[Chem. Pharm. Bull. 33(12)5428—5436(1985)]

Amino Acid Sequences of the Amino- and Carboxyl-Terminal and Reactive Serine Regions of Carboxypeptidase C_{Ua}

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(Received April 13, 1985)

The amino acid sequences of the amino- and carboxyl-terminal regions of carboxypeptidase C_{Ua} were determined to be Ala–Val–Glu–Leu–His–Phe–Ile–His–Asn– and –Arg–His–Met–Glu–Pro–(Ala,Asp,Lys)–Gly–Thr–Ser, respectively. The enzyme was inactivated with the incorporation of 1 mol of ^{3}H-labeled diisopropylfluorophosphate (DFP) per mol of enzyme, and this reagent was found to react with a serine residue in the active site of the enzyme. The primary structure around this reactive serine residue was determined to be Asp–Val–Ala–Gly–Tyr–Asp–Ser–Glu–Trp–Ile–Gln–Leu–Arg–Val–Pro–Cys–Glu–Gly–Asp–Ser*–Gly–Gly–Glu–Leu*–Asp–Lys–Glu–Gly–Met–Ala–Pro–Asn–Gly–Ile–Val–Ser–Asp–Ala–Leu–Phe–Thr–Ser–Arg (Ser*, reactive serine) by sequence analysis of two radioactive peptides released from [^{3}H]DFP-treated carboxypeptidase C_{Ua} on tryptic digestion and cyanogen bromide cleavage.

Keywords——carboxypeptidase; *Citrus unshiu*; active site; primary structure; serine protease

Carboxypeptidases C_{Ua} and C_{Ub} [EC 3.4.16.1], both isolated from the exocarp of mandarin orange (*Citrus unshiu* MARC.), are serine proteases having the ability to liberate most amino acids, including proline, from the C-termini of peptide chains. This broad specificity makes the enzymes especially useful for sequence analysis.[1] Carboxypeptidases C_{Ua} and C_{Ub}, possessing molecular weights of 96000 and 112000, respectively, are somewhat different from each other in their amino acid and carbohydrate compositions and kinetic parameters, whereas they are similar in behavior with inhibitors.[2] Both enzymes have one reactive serine residue in the active site and have identical sequences of seven amino acid residues, Glu–Gly–Asp–Ser–Gly–Gly–Glu–Leu, around this serine.[3] This sequence shows homology to those of typical serine proteases having established primary structures. As regards carboxypeptidases, the complete amino acid sequence has been established only for carboxypeptidases A[4] and B,[5] both pancreatic metalloenzymes. No attempt has so far been made to determine the primary structure of carboxypeptidase C type enzymes, except for the sequences of several amino acid residues around the reactive serine of carboxypeptidases C_N,[6] Y,[7] C_{Ua} and C_{Ub}.[3]

The present paper describes the determination of the amino (N)- and carboxyl(C)-terminal sequences and an extended amino acid sequence around the reactive serine residue of carboxypeptidase C_{Ua}. The sequence homology between this enzyme and other serine proteases is discussed.

Experimental

Materials——Carboxypeptidase C_{Ua} was purified from the exocarp of *Citrus unshiu* MARC. as described previously.[1] Benzyloxycarbonyl-L-glutamyl-L-phenylalanine(Z–Glu–Phe) was obtained from the Peptide Research Foundation, Osaka. Diisopropylfluorophosphate (DFP), L-(1-tosylamide-2-phenyl)ethyl chloromethyl ketone (TPCK), and trypsin (from bovine pancreas, 3 × crystallized) were purchased from Sigma Chemical Co., St. Louis,

Mo. Cyanogen bromide (CNBr) was obtained from Kishida Chemical Industries Co., Osaka. [^{3}H]DFP (specific activity, 6.5 mCi/μmol) was a product of the Radiochemical Centre, Amersham, England. This was diluted with unlabeled DFP to 2.94 μCi/μmol before use. Phenylthiohydantoin (PTH)-amino acids, standard amino acids, and reagents for amino acid sequence analysis were obtained from Wako Pure Chemical Industries, Osaka. Other reagents and organic solvents used were of analytical grade.

