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The Amino Acid Sequence around Four Cysteine Residues in Trout Actin

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Four unique carboxymethylcysteine-containing peptides were isolated from tryptic and chymotryptic digests of trout muscle actin carboxymethylated with iodo[2-^{14}C]acetic acid in 6M-guanidinium chloride. The amino acid sequences of these peptides were determined and showed a high degree of homology with the corresponding sequences from rabbit actin. One of the radioactive peptides was the *C*-terminal peptide and another sequence probably contained the cysteine residue from the *N*-terminal region of the protein.

Of the five cysteine residues present in rabbit skeletal actin (Adelstein & Kuehl, 1970; Elzinga, 1970) three appear to be on the surface of the molecule (Lusty & Fasold, 1969). One of these surface cysteine residues appears to be in the vicinity of the myosin-binding site, whereas another may be close to the site for actin polymerization (Lusty & Fasold, 1969). Johnson & Perry (1968) have reported the amino acid sequence of two tryptic cysteinyl peptides from rabbit actin, together with the amino acid composition of a third, possibly derived from the *N*-terminus, but could find no other cysteinyl sequences.

The role of cysteine residues in actin activity is uncertain. Studies with various thiol reagents have suggested that they may be involved in the polymerization of G-actin (Kuschinsky & Turba, 1951; Drabikowski & Gergely, 1963; Kuehl & Gergely, 1969), nucleotide binding (Strohman & Samorodin, 1962; Katz, 1963; Kuehl & Gergely, 1969) and its combination with myosin (Perry & Cotterill, 1964; Bailin & Barany, 1967). However, the work of Lusty & Fasold (1969) indicates that their role may be structural, rather than participation in the polymerization reaction.

The present work was undertaken to try to obtain further information on the number, location and function of thiol groups in actin.

MATERIALS AND METHODS

Reagents and enzymes. ATP (disodium salt), 2-mercaptoethanol, iodoacetic acid, dithiothreitol, trypsin (twice recrystallized), chymotrypsin (thrice recrystallized), carboxypeptidases A- and B-DFP (enzymes treated with di-isopropyl phosphorofluoridate, dialysed and recrystallized), subtilopeptidase A, and DCC-trypsin (trypsin treated with diphenylcarbamoyl chloride to minimize chymotryptic activity) were all purchased from Sigma (London) Chemical Co., London S.W.6, U.K. Solutions of iodoacetic acid were dried *in vacuo* before use to remove free iodine. Thermolysin was a gift from Dr R. P. Ambler. Puriss-grade phenyl isothiocyanate (redistilled before use and stored at −20°C), anhydrous trifluoroacetic acid and *N*-ethylmorpholine (redistilled before use) were obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. Iodo[2-^{14}C]acetic acid was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. and diluted with carrier iodoacetic acid to a specific radioactivity of 0.2mCi/mmol before use. Guanidinium chloride was obtained from British Drug Houses Ltd., Poole, Dorset, U.K. in a grade specially purified for biochemical work. AnalaR-grade pyridine was redistilled from ninhydrin (1g/l) before use. All other chemicals were of analytical grade.

Trout. Brown trout were obtained from the Welham Park Fish Hatchery, Malton, Yorks., U.K.

Protein. Trout actin was prepared by the method of Seraydarian, Briskey & Mommaerts (1967) with the inclusion of a gel-filtration step on Sephadex G-200 after the final depolymerization (Adelstein, Godfrey & Kielley, 1963). Protein was measured by using the biuret reagent of Gornall, Bardawill & David (1949), standardized against dried trout actin samples. A molecular weight of 45000 for the native protein was assumed throughout (Johnson & Perry, 1968).

Reduction and carboxymethylation of protein thiol groups. Trout actin (5μmol) was dissolved in 6M-guanidinium chloride (50ml) containing 0.1% (v/v) methylamine, pH 8.0. Dithiothreitol (10μmol) was then added, and the solution kept under N_2 at 25°C. After 4h, iodo[2-^{14}C]acetic acid (25μmol), neutralized with NaOH, was added and the solution left in the dark, under N_2, at 25°C, pH 8.0, for 30min. The reagents were then separated from the carboxymethylated protein by gel filtration through a

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column (60cm×2.5cm) of Sephadex G-25 eluted with 0.1M-NH_4HCO_3 containing 0.2% (v/v) thiodiglycol.

Radioactivity measurements. Protein samples (5μl) were removed during the carboxymethylation procedure and the protein was precipitated on to glass-fibre discs with 15% (w/v) trichloroacetic acid. Excesses of reagents were removed by extensive washing with ether–ethanol (1:1, v/v), and the discs then air-dried at 45°C. Each disc was placed in a vial and toluene (5ml), containing 2,5-diphenyloxazole (0.4%, w/v), was added. The radioactivities of samples were counted in a Nuclear–Chicago Unilux III scintillation counter. Counting efficiency of an iodo[2-^{14}C]acetate standard in this system was 70%. For monitoring column effluents, samples (100μl) of each fraction were transferred to glass-fibre discs and their radioactivities were counted in the same manner.

