 75 ml of buffer A; 300 ml of a linear gradient obtained with 150 ml of buffer A and 150 ml of buffer B (50 mM pyridinium acetate pH 3.75); 300 ml of a linear gradient obtained with 150 ml of buffer B and 150 ml of buffer C (2 M pyridinium acetate pH 5.0); and, finally, buffer C alone. The fractions were analysed by liquid scintillation (graph a) and by alkaline fluorescamine (graph b). Flow rate : 16.3 ml/h ; volume of the fractions : 3.4 ml

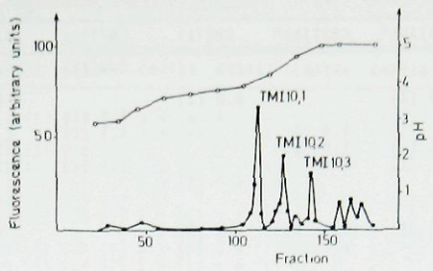

Fig. 4M. Fractionation of peptides TMI10 (Fig. 3M) after regeneration of the methionine residues by treatment with EtSH.
For conditions, see Fig. 3M.

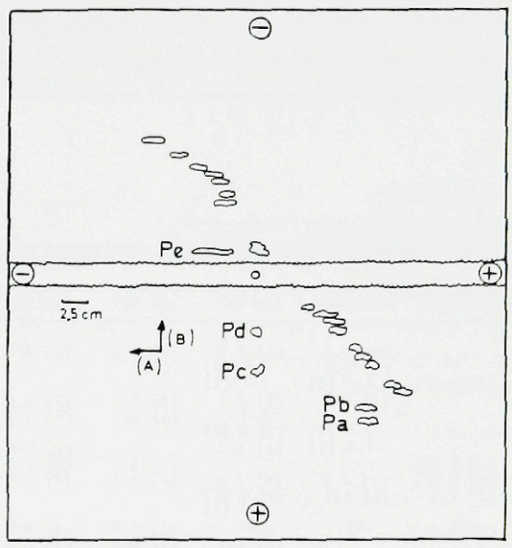

Fig. 5M. Allocation of the disulfide bridges in the native protein.
The protein was digested by pepsin and the digest submitted to analytic paper electrophoresis at pH 6.5 in direction A. After performic oxidation, the paper strip was submitted to a second paper electrophoresis at the same pH in direction B. The peptides were detected by fluorescamine.

Table 1M. Data on tryptic peptides of CB2. Figures in parentheses were obtained by sequencing. ND : not determined; i : ion-exchange chromatography; G50 : filtration on Sephadex G-50; G25 : filtration on Sephadex G-25; P6 : filtration on Bio-Gel P6; P4 : filtration on Bio-Gel P4; P2 : filtration on Bio-Gel P2; 6.5 : electrophoresis at pH 6.5 ; 3.5 : electrophoresis at pH 3.5 ; C : descending paper chromatography; 6.5 D : diagonal electrophoresis (pH 6.5) after performic oxidation.

Amino acid	CB2T1bca*	CB2T1bcb*	CB2T1bdb*	CB2T1eea*	CB2T1eeb*
Lys				1.0 (1)	1.0 (1)
His					
Arg	1.0 (1)	1.0 (1)	1.0 (1)		
CmCys	0.3 (1)	0.4 (1)	0.5 (1)		
Asp	1.5 (1)	4.6 (5)	4.7 (5)	1.2 (1)	1.6 (2)
Thr	2.1 (2)	2.1 (2)	2.0 (2)	1.8 (2)	1.0 (1)
Ser	3.9 (4)				0.7 (1)
Glu	2.0 (2)	2.6 (3)	3.0 (3)	2.1 (2)	1.2 (1)
Pro		0.3 (1)	0.5 (1)	2.4 (2)	0.5 (1)
Gly	4.1 (4)			4.6 (5)	2.9 (3)
Ala	1.2 (1)	1.2 (1)	1.2 (1)	4.4 (4)	4.2 (4)
Val	1.0 (1)	1.0 (1)	1.1 (1)	0.9 (1)	
Ile		0.7 (1)	0.8 (1)	1.0 (1)	0.8 (1)
Leu	1.3 (1)	2.0 (2)	2.3 (2)	1.1 (1)	0.9 (1)
Tyr	0.7 (1)	1.4 (2)	1.3 (2)	0.5 (1)	(1)
Phe		1.0 (1)	1.1 (1)	1.1 (1)	1.3 (2)
Trp	ND (1)	ND	ND	ND	ND
Hse					
Mobility (pH 6.5)	- 0.37	- 0.37	- 0.37	0.00	0.00
N-terminal	Asp	Ile	Ile	Val	Phe
Yield	9.3 %	10.2 %	6.3 %	11.9 %	17.1 %
Position	1 → 20	72 → 92	72 → 92	26 → 47	53 → 71
Number of residues	20	21	21	22	19
Procedure of isolation	G25;6.5; 3.5; C	G25;6.5; 3.5; C	G25;6.5; 3.5; C	G25;6.5; 3.5; C	G25;6.5; 3.5; C