Enzyme Assay——Carboxypeptidase activity was assayed by the method described previously[8] with Z-Glu-Phe as a substrate. The protein concentration of enzyme solutions was determined by the Folin-Lowry method at 660 nm[9] with bovine serum albumin as a protein standard.

Preparation of TPCK-trypsin——Since the preparation of trypsin showed a weak chymotryptic activity, it was treated with TPCK, a specific inhibitor of chymotrypsin, according to the method of Ong *et al.*[10]

Preparation of [^{3}H]DFP-Treated Carboxypeptidase C_{Ua}——The enzyme (16 mg, 167 nmol) was incubated with 10 μl of [^{3}H]DFP (1.7 μmol, 5 μCi) for 60 min at 35 °C in 3 ml of 0.1 M citrate buffer, pH 5.5. In order to inhibit the enzyme completely, 10 μl of unlabeled DFP (1.7 μmol) was added to the mixture, and the incubation was continued for a further 60 min at 35 °C. The mixture was then passed through a column (2 × 38 cm) of Sephadex G-25 (coarse) with distilled water as an eluant in order to remove the excess reagents. Fractions containing the ^{3}H-labeled protein were collected and lyophilized after determination of enzymatic activity and of radioactivity with an Aloka LSC-502 liquid scintillation counter.

Cyanogen Bromide Cleavage and Peptide Separation——The reduced and carboxymethylated ^{3}H-labeled enzyme (7 mg, 73 nmol) was cleaved with 2% CNBr in 70% formic acid (1.4 ml) for 24 h at 4 °C in a sealed glass tube filled with N_2 gas. The reaction mixture was then diluted with water and lyophilized to remove the excess reagents. The resultant ^{3}H-labeled peptides were separated on a column (1 × 95 cm) of Sephadex G-50 (superfine), equilibrated and eluted with 0.1 M NH_4HCO_3, pH 8.0.

Tryptic Digestion and Peptide Separation——The reduced and carboxymethylated ^{3}H-labeled enzyme (7 mg, 73 nmol) was incubated with TPCK-trypsin (36 μg, 1.5 nmol) for 6 h at 35 °C in 0.5 ml of 0.1 M NH_4HCO_3, pH 8.0, containing 0.1% thiodiglycol. The reaction mixture was directly applied to a column (1 × 100 cm) of Sephadex G-50 (superfine) which was equilibrated and eluted with the same buffer.

Gel Electrophoresis——The molecular weights of peptides were determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis according to the methods of Weber and Osborn.[11] Electrophoresis was performed on 10% polyacrylamide gel, pH 7.2, containing 0.1% SDS at room temperature and a current of 8 mA/tube with bromphenol blue as a leading ion. Standard proteins used were bovine plasma albumin, ovalbumin, trypsin, cytochrome c, and insulin B chain.

Amino Acid Analysis——Amino acid analyses were performed on a Hitachi KLA-5 single column amino acid analyzer after hydrolysis of peptides in 0.5 ml of 6 N HCl, under vacuum, for 22 h at 110 °C. The tryptophan content was determined from the results of sequence analysis.

Sequence Determination——The N-terminal sequence analyses of carboxypeptidase C_{Ua} and the ^{3}H-labeled peptides were performed with a JEOL JAS-47K sequence analyzer in the presence of polybrene according to the method of Edman and Begg.[12] Before sample addition, polybrene was purified in the spinning cup by running two complete cycles. Samples (27—50 nmol) dissolved in 0.5 ml of sequential grade water were then fixed on the wall of the spinning cup and automatic sequencing was commenced.[13] The resultant PTH-amino acids were determined on a Waters high performance liquid chromatography (HPLC) system and by thin layer chromatography (TLC) as described previously.[13]