Amino acid analysis. Samples of protein and peptides were hydrolysed in sealed evacuated tubes for 24h at 110°C with 6.7M-HCl. The HCl was removed *in vacuo* over fresh NaOH pellets and the amino acids were analysed with a Technicon AutoAnalyzer or a Beckman Unichrom analyser. Protein serine and threonine values were corrected for hydrolytic losses of 6% and 4% respectively. Tryptophan was determined by the spectrophotometric technique of Edelhoch (1967).

Digestion with trypsin and chymotrypsin. Carboxymethylated protein (5mg/ml) in 0.1M-NH_4HCO_3, pH8.0, was digested with trypsin or chymotrypsin (0.1mg/ml) at 25°C. After 3h a second addition of enzyme was made to give a final concentration of 0.2mg/ml. Total digestion time was 6h, reaction being stopped by addition of acetic acid to pH4.0, and the mixture was then freeze-dried.

Separation of peptides. Preparation of peptide 'maps' was as described by Ambler (1963). Paper chromatography was performed in the descending system (butan-1-ol–acetic acid–water–pyridine, 15:3:12:10, by vol.) described by Waley & Watson (1953). Peptides were detected on paper by the ninhydrin–cadmium reagent of Heilmann, Barrollier & Watzke (1957) or the chlorination method of Rydon & Smith (1952). Radioactive peptides were detected by cutting the positively stained spots from the paper and counting them for radioactivity as described above.

For preparative separation of radioactive peptides, 250mg of a tryptic or chymotryptic digest of *S*-carboxymethylated actin dissolved in 0.5M-acetic acid (25ml) was applied to a column (150cm×2.5cm) of Bio-Gel P-4. The column was developed at 12ml/h with 0.5M-acetic acid and fractions (8ml) were collected. Further purification was achieved on a column (25cm×0.6cm) of Technicon Chromobeads A sulphonated polystyrene resin. The peptides were eluted at 50°C with buffer from a three-chamber linear-gradient maker, the chambers of which contained 60ml of: 0.05M-pyridine acetate buffer, pH2.5; 0.5M-pyridine acetate buffer, pH3.75; 2M-pyridine acetate buffer, pH5.0. Fractions (4ml) were collected at a flow-rate of 20ml/h. Small columns of Sephadex G-10 (18cm×0.8cm) and Sephadex G-15 (30cm×1cm), both eluted with 0.5M-acetic acid at 10ml/h, were also used for peptide purification. To minimize decomposition of carboxymethylcysteine, all buffers contained 0.2% (v/v) thiodiglycol (Li & Vallee, 1964).

Enzymic digestion of peptides. To the peptide (1μmol) in 1% (w/v) NH_4HCO_3, pH8.0 (1.0ml), was added trypsin or subtilisin (0.01%, w/w). After incubation at 37°C for 4h the solution was acidified with acetic acid to pH4.5 and the mixture was freeze-dried. Peptides were digested with thermolysin as described by Ambler & Meadway (1968).

End-group determination. The *N*-terminal residues of both the carboxymethylated protein and the peptides were determined by the 'dansyl' technique (Gray, 1967), except that the DNS-peptides were hydrolysed for only 4h (Gros & Labouesse, 1969). The *N*-terminal residues of thermolytic peptides were hydrolysed for 18h after dansylation to ensure complete hydrolysis. DNS-amino acids were identified on polyamide layers as described by Woods & Wang (1967) by using the ethyl acetate–methanol–acetic acid (20:1:1, by vol.) solvent of Crawshaw, Jessup & Ramwell (1967) as an additional second-dimension solvent.

Carboxypeptidase digestions were performed as described by Ambler (1967) with identification of the released amino acids as their DNS derivatives.

Sequence determination. Dansyl–Edman degradations were performed as described by Gray (1967) with identification of the DNS-amino acids on polyamide layers as described above. The sequence of peptide T-1-Th-1 was determined by mass spectroscopy of the acetylated permethylated derivative (Vilkas & Lederer, 1968) with an AE1 MS-902 mass spectrometer. Amide groups were assigned according to the electrophoretic mobility of the peptide at pH6.5 (Offord, 1966), the mobility of aspartic acid being defined as −1.00.

RESULTS AND DISCUSSION

The amino acid composition of carboxymethylated trout actin is given in Table 1. The values are expressed as residues per molecule of mol.wt. 45000 (Johnson & Perry, 1968). A comparison with the results of Adelstein & Kuehl (1970) for rabbit actin shows significant differences only in the methionine and tryptophan content. No free *N*-terminal amino group was found for trout actin but carboxypeptidase A released phenylalanine from the *C*-terminus. Incorporation of radioactivity into the protein from iodo[2-^{14}C]acetate was halted after 30min (Fig. 1) and was equivalent to the labelling of 4.3 cysteine residues per molecule of protein.

