C-TERMINAL GROUPS IN MYOSIN, TROPOMYOSIN AND ACTIN

by

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The intense study which has been made during the last decade of the properties and interactions of the structural proteins of muscle has made desirable a greater knowledge of their molecular structure. A first step in the chemical approach must be the determination of the number of peptide chains in each molecule. BAILEY[1] recently carried out an extensive search for N-terminal groups in myosin and tropomyosin, using the fluorodinitrobenzene (FDNB) method of SANGER[2], with negative results. Whether these surprising results which have also been obtained for other proteins, are indeed due to an absence of N-terminal groups, or whether they are due to limitations of the method cannot yet be decided.

It was thought that an investigation of C-terminal groups in the muscle proteins would be of interest. Two different methods have been used in the present work. The carboxypeptidase method first used by LENS[3] for insulin has since proved its value for a number of proteins. More recently AKABORI, OHNO AND NARITA[4] have introduced a method involving a digestion of the protein with anhydrous hydrazine, leading to the liberation of only C-terminal residues as free amino acids. A combination of these methods has given concordant results and has served to overcome the uncertainties inherent in each. It has been shown that myosin, tropomyosin and actin all contain C-terminal groups in spite of the apparent lack of N-terminal groups. In the case of tropomyosin and actin, it has been possible to deduce the C-terminal sequence from the carboxypeptidase results.

An application of SANGER's N-terminal method to purified actin has yielded negative results in agreement with unpublished results of BAILEY and in line with his results for myosin and tropomyosin.

The values for the molecular weight and axial ratio of tropomyosin, together with its α-type X-ray pattern, indicate a double chain structure. An attempt has therefore been made at oxidative fission of the molecule with performic acid, but although oxidation of disulphide links was achieved, no fission occurred.

EXPERIMENTAL

Myosin was prepared by the method of TSAO[5], the 39–50% ammonium sulphate fraction being used.

Tropomyosin was prepared by the procedure of BAILEY[6] as far as the first ammonium sulphate

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References p. 542.

fractionation and dialysis. The protein was then precipitated with alcohol in the presence of 0.1 *M* KCl and dried down in ethanol and acetone. A second ammonium sulphate fractionation as described by BAILEY was then carried out, followed by the same drying-down procedure.

Actin was prepared according to TSAO AND BAILEY[7]. Electrophoresis of the product showed the presence of a small amount of tropomyosin, which was removed by the acetone fractionation procedure of TSAO[8].

Two-dimensional paper chromatography was carried out using phenol-ammonia and the butanol-acetic acid solvent of PARTRIDGE[9]. For resolution of the pairs leucine–*iso*leucine and valine–methionine which are not resolved by the above solvents, tert-amyl alcohol-water has been used.

Carboxypeptidase experiments

In all experiments a pure sample of crystalline carboxypeptidase was used, in the form of a suspension containing 2.4 mg/ml of protein. The addition of di-*iso*propylfluorophosphonate (DFP) was found to be unnecessary.

Tropomyosin. Two distinct methods were evolved for examining the order of liberation of amino acids.

a. *Consecutive treatment method.* A solution of tropomyosin (37 mg) in 0.05 *M* KCl (3.2 ml) was adjusted to pH 8.0 and brought to 37°. Carboxypeptidase (0.01 ml) was added and reaction allowed to proceed for 15 minutes. Dowex-50 (100 mg, hydrogen form) was added and the whole shaken for 1 ½ hours at room temperature. The protein solution was sucked off from the resin beads, re-adjusted to pH 8.0, and retreated for 30 minutes with enzyme, as before. After repetition of the resin treatment the cycle was repeated with successive incubation times of 30, 60, and 60 minutes. The amino acids from each incubation were eluted from the washed resin by shaking with 5 *N* NH_4OH (0.7 ml). After evaporation the samples were run two-dimensionally on paper. The results are shown in Table I. Earlier experiments had shown that the first amino acid to be liberated was isoleucine, not leucine.