Table 1M (continued a)

Amino acid	CB2T1hb*	CB2T1hc*	CB2T1i	CB2T2cc	CB2T2fb*
Lys				0.9 (1)	
His					
Arg	0.9 (1)		0.8 (1)		0.8 (1)
CmCys				0.3 (1)	
Asp		1.0 (1)		1.9 (2)	
Thr	1.0 (1)	1.0 (1)			1.1 (1)
Ser	1.0 (1)	0.4		1.7 (2)	1.1 (1)
Glu			1.3 (1)		
Pro					
Gly				2.5 (2)	
Ala	3.2 (3)	2.3 (2)	1.9 (2)		2.9 (3)
Val	0.9 (1)	0.8 (1)	1.1 (1)		1.1 (1)
Ile					
Leu		1.0 (1)			
Tyr					
Phe					
Trp	ND	ND	ND	ND (1)	ND
Hse		0.9 (1)			
Mobility (pH 6.5)	+ 0.43	+ 0.43	+ 0.48	- 0.24	+ 0.46
N-terminal	Val	Ala	Ala	CmCys	Val
Yield	10.7 %	2.1 %	5.1 %	6.1 %	3.4 %
Position	102→108	109→115	48→52	93→101	102→108
Number of residues	7	7	5	9	7
Procedure of isolation	G25;6.5; C	G25;6.5; C	G25;6.5	G25;6.5; 3.5	G25;6.5; C

Table 1M (continued b)

Amino acid	CB2T2fc*	CB2T2g	CB2T3b
Lys			
His			
Arg	0.8 (1)	0.9 (1)	
CmCys			
Asp			
Thr			
Ser			1.0 (1)
Glu	2.2 (2)	1.0 (1)	1.1 (1)
Pro			
Gly			0.4
Ala		2.2 (2)	1.2 (1)
Val		1.0 (1)	
Ile	0.7 (1)		
Leu	0.7 (1)		
Tyr			1.0 (1)
Phe			0.8 (1)
Trp	ND	ND	ND
Hse			
Mobility (pH 6.5)	+ 0.46	+ 0.54	0.00
N-terminal	Gln	Ala	Phe
Yield	3.3 %	31.6 %	7.4 %
Position	21→25	48→52	53→57
Number of residues	5	5	5
Procedure of isolation	G25;6.5; C	G25;6.5	G25;6.5

Table 2M. Data on chymotryptic peptides of CB2. For all details, see Table 1M.

Amino acid	CB2C1b	CB2C1fb	CB2C1fe	CB2C1h	CB2C1ja*	CB2C1n
Lys					0.7 (1)	
His						
Arg			0.9 (1)	1.0 (1)		1.0 (1)
CmCys	0.3 (1)					
Asp	3.7 (4)	0.9 (1)		0.3		0.3
Thr	1.0 (1)	0.7 (1)		0.7 (1)	0.5 (1)	1.0 (1)
Ser			2.8 (3)	2.4 (4)	1.3 (2)	0.3
Glu	1.9 (2)		2.9 (3)	3.0 (3)		1.8 (2)
Pro	1.1 (1)	0.9 (1)				1.2 (1)
Gly		3.0 (3)	2.1 (2)	2.2 (3)	2.3 (2)	3.1 (3)
Ala		3.0 (3)	1.1 (1)	1.1 (1)	2.1 (2)	1.6 (2)
Val	1.1 (1)		1.0 (1)	0.7 (1)	0.7 (1)	1.1 (1)
Ile		0.9 (1)				1.0 (1)
Leu	1.2 (1)	0.8 (1)	1.0 (1)	1.3 (2)		1.0 (1)
Tyr						1.0 (1)
Phe	1.0 (1)	0.8 (1)				
Trp	ND	ND	ND	ND	ND	ND
Hse						
Mobility (pH 6.5)	- 0.74	- 0.28	- 0.28	- 0.17	0.00	+ 0.24
N-terminal	Gln	Gly	Ser	Ser	Ser	Gln
Yield	30.8 %	14.6 %	7.6 %	25.5 %	2.1 %	16.9 %
Position	74→85	58→69	11→22	7→22	98→106	23→36
Number of residues	12	12	12	16	9	14
Procedure of isolation	G25;6.5	G25;6.5; 3.5	G25;6.5; 3.5	G25;6.5	G25;6.5; C	G25;6.5

Table 2M (continued a)