Tryptic peptides

Digestion with trypsin was incomplete after 6h, only 26–27 spots being found on peptide maps instead of the 46–47 spots expected from the lysine+arginine content. Three of these spots, including a ninhydrin-negative spot, were strongly radioactive (Figs. 2*a* and 2*b*). Partial solution of the insoluble core-material was obtained by treatment with DCC-trypsin [18h, 25°C, enzyme/substrate ratio 1:25 (w/w)], but no new radioactive peptides were produced. To characterize them further, the radioactive carboxymethylcysteine-containing pep-

Table 1. *Amino acid content of carboxymethylated actin*

The present results are the means of analyses of three different actin preparations.

Amino acid	Present work Trout actin (mol/45000g)	Adelstein & Kuehl (1970) Rabbit actin* (mol/45000g)
CMCys	5.2	5.3
Asp	38.2	38.6
Thr	30.5	27.9
Ser	26.3	23.3
Glu	44.1	44.5
Pro	22.7	20.9
Gly	30.4	31.5
Ala	32.5	32.3
Val	23.3	21.8
Met	10.2	16.9
Ile	27.2	28.6
Leu	31.9	29.2
Tyr	16.5	17.0
Phe	13.3	13.7
Lys	24.6	21.0
His	10.0	8.1
3-MethylHis	~1.0	0.97
Arg	21.9	20.7
Trp	3.2	4.3

* Not corrected for hydrolytic losses.

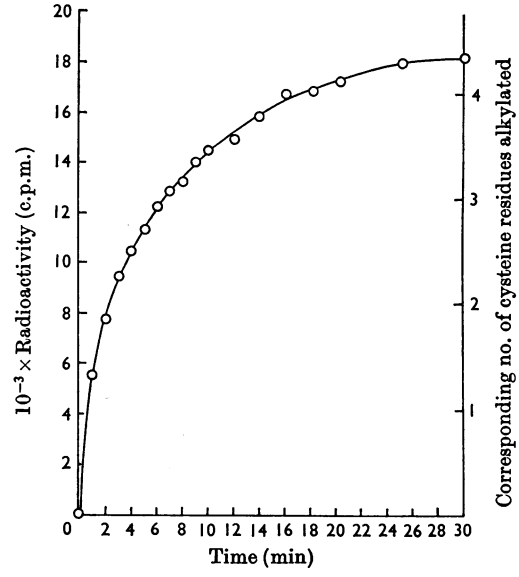

Fig. 1. Course of incorporation of radioactivity into denatured trout actin. Denatured, reduced actin (250mg) was incubated with iodo[2-^{14}C]acetate (0.1mCi, pH8.0) for 30min. Samples (5μl) were withdrawn and counted for radioactivity as described in the Materials and Methods section.

tides were isolated and analysed. The tryptic digest was first fractionated by gel filtration on Bio-Gel P-4 in 0.5M-acetic acid, which gave almost all the radioactivity in three peaks (Fig. 3). Electrophoresis indicated that these corresponded to three radioactive peptides, T-1, T-2 and T-3. The purification procedures used for these peptides and their electrophoretic mobilities are shown in Table 2. The amino acid compositions of the purified peptides are given in Table 3. Peptides T-1, T-2 and T-3 were homogeneous on paper chromatography and electrophoresis at pH3.5 and 6.5, and their properties in the systems used allowed the assignments shown in Fig. 2. Of the total radioactivity incorporated into the protein from iodo[2-^{14}C]-acetate, 72% was applied to the column, the remainder having been precipitated from the tryptic digest. Of this 72%, 90% was recovered from the column in terms of the ratio of counts applied to counts recovered.

Peptide T-1. No free α-amino acid group could be detected in this peptide, and advantage was taken of its virtual lack of retardation on the ion-exchange column to effect its purification. Carboxypeptidase B released lysine from the purified peptide. Digestion of peptide T-1 (1.5μ mol) with thermolysin gave six fragments, which were partially separated by ion-exchange chromatography (Fig. 4). Fractions 7–9, 20–23 and 26–29 corresponded to the pure peptides, T-1-Th-4, T-1-Th-5 and T-1-Th-6. Fractions 1–6 were rechromatographed on Sephadex G-15, which separated out the radioactive peptide, T-1-Th-2. The material more retarded on Sephadex G-15 was passed through Sephadex G-10 to separate the remaining two fragments T-1-Th-1 and T-1-Th-3. The amino acid content of the two major fragments, T-1-Th-2 and T-1-Th-4, is given in Table 4. The sequence of the ninhydrin-negative peptide, T-1-Th-1, was determined by mass spectrometry and the peptide was found to have an *N*-terminal pyrrolidonecarboxylic acid residue. The major sequence ions derived from the permethylated peptide are shown in Table 5. The complete sequences of all the thermolytic fragments of peptide T-1 are shown in Table 6. The electrophoretic mobility of peptide T-1-Th-2 at pH6.5 (−0.66) indicated one amide group to be present. An attempt to assign this amide group to a particular aspartic acid residue by digestion of peptide T-1-Th-2 (100nmol) with subtilisin failed, as none of the fragments obtained had a unique aspartate or asparagine residue.