For C-termianl sequence analysis, the enzyme, which had been inactivated by lyophilization, and the ^{3}H-labeled peptides were hydrolyzed with intact carboxypeptidase C_{Ua}.[1] The hydrolysis was stopped by the addition of an equal volume of 20% trichloroacetic acid at the times indicated in Table I. The reaction mixtures were then centrifuged, and the concentrations of free amino acids in the supernatants were determined on the amino acid analyzer. The C-terminal amino acid of the enzyme was also determined also by using anhydrous hydrazine according to the method of Akabori *et al.*[14]

Results

Preparation of [^{3}H]DFP-Treated Carboxypeptidase C_{Ua}

Incorporation of ^{3}H was determined on aliquots of [^{3}H]DFP-treated carboxypeptidase C_{Ua} by determination of radioactivity after removal of excess reagents by gel filtration on Sephadex G-25, as shown in Fig. 1. Carboxypeptidase C_{Ua} was inactivated with the incorporation of 1 mol of [^{3}H]DFP per mol of enzyme. The yield of [^{3}H]DFP-treated carboxypeptidase C_{Ua} was 90%. The ^{3}H-labeled enzyme obtained was reduced and carboxymethylated by the method of Link and Stark.[15] This preparation was subjected to the following CNBr cleavage or tryptic digestion.

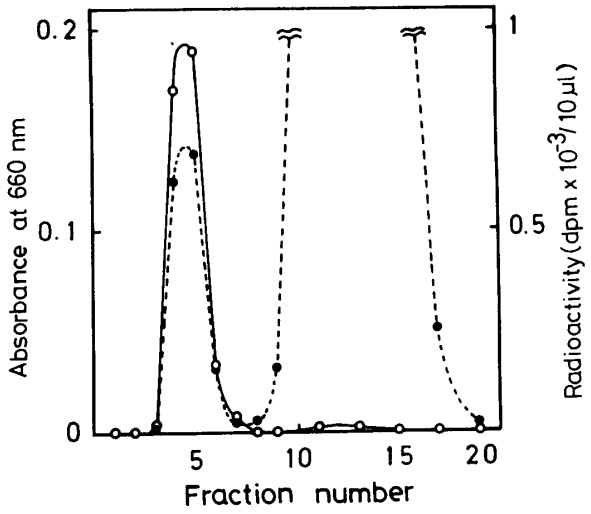

Fig. 1. Chromatography of [^{3}H]DFP-Treated Carboxypeptidase C_{Ua} on Sephadex G-25

The enzyme (16 mg, 167 nmol) was incubated in 3 ml of 0.1 M citrate buffer, pH 5.5, containing 1.7 µmol of [^{3}H]DFP (5 µCi) for 60 min at 35 °C. After the addition of unlabeled DFP (1.7 µmol, 10 µl), the mixture was incubated for a further 60 min and then applied to a column (2 × 38 cm) of Sephadex G-25 (coarse), and eluted with deionized water at 10 ml/h. The effluent was collected in 5-ml fractions. ○—○, absorbance at 660 nm; ●---●, radioactivity (dpm).

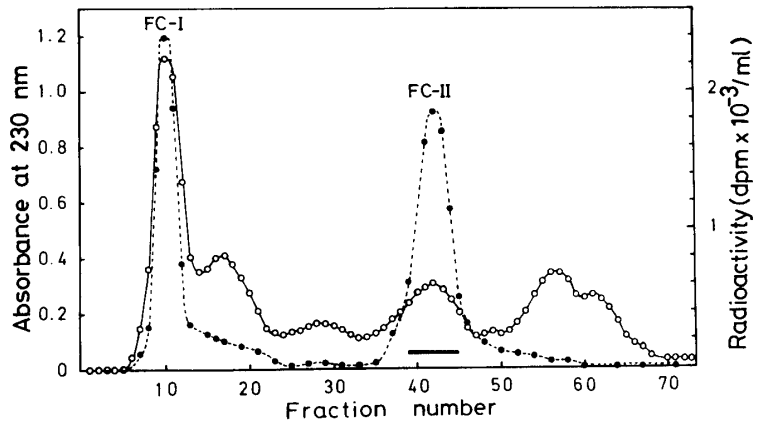

Fig. 2. Chromatography of CNBr Cleavage Product on Sephadex G-50

CNBr cleavage reaction was performed by the method described under Experimental. The reaction mixture was lyophilized and applied to a column (1 × 95 cm) of Sephadex G-50 (superfine), eluted with 0.1 M NH_4HCO_3, pH 8.0 at 6 ml/h. The effluent was collected in 1-ml fractions. ○—○, absorbance at 660 nm; ●---●, radioactivity (dpm).