Amino acid	CB2C1qb*	CB2C1qc*	CB2C1rg*	CB2C1s	CB2C1ta*	CB2C2c
Lys		1.0 (1)		1.0 (1)		
His						
Arg	0.9 (1)			0.4	1.1 (1)	
CmCys						
Asp	0.9 (1)	0.1		0.3	1.3 (1)	
Thr	0.9 (1)	0.7 (1)	0.4 (1)	1.2 (1)		
Ser		0.4				
Glu		0.9 (1)				1.0 (1)
Pro		1.0 (1)		1.0 (1)		
Gly	0.3	1.3 (1)		1.1 (1)	0.3	
Ala	2.3 (2)	2.8 (3)	1.1 (1)	2.9 (3)	1.4 (2)	1.0 (1)
Val		1.0 (1)	1.0 (1)	0.2		0.1
Ile						
Leu			1.0 (1)			1.0 (1)
Tyr						
Phe						
Trp	ND	ND	ND	ND	ND	ND
Hse			0.4 (1)			
Mobility (pH 6.5)	+ 0.45	+ 0.45	+ 0.52	+ 0.57	+ 0.60	- 0.55
N-terminal	Thr	Gly	Ala	Gly	Ala	Ala
Yield	7.7 %	10.0 %	2.9 %	10.2 %	2.3 %	14.2 %
Position	106→110	43→51	111→115	43→49	107→110	88→90
Number of residues	5	9	5	7	4	3
Procedure of isolation	G25;6.5; C	G25;6.5; C	G25;6.5; C	G25;6.5	G25;6.5; C	G25;6.5

Table 2M (continued b)

Amino acid	CB2C2f	CB2C2l	CB2C3a	CB2C3b	CB2C3d	CB2C3e
Lys	0.4	1.2 (1)				
His						
Arg				0.6 (1)		
CmCys			0.5 (1)	0.4 (1)		
Asp	1.0 (1)	1.1 (1)	0.9 (1)	2.8 (3)	0.3	1.0 (1)
Thr	0.1					0.3
Ser	0.2			0.6 (1)	0.9 (1)	0.1
Glu	1.0 (1)			0.9 (1)	1.2 (1)	1.3 (1)
Pro						
Gly	1.0 (1)		1.2 (1)	0.5		0.9 (1)
Ala	1.0 (1)			1.1 (1)	1.2 (1)	1.0 (1)
Val						
Ile	1.1 (1)	0.9 (1)				0.9 (1)
Leu				1.3 (1)		0.4
Tyr		0.8 (1)	1.0 (1)		0.7 (1)	0.3
Phe	0.9 (1)					0.9 (1)
Trp	ND	ND	ND	ND (1)	ND	ND
Hse						
Mobility (pH 6.5)	- 0.38	+ 0.37	- 0.90	- 0.64	- 0.53	- 0.37
N-terminal	Ala	Asn	Asp	Ala	Gln	Ala
Yield	16.0 %	9.6 %	29.5 %	2.8 %	6.8 %	20.8 %
Position	37→42	70→73	1→4	88→97	54→57	37→42
Number of residues	6	4	4	10	4	6
Procedure of isolation	G25;6.5	G25;6.5	G25;6.5	G25;6.5	G25;6.5	G25;6.5

Table 2M (continued c)

Amino acid	CB2C3fa	CB2C3ic*	CB2C3n	CB2C4e	CB2C5f
Lys					
His					
Arg	0.9 (1)		1.0 (1)		
CmCys	0.4 (1)				
Asp	2.9 (3)				
Thr				1.1 (1)	1.0 (1)
Ser	1.2 (1)	1.0 (1)			
Glu		1.3 (1)			
Pro					
Gly	0.5				
Ala		1.1 (1)			
Val					
Ile					
Leu					
Tyr		0.6 (1)		1.0 (1)	
Phe			1.0 (1)		
Trp	ND (1)	ND	ND	ND	ND (1)
Hse					
Mobility (pH 6.5)	- 0.29	0.00	+ 0.72	0.00	0.00
N-terminal	Asn	Gln	Arg	Thr	Thr
Yield	4.4 %	7.4 %	23.5 %	8.0 %	9.4 %
Position	91→97	54→57	52→53	86→87	5→6
Number of residues	7	4	2	2	2
Procedure of isolation	G25;6.5; 3.5	G25;6.5; C	G25;6.5	G25;6.5	G25;6.5

Table 3M. Data on *Armillaria mellea* digest of CB2. For all details, see Table 1M.