Peptide T-2. Ion-exchange chromatography failed to give a complete separation of this peptide

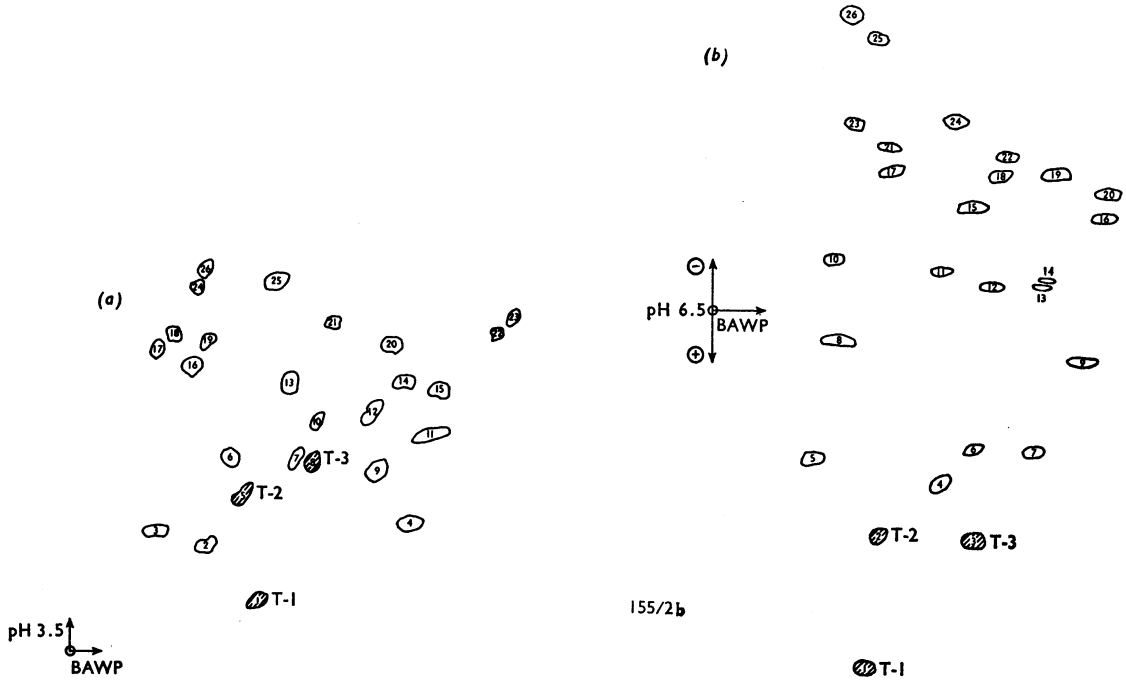

Fig. 2. Peptide 'maps' of tryptic digests of carboxymethylated actin. The open spots represent peptides present in the tryptic digests; shaded spots were radioactive carboxymethylcysteine-containing peptides. On both 'maps' spot no. 1 was ninhydrin-negative. After application of approx. 1 mg of peptide material at 'O', descending chromatography was performed with butan-1-ol–acetic acid–water–pyridine (15:1:12:10, by vol.) along the axis marked BAWP. Electrophoresis was then performed at right angles (*a*) in pH 3.5 buffer [1% (v/v) pyridine–10% (v/v) acetic acid] at 3 kV or (*b*) in pH 6.5 buffer [10% (v/v) pyridine–0.3% (v/v) acetic acid] at 2 kV.