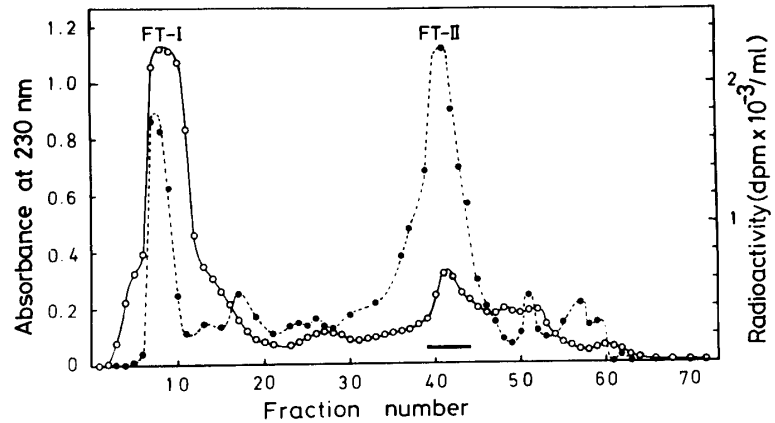

Fig. 3. Chromatography of Tryptic Digest on Sephadex G-50

Tryptic digestion was carried out as described under Experimental. The digest was applied directly to a column (1 × 100 cm) of Sephadex G-50 (superfine), and eluted with 0.1 M NH_4HCO_3, pH 8.0, at 6 ml/h. The effluent was collected in 1-ml fractions. ○—○, absorbance at 660 nm; ●---●, radioactivity (dpm).

Separation of ^{3}H-Labeled Peptides

^{3}H-Labeled peptides were each separated from the CNBr cleavage products and the tryptic digest of ^{3}H-labeled carboxypeptidase C_{Ua} by gel filtration on Sephadex G-50.

Figure 2 shows the result of chromatography of CNBr cleavage products on Sephadex G-50. Two main radioactive fractions, FC-I and -II, were obtained with five less radioactive fractions. Fraction FC-II (shown by a horizontal bar) was further purified by rechromatography, giving one radioactive peak which was named CB-peptide.

Figure 3 shows the result of chromatography of tryptic digest on Sephadex G-50. Two main radioactive fractions, FT-I and -II, were obtained with five less radioactive fractions. Rechromatography of fraction FT-II (shown by a horizontal bar) yielded one radioactive peak, T-peptide.

CB- and T-peptides, both obtained in yields of about 60% on the basis of radioactivity, were lyophilized and subjected to sequence analysis.

FC-I and FT-I seemed to be ^{3}H-labeled carboxypeptidase C_{Ua} which was not changed sufficiently in primary structure by CNBr cleavage and tryptic digestion.

Gel Electrophoresis

The electrophoretic patterns on SDS–polyacrylamide gel showed the homogeneity of

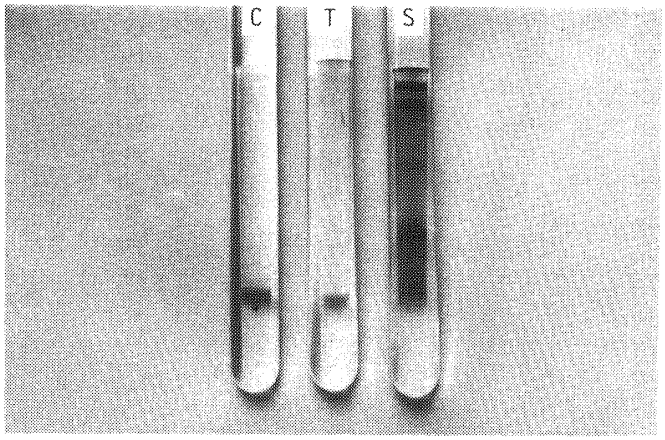

Fig. 4. Polyacrylamide Gel Electrophoresis of ^{3}H-Labeled Peptides Purified from CNBr Cleavage Product and Tryptic Digest

Electrophoresis was carried out with 10% polyacrylamide gel in 0.5 M sodium phosphate buffer, pH 7.2, containing 0.1% SDS. The gel was stained with Amido-black 10 B after electrophoresis.