Amino acid	CB2AM2a	CB2AM2b	CB2AM2d	CB2AM2e	CB2AM2f	CB2AM3
Lys	0.8 (1)	0.6	0.7 (1)	1.3 (1)	1.3 (1)	0.8 (1)
His						
Arg	0.9 (1)	1.8 (2)	0.7 (1)	0.9 (1)	1.3 (1)	1.0 (1)
CmCys	1.4 (2)	0.5 (1)				
Asp	7.2 (7)	4.2 (2)	1.9 (2)	1.1 (1)	1.0 (1)	1.8 (2)
Thr	2.2 (2)	5.2 (4)	1.0 (1)	2.1 (2)	1.5 (2)	0.9 (1)
Ser	1.8 (2)	4.8 (4)	0.9 (1)	1.0 (1)	0.9 (1)	0.9 (1)
Glu	2.9 (3)	6.0 (6)	1.8 (2)			1.8 (2)
Pro	0.7 (1)	1.4 (2)	0.7 (1)			0.7 (1)
Gly	1.8 (2)	8.1 (9)	2.5 (3)	0.2	0.3	2.6 (3)
Ala	1.0 (1)	5.6 (5)	6.3 (6)	4.6 (5)	5.0 (5)	6.3 (6)
Val	1.0 (1)	2.2 (2)	1.0 (1)	2.1 (2)	1.9 (2)	1.2 (1)
Ile	0.7 (1)	1.7 (2)	0.8 (1)			0.9 (1)
Leu	1.7 (2)	3.0 (3)	0.9 (1)	0.9 (1)	1.0 (1)	1.0 (1)
Tyr	1.5 (2)	1.6 (2)	0.7 (1)			0.8 (1)
Phe	0.9 (1)	1.2 (1)	1.5 (2)			1.8 (2)
Trp	ND (1)	ND (1)	ND	ND	ND	ND
Hse				0.3 (1)	0.7 (1)	
Mobility (pH 6.5)	- 0.55	- 0.22	+ 0.17	+ 0.45	+ 0.65	+ 0.17
N-terminal	Lys	Asn	Lys	Lys	Lys	Lys
Yield	12.3 %	0.5 %	43.6 %	1.1 %	0.4 %	16.6 %
Position	71→100	1→46	47→70	101→115	101→115	47→70
Number of residues	30	46	24	15	15	24
Procedure of isolation	G50;6.5	G50;6.5	G50;6.5	G50;6.5	G50;6.5	G50

Table 4M. Data on *Staphylococcus aureus* protease digest of CB2AM2a. For all details, see Table 1M.

Amino acid	CB2AM2aSAa	CB2AM2aSAb
Lys	1.0 (1)	
His		
Arg		1.0 (1)
CmCys	0.5 (1)	0.6 (1)
Asp	3.9 (4)	3.3 (3)
Thr	1.9 (2)	
Ser		1.3 (2)
Glu	3.1 (3)	
Pro	1.0 (1)	
Gly	0.4	1.9 (2)
Ala	1.0 (1)	
Val	0.8 (1)	
Ile	1.0 (1)	
Leu	1.4 (1)	0.9 (1)
Tyr	2.0 (2)	
Phe	0.8 (1)	
Trp	ND	ND (1)
Hse		
Mobility (pH 6.5)	- 0.35	- 0.18
N-terminal	Lys	Leu
Yield	31.9 %	40.8 %
Position	71→89	90→100
Number of residues	19	11
Procedure of isolation	6.5	6.5

Table 5M. Time course of degradation of 10 nmol of CB2AM2aSAa (71-89) by carboxypeptidase Y

Time	Amount of amino acid residue released :					
	Asn	Phe	Thr	Tyr	Ala	Glu
min	nmol	nmol	nmol	nmol	nmol	nmol
0	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.49	0.53	0.66	0.95
20	0.00	0.25	0.81	0.84	0.74	1.00
40	0.00	0.81	1.03	0.95	1.03	1.07
60	0.00	0.94	1.02	0.94	1.00	1.09

Table 6M. Data on tryptic peptides of CB3. For all details, see Table 1M.