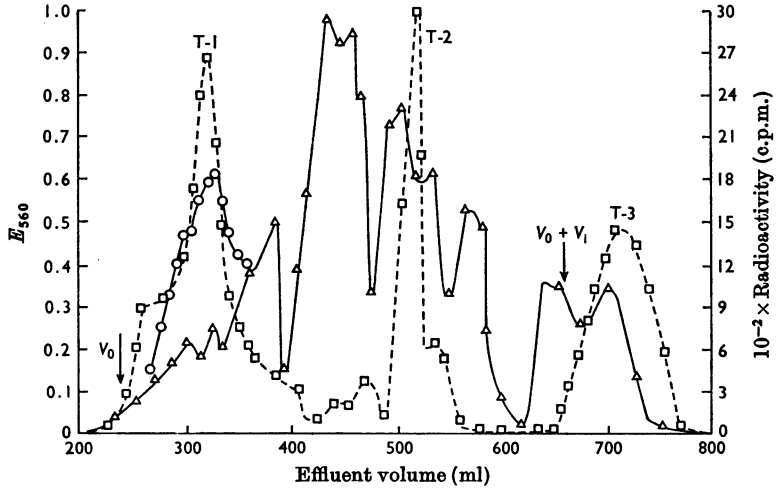

Fig. 3. Profile of the tryptic peptides of [^{14}C]carboxymethylated actin on Bio-Gel P-4 eluted with 0.5 M-acetic acid. □, Radioactivity (c.p.m.) in samples; △, E_{560} after reaction between ninhydrin and unhydrolysed peptide; ○, E_{560} after reaction between ninhydrin and hydrolysed peptide.

from a non-radioactive component. Purification was achieved by rechromatography under the same conditions, by using 150ml of each buffer. Electrophoretic mobility at pH6.5 (−0.59) coupled with five cycles of dansyl–Edman degradation indicated the sequence of peptide T-2 to be:

CMCys*-Asp-Ile-Asp-Ile-Arg

Peptide T-3. This peptide was abnormally retarded on the Bio-Gel P-4 column (Fig. 3) and emerged at a volume of approx. 100ml. Electrophoresis at pH2.1 and 6.5 indicated that the latter portion of this volume contained peptide T-3 in a pure form and this was confirmed by amino acid analysis (Table 3). One cycle of dansyl–Edman degradation established the sequence of peptide T-3 as:

CMCys-Phe

The complete amino acid sequences of two of the tryptic cysteinyl peptides, T-2 and T-3, and the partially ordered fragments of the third peptide, T-1, are shown in Table 7. The amino acid content of peptide T-1, and the amino acid sequences of peptides T-2 and T-3, are identical with those reported by Johnson & Perry (1968) for rabbit actin. Johnson & Perry (1968) suggested that peptide T-1 might be derived from the *N*-terminus. However, the *N*-terminal sequence of rabbit actin has been reported by Alving & Laki (1966) and Gaetjens & Barany (1966) to be:

N-AcetylAsp-Glu-Thr...

with possibly alanine as the next amino acid. As no free α-amino group was detected here it is possible that the *N*-terminus of trout actin was also acetylated and that deacetylation occurred during the purification procedures. This seems unlikely with the mild conditions employed. It is also possible that pyrrolidonecarboxylic acid is *N*-terminal in trout actin, but homology with the corresponding sequence from rabbit actin (M. Elzinga, personal communication) is against this.

Chymotryptic peptides

Digestion with chymotrypsin was far more complete than with trypsin. Seven radioactive peaks were obtained from the Bio-Gel column (Fig. 5), two of which, C-1 and C-2, corresponded to radioactive ninhydrin-negative peptides. Electrophoresis indicated that each radioactive peak contained only one radioactive peptide, except peak C-7, which contained four weakly radioactive peptides in low yield. The purification procedures, electrophoretic mobilities and yields of these peptides are shown in Table 8.

Peptides C-1 and C-2. After further purification by ion-exchange chromatography, peptides C-1 and C-2 were separated on a column (52cm×1.2cm) of Sephadex G-25 eluted with 0.5M-acetic acid. Peptide C-1 was recovered in very low yield (25nmol). Carboxypeptidase A released leucine from peptide C-2 and amino acid analysis of this peptide showed: carboxymethylcysteine, 0.6; aspartic acid, 3.6; threonine, 1.9; serine, 1.1; glutamic acid, 2.1; glycine, 2.0; alanine, 1.2; valine, 0.8; leucine, 1.8 (molar ratios expressed relative to glycine). No free α-amino group could be detected. From this evidence it is possible that peptide C-2 is the chymotryptic equivalent of the putative *N*-terminal tryptic peptide T-1, with chymotrypsin splitting a leucine–valine bond, since its composition is that of peptide T-1, but for its lack of one valine residue and one lysine residue.

* Abbreviation: CMCys (in sequences), carboxymethylcysteine.

Table 2. *Purification procedures, electrophoretic mobilities and overall yields of the tryptic carboxymethylcysteinyl peptides*

For experimental details see the text.

Peptide	Purification procedure after gel filtration	Yield (%)	Mobility (pH6.5)
T-1	Ion-exchange chromatography	70–80	−0.81
T-2	Two cycles, ion-exchange chromatography	40	−0.59
T-3	None	60	−0.60

Table 3. *Amino acid analyses of the acid hydrolysates of peptides T-1, T-2 and T-3*

Results are expressed as molar ratios relative to valine for peptide T-1, isoleucine for peptide T-2, and phenylalanine for peptide T-3.