Samples: C, CB-peptide; T, T-peptide; S, standard proteins (bovine plasma albumin, ovalbumin, trypsin, cytochrome c, and insulin B chain, all 5 μg in 10 μl).

TABLE I. Enzymatic Determination of the C-Terminal Sequence of Carboxypeptidase C_{Ua}

Amino acid	Mol per mol of substrate				
	Incubation time (min)				
	10	30	90	180	360
Serine	0.207	0.362	0.455	0.507	0.655
Threonine	0.117	0.242	0.351	0.409	0.595
Glycine		0.233	0.274	0.377	0.564
Lysine		0.222	0.273	0.341	0.512
Aspartic acid		0.207	0.265	0.333	0.523
Alanine		0.204	0.250	0.322	0.487
Proline				0.317	0.409
Glutamic acid				0.208	0.328
Methionine				0.186	0.313
Histidine				0.150	0.188
Arginine				0.114	0.152

The enzyme (1 mg, 10 nmol), inactivated by lyophilization, was incubated with 34 μg (0.35 nmol) of intact carboxypeptidase C_{Ua} at 35 °C in 0.5 ml of 0.1 M citrate buffer, pH 5.5. The enzymatic reaction was stopped by the addition of 0.5 ml of 20% trichloroacetic acid at the times indicated, and the reaction mixture was centrifuged. The concentrations of the amino acids liberated were determined with 0.5-ml aliquots of the supernatants on an amino acid analyzer.

TABLE II. Automated N-Terminal Sequence Analyses of Carboxypeptidase C_{Ua}, CB-Peptide, and T-Peptides

Position	Carboxypeptidase C_{Ua}		CB-peptide			T-peptide		
	Residue	Yield (nmol)	Residue	Yield[a] (nmol)	(dpm)	Residue	Yield[a] (nmol)	(dpm)
1	Ala	6.3	Asp	39	28	Val	31	32
2	Val	6.9	Val	37	28	Pro	19	30
3	Glu	4.3	Ala	29	31	Cys	14	32
4	Leu	3.0	Gly	24	29	Glu	25	31
5	His	4.0	Tyr	24	32	Gly	24	29
6	Phe	5.3	Asp	28	29	Asp	21	36
7	Ile	4.6	Ser		28	Ser		196
8	His	3.7	Glu	16	32	Gly	22	54
9	Asn	3.0	Trp	20	31	Gly	20	45
10			Ile	22	31	Glu	19	39
11			Gln	11	30	Leu	18	36
12			Leu	17	29	Asp	13	32
13			Arg	9.3	29	Lys	18	33
14			Val	27	30	Glu	24	31
15			Pro	11	31	Gly	17	30
16			Cys	5.5	29	Met	13	31
17			Glu	5.2	31	Ala	16	29
18			Gly	8.7	28	Pro	13	32
19			Asp	6.7	34	Asn	6.2	30
20			Ser		82	Gly	11	30
21			Gly	7.7	47	Ile	11	28
22			Gly	7.7	40	Val	14	29
23			Glu	3.4	33	Ser		29
24			Leu	7.5	31	Asp	4.7	28
25			Asp	1.9	32	Ala	6.0	31
26			Lys	4.9	30	Leu	4.8	30
27						Phe	5.2	32

Carboxypeptidase C_{Ua} (27 nmol) and CB- and T-peptides (both approximately 50 nmol) were applied to a sequence analyzer after the addition of polybrene to the spinning cup. PTH-serine could not be quantitated but was identified by TLC. *a*) The radioactivities of the PTH-amino acids were separately determined with an Aloka LSC-502 liquid scintillation counter using one-tenth volume of the derivatives.

both CB- and T-peptides and gave approximate value of 3000 for their molecular weights (Fig. 4).