Amino acid	CB3T1d	CB3T1h	CB3T1i	CB3T1j	CB3T2a	CB3T2b	CB3T2e
Lys							
His	1.1 (1)	0.9 (1)	3.4 (4)	4.0 (4)			0.6 (1)
Arg	0.2	1.1 (1)	1.4 (1)	1.4 (1)			
CmCys	0.5 (1)	0.6 (1)			0.7 (1)	0.6 (1)	
Asp	1.1 (1)	1.0 (1)	3.9 (4)	3.9 (4)			
Thr	0.2		1.9 (2)	1.8 (2)			
Ser	0.9 (1)	0.6 (1)			2.1 (2)	1.9 (2)	
Glu	1.2 (1)	1.4 (2)	1.3 (1)	0.9 (1)			
Pro			1.7 (2)	1.9 (2)	0.9 (1)	1.1 (1)	
Gly	2.3 (4)	2.8 (4)	6.3 (7)	6.5 (7)	1.3 (1)	1.2 (1)	1.0 (1)
Ala	3.6 (4)	4.9 (7)	1.0 (1)	1.0 (1)	1.1 (1)	1.2 (1)	
Val	0.3		1.2 (1)	1.0 (1)			
Ile			0.9 (1)	1.0 (1)	0.8 (1)	1.0 (1)	
Leu	1.1 (1)	2.3 (2)	0.9 (1)	0.9 (1)			
Tyr	0.3 (1)	1.0 (1)	0.6 (1)	0.5 (1)			0.6 (1)
Phe	0.6 (1)	1.3 (1)	0.9 (1)	0.8 (1)	0.8 (1)	1.0 (1)	
Trp	ND	ND	ND	ND	ND (1)	ND (1)	ND
Hse							
Mobility (pH 6.5)	- 0.25	- 0.06	0.00	+ 0.29	- 0.51	- 0.19	+ 0.53
N-terminal	Tyr	Tyr	Asn	Asn	Phe	Phe	Tyr
Yield	6.2 %	24.2 %	11.4 %	21.8 %	1.6 %	12.9 %	2.4 %
Position	155→170	155→176	177→203	177→203	204→212	204→212	155→157
Number of residues	16	22	27	27	9	9	3
Procedure of isolation	G25;6.5	G25;6.5	G25;6.5	G25;6.5	G25;6.5	G25;6.5	G25;6.5

Table 7M. Data on thermolytic peptides of CB3. For all details, see Table 1M.

Amino acid	CB3TH1c	CB3TH1e	CB3TH2e	CB3TH21	CB3TH3ad*
Lys					
His	2.8 (3)	3.3 (3)	0.9 (1)	0.9 (1)	
Arg				0.9 (1)	
CmCys					0.5 (1)
Asp	2.2 (2)	2.0 (2)	1.0 (1)	1.0 (1)	
Thr	1.7 (2)	1.2 (1)			
Ser			0.9 (1)		1.4 (2)
Glu	0.9 (1)		1.2 (1)		
Pro	1.9 (2)	2.1 (2)			0.9 (1)
Gly	3.3 (3)	3.3 (3)	4.1 (4)	1.0 (1)	1.5 (1)
Ala			3.0 (3)	1.6 (2)	1.2 (1)
Val					
Ile	0.8 (1)	0.9 (1)			0.9 (1)
Leu	0.8 (1)	0.8 (1)	1.3 (1)		
Tyr	0.9 (1)	1.1 (1)	1.0 (1)		
Phe	0.6 (1)				
Trp	ND	ND	ND	ND	ND
Mobility (pH 6.5)	+ 0.13	+ 0.38	0.00	+ 0.81	- 0.37
N-terminal	Phe	Ile	Tyr	Ala	Ser
Yield	1.6 %	15.7 %	1.9 %	7.7 %	2.2 %
Position	180→196	183→196	155→167	174→179	206→212
Number of residues	17	14	13	6	7
Procedure of isolation	G25;6.5	G25;6.5	G25;6.5	G25;6.5	G25;6.5; C

Table 7M (continued a)

Amino acid	CB3TH3ba*	CB3TH3cf	CB3TH4ed*	CB3TH4ef*	CB3TH5b
Lys					
His			0.7 (1)		
Arg		0.9 (1)			
CmCys	0.3 (1)				
Asp		1.0 (1)	1.0 (1)		
Thr					1.0 (1)
Ser	0.8 (1)				
Glu	0.9 (1)			1.1 (1)	1.1 (1)
Pro					
Gly	3.0 (3)	3.0 (3)	1.3 (1)		
Ala	2.0 (2)	1.2 (1)	1.7 (2)	1.0 (1)	
Val		0.9 (1)			
Ile					
Leu	0.7 (1)			1.0 (1)	
Tyr			0.7 (1)		
Phe	0.8 (1)				0.9 (1)
Trp	ND	ND	ND	ND	ND
Mobility (pH 6.5)	- 0.29	0.00	0.00	0.00	- 0.52
N-terminal	Leu	Val	Tyr	Leu	Phe
Yield	2.1 %	9.0 %	4.2 %	10.7 %	8.1 %
Position	161→170	197→203	155→160	171→173	180→182
Number of residues	10	7	6	3	3
Procedure of isolation	G25;6.5; C	G25;6.5; 3.5	G25;6.5; C	G25;6.5; C	G25;6.5

Table 8M. Data on *Staphylococcus aureus* protease digest of CB3. For all details, see Table 1M.