Amino acid	No. of residues		
	Peptide T-1	Peptide T-2	Peptide T-3
CMCys	0.9	1.0	0.9
Asp	4.2	2.0	—
Thr	2.1	—	—
Ser	2.2	—	—
Glu	2.2	—	—
Gly	2.2	—	—
Ala	0.8	—	—
Val	2.0	—	—
Ile	—	2.0	—
Leu	2.1	—	—
Phe	—	—	1.0
Lys	0.8	—	—
Arg	—	0.9	—

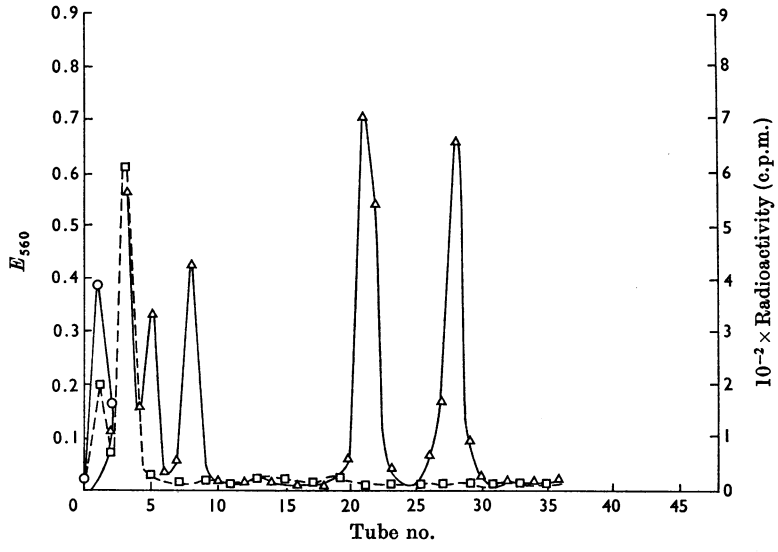

Fig. 4. Profile of the fragments from a thermolysin digest of tryptic peptide T-1 on a sulphonated polystyrene resin column eluted with a pyridine acetate gradient. Fraction size, 4 ml. □, Radioactivity (c.p.m.) in samples; △, E_{560} after reaction between ninhydrin and unhydrolysed peptide; ○, E_{560} after reaction between ninhydrin and hydrolysed peptide.

Table 4. *Amino acid analyses of the two major thermolytic fragments from the tryptic cysteinyl peptide T-1*

Results are expressed as molar ratios relative to leucine for peptide T-1-Th-2 and relative to lysine for peptide T-1-Th-4.

	No. of residues	
Amino acid	Peptide T-1-Th-2	Peptide T-1-Th-4
CMCys	1.1	—
Asp	1.9	—
Ser	1.2	—
Gly	2.3	—
Val	0.9	1.1
Leu	1.0	0.9
Lys	—	1.0

Peptide C-3. This peptide was purified by ion-exchange chromatography followed by removal of a final contaminant on Sephadex G-15. Amino acid analysis of peptide C-3 showed: carboxymethylcysteine, 0.8; aspartic acid, 1.1; glutamic acid, 1.1; proline, 1.2; serine, 0.8; glycine, 1.0; alanine, 1.0; valine, 0.7; leucine, 0.8; phenylalanine, 0.9; histidine, 1.1; lysine, 0.7; arginine, 0.9 (molar ratios expressed relative to glycine). Carboxypeptidase A released phenylalanine and traces of carboxymethylcysteine; dansyl–Edman degrada-

Table 5. *Sequence ions found for the acetylated permethylated thermolytic peptide T-1-Th-1*

The source temperature was 275°C. pGlu, pyrrolidone carboxylic acid.

m/e value	Sequence
126	pGlu
255	pGlu-Thr
384	pGlu-Thr-Thr
469	pGlu-Thr-Thr-Ala
596	pGlu-Thr-Thr-Ala-Leu

Table 6. *Amino acid sequences of thermolytic fragments of the tryptic cysteinyl peptide T-1*

The symbol (→) denotes a residue established by dansyl–Edman degradation; the symbol (←) denotes a residue identified by carboxypeptidase digestion. The sequence of peptide T-1-Th-1 was determined by mass spectrometry. pGlu, pyrrolidonecarboxylic acid.

Peptide	Amino acid sequence
T-1-Th-1	pGlu-Thr-Thr-Ala-Leu
T-1-Th-2	Leu-Val-CMCys-Asx-Asx-Gly-Gly-Ser
T-1-Th-3	Asp-Asp-Glu
T-1-Th-4	Thr-Ala
T-1-Th-5	Ala-Leu-Val
T-1-Th-6	Leu-Val-Lys

tion revealed the *N*-terminal sequence of peptide C-3 as:

Asx-Glx-Ala-Gly...