Sequence Analysis

The results of C-terminal sequence analysis with intact carboxypeptidase C_{Ua} are given in Table I, showing that the enzyme has the C-terminal sequence –Arg–His–Met–Glu–Pro–(Ala, Asp, Lys)–Gly–Thr–Ser. The C-terminal residue was also identified as serine by the hydrazinolysis method.[14]

The automated sequence analysis of the enzyme identified up to nine residues from the N-terminus (Table II). The yield of alanine, the N-terminus, was 23%. The results indicate the sequence Ala–Val–Glu–Leu–His–Phe–Ile–His–Asn–. The repetitive yield was calculated to be 97% from the yields of His-5 and His-8.

The results of automated sequence analyses of CB- and T-peptides are also given in Table II. The analyses permitted the identification of 26 and 27 residues in the N-terminal regions of CB- and T-peptides, respectively. The radioactivities of the amino acids from the N-terminal of these peptides were determined separately with an Aloka LSC-502 liquid scintilation

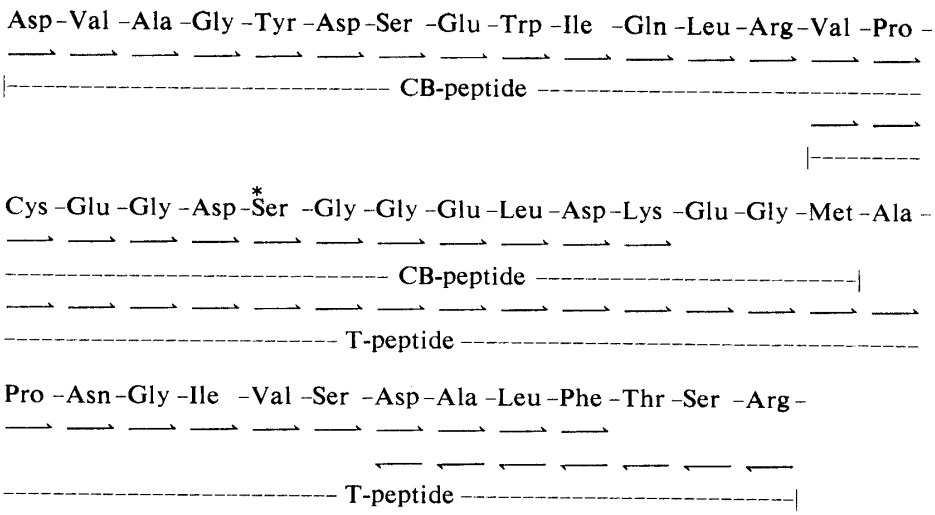

Fig. 5. Amino Acid Sequence around the Reactive Serine Residue of Carboxypeptidase C_{Ua}

Amino acid sequence was determined with a sequence analyzer (⟶) and carboxypeptidase C_{Ua} (⟵). Ser, reactive serine.

TABLE III. Amino Acid Compositions of CB-Peptide and T-Peptide

Amino acids	CB-peptide		T-peptide	
	Residues per mol of peptide	Nearest integer	Residues per mol of peptide	Nearest integer
Tryptophan	—	— (1)	—	— (0)
Lysine	0.7	1 (1)	0.6	1 (1)
Histidine	0	0 (0)	0	0 (0)
Arginine	0.6	1 (1)	0.6	1 (1)
Aspartic acid	4.3	4 (4)	4.2	4 (3)
Threonine	0	0 (0)	0.8	1 (1)
Serine	1.8	2 (2)	2.8	3 (3)
Glutamic acid	5.0	5 (4)	2.7	3 (3)
Proline	1.1	1 (1)	2.2	2 (2)
Glycine	5.3	5 (5)	5.1	5 (5)
Alanine	0.8	1 (1)	1.6	2 (2)
Half-cystine	0.8	1 (1)	0.9	1 (1)
Valine	1.8	2 (2)	1.8	2 (2)
Methionine	1.0	1 (1)	0.9	1 (1)
Isoleucine	0.6	1 (1)	0.6	1 (1)
Leucine	1.6	2 (2)	1.6	2 (2)
Tyrosine	0.9	1 (1)	0	0 (0)
Phenylalanine	0	0 (0)	0.8	1 (1)
Glutamine	0	0 (1)	0	0 (0)
Asparagine	0	0 (0)	0	0 (1)
Total	26.3	28 (29)	27.2	30 (30)