Amino acid	CB3SA1a	CB3SA1be*	CB3SA2ab*	CB3SA2c	CB3SA2d
Lys					
His	2.0 (2)	3.1 (3)			0.9 (1)
Arg	1.2 (1)	1.0 (1)			
CmCys	0.5 (1)	0.5 (1)	0.6 (1)	0.4 (1)	0.7 (1)
Asp	2.0 (2)	2.8 (3)			1.2 (1)
Thr	1.2 (1)	0.9 (1)			
Ser	1.3 (1)	1.6 (2)	0.8 (1)	0.9 (1)	0.9 (1)
Glu	3.2 (3)	0.2		1.3 (1)	1.2 (1)
Pro		3.0 (3)	0.5 (1)		
Gly	4.9 (5)	7.1 (7)	1.2 (1)	2.9 (2)	3.9 (4)
Ala	6.2 (7)	2.3 (2)	0.8 (1)	3.1 (3)	4.0 (4)
Val		0.7 (1)			
Ile		1.7 (2)	0.8 (1)		
Leu	1.8 (2)	0.8 (1)		1.0 (1)	1.6 (2)
Tyr	0.8 (1)	0.3 (1)			0.6 (1)
Phe	2.3 (2)	0.5 (1)		0.9 (1)	1.1 (1)
Trp	ND	ND (1)	ND	ND	ND
Hse					
Mobility (pH 6.5)	0.00	+ 0.08	- 0.48	- 0.28	- 0.25
N-terminal	Tyr	Ile	Ala	Ala	Tyr
Yield	21.9 %	4 %	3.9 %	2.5 %	2.2 %
Position	155→182	183→212	207→212	163→172	155→171
Number of residues	28	30	6	10	17
Procedure of isolation	P4;6.5	P4;6.5; C	P4;6.5; C	P4;6.5	P4;6.5

Table 8M (continued a)

Amino acid	CB3SA2ea*	CB3SA2eb*	CB3SA3a
Lys			
His	0.8 (1)	0.6 (1)	1.1 (1)
Arg	0.9 (1)		
CmCys	0.5 (1)		
Asp	1.3 (1)	1.1 (1)	1.2 (1)
Thr	0.9 (1)		
Ser	0.8 (1)		
Glu	2.6 (3)		
Pro	0.1		
Gly	2.3 (2)	2.8 (3)	2.6 (3)
Ala	3.7 (4)	2.8 (3)	2.7 (3)
Val			
Ile			
Leu	0.9 (1)	1.0 (1)	1.1 (1)
Tyr		0.5 (1)	0.9 (1)
Phe	1.7 (2)		
Trp	ND	ND	ND
Hse			
Mobility (pH 6.5)	- 0.48	0.00	0.00
N-terminal	Ser	Tyr	Tyr
Yield	6.5 %	22.4 %	14.0 %
Position	165→182	155→164	155→164
Number of residues	18	10	10
Procedure of isolation	P4;6.5; C	P4;6.5; C	P4;6.5

Table 9M. Data on chymotryptic peptides of CB3T1j. For all details, see Table 1M.

Amino acid	CB3T1jCd	CB3T1jCf	CB3T1jCh
Lys			
His		3.0 (3)	1.1 (1)
Arg	0.8 (1)		
CmCys			
Asp	0.9 (1)	1.9 (2)	0.8 (1)
Thr		1.5 (2)	
Ser			
Glu		0.8 (1)	
Pro		1.9 (2)	
Gly	3.2 (3)	2.8 (3)	1.2 (1)
Ala	0.9 (1)		
Val	0.6 (1)		
Ile		0.7 (1)	
Leu		0.8 (1)	
Tyr		0.7 (1)	
Phe			0.9 (1)
Trp	ND	ND	ND
Hse			
Mobility (pH 6.5)	0.00	+ 0.13	+ 0.35
N-terminal	Val	Thr	Asn
Yield	6.9 %	26.9 %	13.4 %
Position	197→203	181→196	177→180
Number of residues	7	16	4
Procedure of isolation	6.5	6.5	6.5

Table 10M. Data on chymotryptic peptides of CB4. For all details, see Table 1M.

Amino acid	CB4Cb	CB4Cd
Lys	0.9 (1)	
His		0.9 (1)
Arg		1.6 (2)
CmCys		0.3 (1)
Asp	2.2 (2)	3.0 (3)
Thr	1.0 (1)	0.8 (1)
Ser	0.5	3.3 (4)
Glu		
Pro	0.9 (1)	
Gly	2.9 (3)	2.8 (2)
Ala		1.1 (1)
Val	1.1 (1)	2.0 (2)
Ile	1.1 (1)	
Leu		
Tyr		
Phe	0.7 (1)	
Trp		
Hse		0.7 (1)
Mobility (pH 6.5)	0.00	+ 0.24
N-terminal	Gly	Arg
Yield	40.5 %	36.0 %
Position	126→136	137→154
Number of residues	11	18
Procedure of isolation	6.5	6.5

Table 11M. Data on tryptic limited digest of the SPE-protein. For all details, see Table 1M.