Digestion of peptide C-3 (1μmol) with trypsin and chromatography of the digest on Sephadex G-10 gave two major peptides, C-3-T-1 and C-3-T-2. Amino acid analysis of peptide C-3-T-2 gave: carboxymethylcysteine, 0.8; phenylalanine, 1.0 (molar ratios expressed relative to phenylalanine). Dansylation showed that the *N*-terminus of peptide C-3-T-2 was carboxymethylcysteine which, coupled with the carboxypeptidase evidence above, gave the sequence of this peptide as CMCys-Phe. Peptide C-3-T-1 (0.7μmol) was further digested with thermolysin to give two peptides, which were separated by ion-exchange chromatography. Dansyl–Edman degradation of the first of these, C-3-T-1-Th-1, established the sequence:

Asx-Glx-Ala-Gly-Pro-Ser

The electrophoretic mobility of this peptide at pH 6.5 (−0.68) corresponded to two free side-chain

Table 7. *Amino acid sequences of three tryptic cysteinyl peptides from trout actin*

pGlu, pyrrolidonecarboxylic acid.

Peptide	Amino acid sequence
T-1*	Asp-Asp-Glu
	pGlu-Thr-Thr-Ala-Leu-Val-Cys-Asx-Asx-Gly-Gly-Ser
	Leu-Val-Lys
T-2	Cys-Asp-Ile-Asp-Ile-Arg
T-3	Cys-Phe

* Unordered fragments.

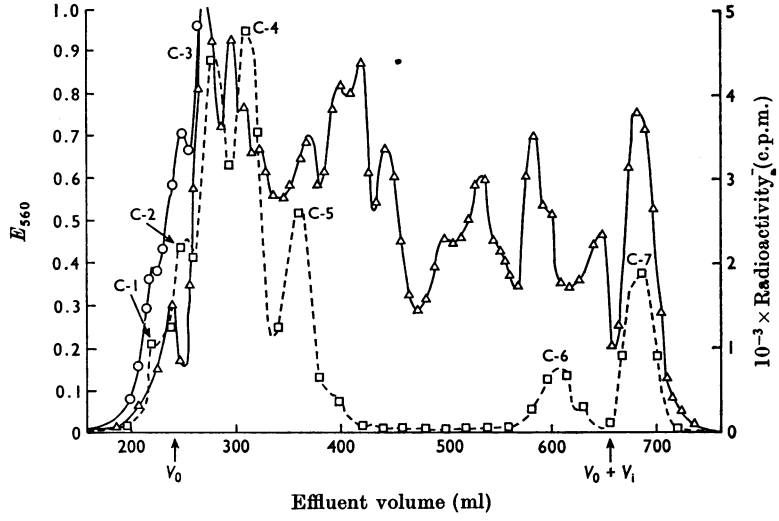

Fig. 5. Elution profile of the chymotryptic peptides of [^{14}C]carboxymethylated actin on Bio-Gel P-4. □, Radioactivity (c.p.m.) in samples; △, E_{560} after reaction between ninhydrin and unhydrolysed peptide; ○, E_{560} after reaction between ninhydrin and hydrolysed peptide.

Table 8. *Purification procedures, yields and electrophoretic mobilities of the chymotryptic carboxymethylcysteinyl peptides*

For experimental details see the text.

Peptide	Effluent volume from Bio-Gel P-4 (ml)	Purification procedure	Yield (%)	Electrophoretic mobility (pH 6.5)
C-1	208–260	Ion-exchange, Sephadex G-25	0.5	−0.51
C-2	208–260	Ion-exchange, Sephadex G-25	4	−1.01
C-3	260–292	Ion-exchange, Sephadex G-15	30	−0.15
C-4	292–334	Ion-exchange, Sephadex G-15, ion-exchange	10	0.00
C-5	334–380	Ion-exchange	16	0.00
C-6	580–620	Ion-exchange	6	+0.41

carboxyl groups. By a similar technique the sequence of the second thermolytic fragment was found to be:

Leu-Val-His-Arg-Lys

Thus the complete sequence of peptide C-3 is:

Asp-Glu-Ala-Gly-Pro-Ser-Leu-Val-His-Arg-Lys-CMCys-Phe

Table 9. *Amino acid sequences of three chymotryptic cysteinyl peptides from trout actin*

Peptide	Amino acid sequence
C-3	Asp-Glu-Ala-Gly-Pro-Ser-Leu-Val-His-Arg-Lys-Cys-Phe
C-4	Lys-Lys-Cys-Asp-Ile-Asp-Ile-Arg-Asp-Leu
C-5	Arg-Cys-Pro-Thr-Ser-Leu-Phe

This peptide appears to be the chymotryptic *C*-terminal peptide, as its *C*-terminal sequence is identical with that of the *C*-terminal tryptic peptide T-3. Also, there is close homology between this peptide and the *C*-terminal peptide from rabbit actin (Elzinga, 1969; see Table 10). No tryptic cleavage of the arginine–lysine bond in this peptide was observed.