The peptides were hydrolyzed in 0.5 ml of 6 N HCl for 24 h at 110 °C. Amino acid analyses of the hydrolysates was performed on an amino acid analyzer. The number of residues in the finally determined sequences are indicated in parentheses.

counter using one-tenth volume of their PTH-derivatives. The PTH-derivative of serine could not be quantitated because it was rapidly destroyed during the analysis, yielding several by-products. The PTH-derivatives of glutamine and asparagine were partially deamidized

during the analysis, giving derivatives of glutamic and aspartic acids, respectively. The repetitive yields were calculated to be 97% for T-peptide from the yields of Val-1 and Val-22.

Carboxypeptidase C_{Ua} released the following amino acids sequentially from the C-terminus of T-peptide on incubation for 120 min at 35 °C: Arg, Ser, Thr, Phe, Leu, Ala, and Asp in amounts of 0.84, 0.66, 0.54, 0.51, 0.38, 0.17, and 0.12 mol, respectively, per mol of peptide.

The results of amino acid sequence analysis of the ^{3}H-labeled peptides are shown in Fig. 5. The primary structure around the reactive serine residue of carboxypeptidase C_{Ua} was thus determined to be (Met)–Asp–Val–Ala–Gly–Tyr–Asp–Ser–Glu–Trp–Ile–Gln–Leu–Arg–Val–Pro–Cys–Glu–Gly–Asp–$\overset{*}{\text{Ser}}$–Gly–Gly–Glu–Leu–Asp–Lys–Glu–Gly–Met–Ala–Pro–Asn–Gly–Ile–Val–Ser–Asp–Ala–Leu–Phe–Thr–Ser–Arg ($\overset{*}{\text{Ser}}$, reactive serine).

Table III shows the amino acid compositions of CB- and T-peptides. The half-cystine and methionine contents were determined as S-carboxymethylcysteine and homoserine, respectively. Tryptophan was not determined. The amino acid compositions were in good agreement with those determined by sequence analysis. The molecular weights of CB- and T-peptides were calculated to be 3155 and 3051, respectively, from these amino acid compositions.

Discussion

CB- and T-peptides, each containing a reactive serine residue, were separated and purified by chromatography on Sephadex G-50 (superfine). Both peptides were judged to be sufficiently homogeneous to allow sequence analysis from the results of SDS–polyacrylamide gel electrophoresis. The approximate value of 3000 determined by SDS–polyacrylamide gel electrophoresis for the molecular weights of both CB- and T-peptides was in good agreement with those of 3155 and 3051, respectively, calculated from their amino acid compositions.

Automated sequence analyses provided identification of the first 26 and 27 residues of CB- and T-peptides, respectively, whereas only nine residues were identified for carboxypeptidase C_{Ua} (Table II). These results indicate that the analysis proceeded with good efficiency for the peptides having low molecular weights, 3155 and 3051, in the presence of polybrene. On the other hand, the coupling and cleavage reactions seem to have proceeded insufficiently in the sequence analyzer with carboxypeptidase C_{Ua} having a high molecular weight, 96000, even in the presence of polybrene.

The C-terminal sequence of T-peptide determined by sequential hydrolysis with carboxypeptidase C_{Ua} overlapped the sequence obtained with the sequence analyzer.