b. *Time curve method.* A solution of tropomyosin (15 ml, 4.76 mg/ml) containing toluene was incubated with enzyme (0.075 ml) at 37°. Aliquots (2 ml) were removed at intervals of ¼, ¾, 1¼, 2¼, 3¾ and 6 hours. Each was shaken with Zeocarb-225 (100 mg) for 1 hour, the resin was washed twice with water and eluted successively with 0.5 ml and 0.3 ml of 5 *N* NH_4OH. The combined eluates were evaporated in 5 ml beakers and applied to Whatman No. 1 paper together with standard spots of the amino acids found in the above experiment. The papers were run simultaneously in butanol-acetic acid for 2 nights, dried overnight at room temperature, sprayed with 0.5% ninhydrin in ethanol and allowed to stand in a dark room for 18 hours. After making a photostat copy of the chromatogram the spots were cut out, eluted with 50% ethanol and the volume of eluate made up to 5 ml. The optical density was measured in the Spekker absorptiometer using a Spectrum Yellow filter and 0.5 cm cells. All values were corrected for the paper blank (in subsequent experiments washed paper was used because of its lower blanks). Resin and enzyme blanks were negligible but a small amount of serine was found in a tropomyosin blank. Linear standard plots were obtained for the known amino acids, and were used to calculate the moles of amino acid liberated per unit weight (53,000) of protein. It should be noted that in the above technique for quantitative paper chromatography the colour yield from standards varied somewhat from one experiment to the next, probably due to the humidity effect recorded by WELLINGTON[10]. A set of standard spots was, therefore, always run and developed simultaneously with the unknowns in each experiment. Results are shown graphically in Fig. 1.

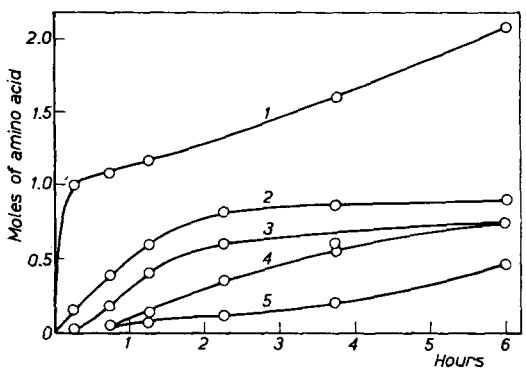

Fig. 1. The liberation of amino acids from tropomyosin by carboxypeptidase. Curve 1. *iso*leucine; 2. serine; 3. threonine; 4. methionine; 5. alanine.

In another experiment under the same conditions but using a different tropomyosin preparation, 2.5 moles of "*iso*leucine" were liberated in 9¾ hours and 4.6 mols in 22 hours. Chromatograms in tert-amyl alcohol showed that at 6 ½ hours *iso*leucine but no leucine was present, while at 9¾ hours a small amount of leucine had appeared. In another prolonged incubation considerable amounts of leucine appeared. Methionine but only traces of valine were present in all. A two-dimensional chromatogram of the 6 ½ hour sample showed strong *iso*leucine, serine, threonine, methionine and alanine spots, light aspartic acid, asparagine and lysine spots, and faint glutamic acid, arginine and tyrosine spots. A two-dimensional chromatogram of a 40-hour incubation at 25° in the presence of DFP showed strong

References p. 542.

"*iso*leucine", serine, threonine, "methionine", alanine, aspartic acid, lysine and asparagine spots, a light arginine spot and faint glutamic acid, glutamine, glycine and tyrosine spots.

Myosin. A solution of myosin in 0.3 *M* KCl was adjusted to pH 8.0. A sample of this solution (34 ml, 21 mg/ml) was brought to 37° and treated with carboxypeptidase (0.25 ml). To 4 ml aliquots removed at ¼, ½, 1, 1½, 3¼, 5½ and 22½ hours, was added Zeocarb-225 (700 mg) and water (8 ml). After shaking for 2 hours the resin was washed three times with water and eluted twice with 5 *N* NH_4OH (2 ml). The ammonia was evaporated and the residue divided into 2 parts, one for quantitative chromatography as described above and one for two-dimensional or tert-amyl alcohol chromatography.

The most rapidly liberated amino acid was *iso*leucine. It was not possible to distinguish between the amino acids which appeared next. Leucine, alanine and valine were all rapidly liberated at comparable rates, equal approximately to half that for *iso*leucine. After 22½ hours the amounts liberated corresponded approximately to one end-group in a particle weight of 300,000 (*iso*leucine), 500,000 (alanine), 600,000 (valine), and 800,000 (leucine).

A two-dimensional chromatogram of the 22½ hour sample together with one in tert-amyl alcohol showed large amounts of *iso*leucine, alanine, valine, leucine, lesser amounts of threonine, lysine, histidine, small amounts of glutamic acid, serine and arginine and traces of tyrosine and glycine.

Similar results were obtained with a number of different myosin preparations in qualitative experiments.