Peptide C-4. Ion-exchange chromatography followed by gel filtration on Sephadex G-15 did not completely separate peptide C-4 from non-radioactive material. Purification was achieved by passage through Technicon resin by using a four-chamber buffer gradient. The first chamber contained 0.025 M-pyridine acetate buffer, pH 3.98 (50ml), the second contained 0.05 M-pyridine acetate buffer, pH 3.98 (50ml), the third contained 0.1 M-

Table 10. *Amino acid sequences around four cysteine residues in trout actin, including the* N*-terminal and* C*-terminal sequences*

A comparison with the results of other workers for rabbit actin is included. pGlu, pyrrolidonecarboxylic acid; Ac, acetyl.

Location of sequence	Species	Amino acid sequence
Probable N-terminus (unordered fragments)	Trout	Asp-Asp-Glu, pGlu-Thr-Thr-Ala-Leu-Val-Cys-Asx-Asx-Gly-Gly- ←——T-1——→
		Ser, Leu-Val-Lys ——→
N-Terminus	Rabbit*	Ac-Asp-Glu-Thr
	Trout	Lys-Lys-Cys-Asp-Ile-Asp-Ile-Arg-Asp-Leu ←——C-4——→
		←——T-2——→
	Rabbit†	Cys-Asp-Ile-Asp-Ile-Arg
	Trout	Arg-Cys-Pro-Thr-Ser-Leu-Phe ←——C-5——→
C-Terminus	Trout	Asp-Glu-Ala-Gly-Pro-Ser-Leu-Val-His-Arg-Lys-Cys-Phe ←——C-3——→
		←T-3→
C-Terminus	Rabbit‡	Asp-Glu-Ala-Gly-Pro-Ser-Leu-Val-His-Arg-Lys-Cys-Phe

* Alving & Laki (1966); Gaetjens & Barany (1966).
† Johnson & Perry (1968).
‡ Elzinga (1969).

pyridine acetate buffer, pH 3.98 (50 ml), and the fourth contained 1.0 M-pyridine acetate buffer, pH 6.12 (50 ml). Amino acid analysis of peptide C-4 showed carboxymethylcysteine, 0.9; aspartic acid, 3.2; isoleucine, 1.9; leucine, 1.0; lysine, 1.8; arginine, 0.9 (molar ratios expressed relative to leucine). Dansyl–Edman degradation of this peptide revealed the partial sequence:

Lys-Lys-CMCys-Asx-Ile-Asx

Carboxypeptidase A released leucine, followed much more slowly by aspartic acid or asparagine. Electrophoretic mobility at pH 6.5 (−0.22) indicated that no amides were present. By comparison with the sequence of tryptic peptide T-2 the sequence of peptide C-4 may be written as:

Lys-Lys-CMCys-Asp-Ile-Asp-Ile-Arg-Asp-Leu

Peptide C-5. This peptide was separated from non-radioactive ninhydrin-positive material by ion-exchange chromatography. A yellow oily contaminant was extracted with methanol, and peptide C-5 was then further purified by passage through Sephadex G-15. Amino acid analysis of the purified peptide showed: carboxymethylcysteine, 0.7; threonine, 0.8; serine, 0.7; proline, 0.9; leucine, 1.0; phenylalanine, 1.1; arginine, 1.2 (molar ratios expressed relative to leucine). Carboxypeptidase A released phenylalanine and leucine from the *C*-terminus, and the complete sequence of this peptide was revealed by dansyl–Edman degradation as:

Arg-CMCys-Pro-Thr-Ser-Leu-Phe

Peptide C-6. This peptide was recovered in relatively low yield (48%) after purification on Chromobeads A. Results from carboxypeptidase A digestion were unclear, but dansyl–Edman degradation gave a partial sequence for peptide C-6:

Arg-Lys-CMCys...

The electrophoretic mobility of peptide C-6 at pH 6.5 (+0.35) corresponded to a singly charged tetrapeptide and it appeared that peptide C-6 was a minor *C*-terminal fragment with sequence:

Arg-Lys-CMCys-Phe

The complete amino acid sequences of three chymotryptic cysteinyl peptides from trout actin are shown in Table 9. Peptide C-5 was the only chymotryptic cysteinyl peptide for which no tryptic counterpart could be found, and this hydrophobic peptide probably formed part of the insoluble core-material not digested by trypsin. It is possible that the fifth cysteinyl sequence was lost in minor peptides such as those found in peak C-7 on the Bio-Gel chromatogram (Fig. 5). None of these minor peptides was in sufficient yield to permit any sequence information to be obtained. No evidence was found for the presence of any undigested chymotryptic core-material, either on 'fingerprints' or by centrifugation of the digest.

A comparison of the cysteinyl sequences presented here and those published previously from rabbit actin is given in Table 10. There is considerable conservation of sequence around the cysteine residues, the principal difference being at the *N*-terminal region of the protein.

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