The amino acid sequences of CB- and T-peptides, both containing a reactive serine residue, overlapped each other, and thus the sequence around this serine residue of carboxypeptidase C_{Ua} was determined. Figure 6 shows the extended amino acid sequences around the reactive serine residues of typical serine proteases including carboxypeptidase C_{Ua}.

The sequence of eight amino acid residues around the serine residue of carboxypeptidase C_{Ua} is similar to those of trypsin,[16] chymotrypsin,[17] elastase,[18] and thrombin.[19] Besides the serine residue, they have an aspartic acid residue and a histidine residue in the catalytic site to form the proton relay system, as proposed by Kraut.[20] The overall sequences around the reactive serine residues of the typical serine proteases listed, however, show only about 30% homology to that of carboxypeptidase C_{Ua}.

These structural differences occur in the regions of carboxypeptidase C_{Ua} which are assumed to participate in substrate binding, suggesting that carboxypeptidase C_{Ua} differs considerably in its interaction with substrates from chymotrypsin, trypsin, and related enzymes. This is supported by the fact that TPCK and L-(1-tosylamide-2-lysyl)ethyl chlo-

CPase C_{Ua}	— — M D V A G Y D S E — — W I Q L R — V P C E G D S* G G E L
	180 (M), 190 (S)
CA bovine	K I K D A M I C A G — — — A S G V — — S S C M G D S* G G P L
CB bovine	R V T D V M I C A G — — — A S G V — — S S C M G D S* G G P L
	170 (G), 180 (Q)
Tr bovine	Q I T S N M F C A G Y — — L E G G K — D S C Q G D S* G G P V
Tr porcine	Q I T G N M I C V G F — — L E G G K — D S C Q G D S* G G P V
Tr dogfish	M I T N N M M C B G Y — — M E G G K — D S C Q G D S* G G P V
Tr St.	— V A N E E I C A G Y — P D T G G V — D T C Q G D S* G G P M
E porcine	K I K D A M V C A G — — — G N G V R — S G C Q G D S* G G P L
Th bovine	R I T B B M F C A G Y K P G E G K R G D A C E G D S* G G P F

CPase C_{Ua}	D — — — K E G — — — M — A P N G I V S D A L F — T S R
	200 (V), 210 (V), 220 (C)
CA bovine	V C — K K N G — — — A W T L V G I V S W G S S — T C S — T S
CB bovine	V C — Q K N G — — — A W T L A G I V S W G S S — T C S — T S
	190 (S), 200 (G)
Tr bovine	V C — — — S G — — — K — — L Q G I V S W G S — — G C A Q K N
Tr porcine	V C — — — N G — — — Q — — L Q G I V S W G Y — — G C A Q K N
Tr dogfish	V C — — — N G — — — M — — L Q G I V S W G Y — — G C A E R D
Tr St.	P R — K D N A — — D E W I Q V G I V S W G Y — — G C A R P G
E porcine	H C — L V N G — — — Q Y A V H G V T S F V S R L G C N V T R
Th bovine	V M — — K S P Y N N R W Y Q M G I V S W G E — — G C D R N G

Fig. 6. Comaprison of the Sequence around the Reactive Serine Residue of Carboxypeptidase C_{Ua} with Those of Typical Serine Proteases

The sequences of the serine proteases, other than that of carboxypeptidase C_{Ua}, were compiled by de Haen *et al.*[21] Residues are numbered according to the chymotrypsin numbering system. Deletions (—) were inserted to obtain maximum homology. S*, reactive serine; CPase C_{Ua}, carboxypeptidase C_{Ua}; CA bovine, bovine chymotrypsin A; CB bovine, bovine chymotrypsin B; Tr bovine, bovine tyrpsin; Tr porcine, porcine trypsin; Tr dogfish, dogfish trypsin; Tr St., *St. griseus* trypsin; E porcine, porcine elastase; Th bovine, bovine thrombin.

romethyl ketone, specific inhibitors of chymotrypsin and trypsin, respectively, as well as soybean trypsin inhibitor, exert no influence on the activity of carboxypeptidase C_{Ua}, though direct proof of the interaction between the enzyme and substrates remains to be obtained.

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