Amino acid	TL1	TL2fb*	TL2g	TL2h	TL3a	TL3gc
Lys	0.8 (1)	0.8 (1)				
His		1.0 (1)	2.6 (4)	3.8 (4)		
Arg	0.9 (1)	0.8 (1)	1.2 (1)	1.0 (1)	1.0 (1)	1.0 (1)
PeCys	ND	ND	ND	ND	ND (1)	ND
Asp	0.9 (1)	2.1 (2)	3.9 (4)	3.6 (4)	1.3 (1)	
Thr	1.8 (2)	0.9 (1)	1.9 (2)	1.8 (2)	1.8 (2)	
Ser		0.7	1.0		3.4 (4)	
Glu	2.9 (3)		1.1 (1)	1.2 (1)	2.2 (2)	2.0 (2)
Pro	1.7 (2)	0.5 (1)	1.9 (2)	1.9 (2)		
Gly	5.1 (5)	2.7 (3)	6.2 (7)	6.3 (7)	3.4 (4)	
Ala	5.5 (6)	1.1 (1)	1.3 (1)	1.0 (1)	1.2 (1)	
Val	1.9 (2)	1.0 (1)	1.1 (1)	0.8 (1)	0.9 (1)	
Ile	0.9 (1)	0.7 (1)	0.9 (1)	0.8 (1)		0.8 (1)
Leu	1.1 (1)		1.1 (1)	1.1 (1)	1.0 (1)	1.2 (1)
Tyr	1.0 (1)		0.9 (1)	0.9 (1)	0.8 (1)	
Phe	1.0 (1)	0.6 (1)	1.0 (1)	0.9 (1)		
Trp	ND	ND	ND	ND	ND (1)	ND
Met		0.3 (1)				
Mobility (pH 6.5)		+ 0.10	+ 0.19	+ 0.24	- 0.32	+ 0.59
N-terminal	Val	His	Asn	Asn	Asn	Gln
Yield	76.5 %	9.9 %	25.5 %	30.6 %	8.6 %	25.6 %
Position	26→52	123→137	177→203	177→203	1→20	21→25
Number of residues	27	15	27	27	20	5
Procedure of isolation	P6	P6;6.5; C	P6;6.5	P6;6.5	P6;6.5	P6;6.5; 3.5

Table 11M (continued a)

Amino acid	TL3gd	TL4ac	TL4b	TL5
Lys				
His	1.5 (2)			
Arg				
PeCys	ND	ND	ND (1)	ND
Asp				0.3
Thr				
Ser		1.1 (1)	1.5 (2)	1.0 (1)
Glu		1.2 (1)		0.9 (1)
Pro			1.0 (1)	0.3
Gly	1.3 (1)		0.9 (1)	0.4
Ala		1.0 (1)	0.9 (1)	1.2 (1)
Val				
Ile			0.9 (1)	
Leu				
Tyr	0.8 (1)	0.8 (1)		0.9 (1)
Phe		0.9 (1)	0.9 (1)	1.1 (1)
Trp	ND	ND	ND (1)	ND
Met	0.5 (1)			
Mobility (pH 6.5)	+ 0.59	0.00	+ 0.13	0.00
N-terminal	His	Phe	Phe	Phe
Yield	3.5 %	2.9 %	39.0 %	24.3 %
Position	153→157	53→57	204→212	53→57
Number of residues	5	5	9	5
Procedure of isolation	P6;6.5; 3.5	P6;6.5; 3.5	P6;6.5	P6;6.5

Table 12M. Data on tryptic peptides of TL1. For all details, see Table 1M.

Amino acid	TL1Ta	TL1Tc
Lys	1.0 (1)	0.3
His		
Arg		0.7 (1)
PeCys	ND	ND
Asp	1.3 (1)	
Thr	1.9 (2)	
Ser	1.2	
Glu	1.7 (2)	1.0 (1)
Pro	2.0 (2)	
Gly	4.2 (5)	0.6
Ala	4.1 (4)	1.7 (2)
Val	1.3 (1)	0.9 (1)
Ile	1.2 (1)	
Leu	0.7 (1)	
Tyr	0.6 (1)	
Phe	0.9 (1)	
Trp	ND	ND
Mobility (pH 6.5)	0.00	+ 0.63
N-terminal	Val	Ala
Yield	40.0 %	35.0 %
Position	26→47	48→52
Number of residues	22	5
Procedure of isolation	6.5	6.5

Table 13M. Data on tryptic peptides of SCM-protein followed by radioactive labelling of methionine (TMI experiment). For all details, see Table 1M.

Amino acid	TMI3c	TMI3g	TMI4b	TMI4c	TMI5c	TMI8b	TMI10,1
Lys		1.0 (1)		1.0 (1)			
His						2.5 (4)	
Arg	1.0 (1)					1.0 (1)	1.0 (1)
CmCys	0.2 (1)		0.3 (1)				
Asp	1.6 (1)	2.2 (2)		1.9 (2)		3.7 (4)	
Thr	1.9 (2)	0.7 (1)		0.9 (1)		1.7 (2)	
Ser	3.6 (4)		1.8 (2)	1.2 (1)	1.0 (1)		
Glu	2.3 (2)	0.4		1.1 (1)	1.1 (1)	1.1 (1)	1.1 (1)
Pro		0.8 (1)	1.1 (1)	0.9 (1)		1.7 (2)	
Gly	3.5 (4)	2.5 (3)	0.6 (1)	2.8 (3)	0.3	5.9 (7)	
Ala	1.2 (1)	2.9 (3)	0.6 (1)	4.1 (4)	1.1 (1)	1.2 (1)	1.1 (1)
Val	1.0 (1)					1.1 (1)	
Ile		0.9 (1)	0.6 (1)	0.9 (1)		0.9 (1)	
Leu	1.1 (1)	0.9 (1)		0.9 (1)		0.9 (1)	0.8 (1)
Tyr	0.8 (1)			0.6 (1)	0.8 (1)	0.6 (1)	
Phe		0.8 (1)	0.5 (1)	1.8 (2)	1.0 (1)	1.0 (1)	
Trp	ND (1)	ND	ND (1)	ND	ND	ND	ND
Met							0.9 (1)
Mobility (pH 6.5)	ND	ND	ND	ND	ND	ND	ND
N-terminal	Asp	Gly	Phe	Phe	Phe	Asn	Leu
Yield	19.6 %	32.0 %	58.6 %	6.3 %	11.4 %	42.4 %	6.4 %
Position	1→20	58→71	204→212	53→71	53→57	177→203	118→122
Number of residues	20	14	9	19	5	27	5
Procedure of isolation	i; 3.5	i; 3.5	i; 3.5	i; 3.5	i; 3.5	i; 3.5	i; i

Table 14M. Data on tryptic peptides of SCM-protein followed by radioactive labelling of methionine (TMS experiment). For all details, see Table 1M.

Amino acid	TMS1,2c	TMS2,2c	TMS2,2d
Lys		0.8 (1)	
His			
Arg	0.9 (1)		0.9 (1)
CmCys	0.2 (1)		
Asp	3.0 (3)	1.1 (1)	
Thr	1.1 (1)	1.1 (1)	
Ser	3.1 (4)	0.3	
Glu			1.1 (1)
Pro			
Gly	2.7 (2)	0.3	
Ala	1.0 (1)	1.9 (2)	1.0 (1)
Val	2.4 (2)	1.2 (1)	
Ile			
Leu		0.9 (1)	0.7 (1)
Tyr			
Phe			
Trp	ND	ND (1)	ND
Met		0.6 (1)	0.6 (1)
Mobility (pH 6.5)	0.00	+ 0.29	+ 0.40
N-terminal	Ser	Ala	Leu
Yield	7.2 %	4.0 %	10.6 %
Position	138→152	109→117	118→122
Number of residues	15	9	5
Procedure of isolation	P6;G25; 6.5	P6;P2; 6.5	P6;P2; 6.5

Table 15M. Data on peptic peptides containing cysteic acid. For all details, see Table 1M.

Amino acid	Pa	Pb	Pc	Pd	Pea	Peb
Lys						0.5 (1)
His						
Arg					1.1 (1)	0.6 (1)
Cy a	0.8 (1)	1.0 (1)	0.9 (1)	0.9 (1)	1.0 (1)	0.5 (1)
Asp	3.0 (3)	3.2 (3)			3.2 (3)	2.7 (3)
Thr	1.2 (1)	1.1 (1)			1.4 (1)	0.6 (1)
Ser			0.7 (2)	0.5	2.6 (3)	2.8 (3)
Glu				0.9 (1)	0.6	0.4
Pro	0.5 (1)	0.5 (1)	0.5 (1)			
Gly			0.7 (1)	0.6	3.9 (4)	2.3 (2)
Ala			1.6 (1)	2.1 (2)	0.8 (1)	1.5 (2)
Val	0.4 (1)	1.0 (1)			2.5 (2)	0.7 (1)
Ile						
Leu				1.1 (1)	0.4	
Tyr						
Phe		0.5 (1)	0.5 (1)	0.4 (1)	0.2 (1)	
Trp	ND	ND	ND (1)	ND	ND	ND (1)
$MetO_2$						
Mobility (pH 6.5)[a]	- 0.90	- 0.80	- 0.60	- 0.40	0.00	0.00
N-terminal	Asp	Asp	Phe	Phe	Gly	Asn
Yield	2.9 %	6.2 %	3.3 %	2.1 %	2.2 %	1.7 %
Position	78→84	78→85	204→211	168→173	134→150	91→106
Number of residues	7	8	8	6	17	16
Procedure of isolation	6.5 D	6.5 D	6.5 D	6.5 D	6.5 D; 3.5	6.5 D; 3.5

a) After performic oxidation.