Actin. A solution of actin (3.8 ml, 10 mg/ml) in 0.05 *M* KCl was shaken with Zeocarb-225 (200 mg) for one hour. The protein solution was sucked off, adjusted to pH 8.0 and incubated with enzyme (0.01 ml) for 2 minutes at 20°. The resin treatment was then repeated followed by successive incubations with the same amount of enzyme at 37° for 2,2, 4, 20 and 40 minutes. The amino acids eluted from each batch of resin were chromatographed two-dimensionally to give the result shown in Table II. Chromatography in tert-amyl alcohol of a portion of the 40-minute sample showed the presence of *iso*leucine, not leucine.

A fresh sample of the same actin solution (1.24 ml) was incubated for 2 hours at 37° with enzyme (0.02 ml) and worked up as before. The product was run in butanol-acetic acid alongside standard (24 μg) spots of phenylalanine and *iso*leucine. The actin gave spots of phenylalanine and *iso*leucine each judged to be equivalent to 20 μg (23 μg and 29 μg respectively required for one end-group per unit weight of 70,000).

The hydrazine method

Preparation of anhydrous hydrazine. Hydrazine hydrate (1040 ml, 60%) was distilled through a Quickfit column (20 × 1.5 cm) of glass helices until a constant boiling residue remained (b.p. 119.5° 360 ml, 97% hydrate). This residue was distilled *in vacuo* from NaOH pellets (300 g) using a heating mantle, and the product collected in fractions (97–93% hydrazine). A second distillation from sodium hydroxide (30 g) (bath 95–105°, pressure 26–16 cm mercury) gave hydrazine (130 g) ranging in quality from 99.1–98.6%. The fractions were sealed in 5 ml ampoules. Assays were carried out by the iodate titration of KOLTHOFF[11].

General procedure. The protein (100 mg) was introduced into a weighed tube with a partly drawn out neck, dried overnight in a vacuum desiccator, weighed and sealed with hydrazine (2–3 ml, 99.1%). The tube was heated for 8 hours at 100° and the contents evaporated in a vacuum desiccator over sulphuric acid. A solution of the residue in water (10 ml) was shaken overnight at 0° with the carboxylic resin Amberlite IRC-50 (1 g), decanted and filtered. After washing the resin with water (2 ml) the combined aqueous fraction was shaken with benzaldehyde (0.6 ml) and pyridine (0.06 ml) overnight at room temperature in a glass-stoppered tube. Extraction with ether was carried out four times in the same tube, the aqueous layer centrifuged if necessary, evaporated in a desiccator and the residue used for paper chromatography.

Tropomyosin. Two-dimensional chromatography of the residue obtained as above revealed traces of glutamic acid, serine, glycine and alanine, with two strong spots on a pale purple background in the "leucine position". This fast-running material did not react with ammoniacal silver nitrate. For further resolution of this group the residue was streaked on paper and run in butanol-acetic acid. The fast-running ninhydrin-positive material consisted of three ill-defined bands which were eluted separately with water and re-run in butanol-acetic acid and in tert-amyl alcohol-water. The slowest of these bands gave in both solvents a strong isoleucine spot with some streaked material. The two other bands gave in tert-amyl alcohol-water only streaks at the origin and in butanol-acetic acid a tailed spot faster than leucine. Acid hydrolysis of these two bands gave a small of benzaldehyde and a mixture of amino acids. They probably consist of benzal-hydrazide compounds. The yield of *iso*leucine was about 25% of the theoretical amount for a single end-group.

Actin. A two-dimensional chromatogram of the end-product of the general procedure showed traces of glutamic acid, glycine, and alanine, a small amount of serine and a strong grey spot characteristic of phenylalanine, surrounded by a slight purple background. The yield of phenylalanine was about 25% of the theoretical amount for a single end-group.

References p. 542.

Myosin. When myosin was treated in the manner described for tropomyosin and the product submitted to two-dimensional chromatography small glutamic acid, serine, alanine spots and traces of glycine were obtained as well as some hydrazide spots and a complex group on a purple background around the leucine position. When this latter group was further resolved by chromatography as for tropomyosin a small amount of *iso*leucine only was obtained but this was only comparable with the amount of glutamic acid present.

DNP-Actin

Actin was converted to DNP-actin by the method of SANGER. Hydrolysis was carried out in a sealed tube with 6 *N* HCl for 8 hours at 100°, and the hydrolysate treated in the usual way. The ether-soluble fraction was chromatographed on buffered paper with tert-amyl alcohol-buffer (BLACKBURN AND LOWTHER[12]) and was found to be devoid of DNP-amino acids. Paper chromatography of the aqueous fraction with butanol-acetic acid, followed by elution of the bands and re-running in tert-amyl alcohol-water revealed much ε-DNP lysine and its peptides but no DNP-arginine or bis-DNP-histidine.

Oxidation of tropomyosin

Tropomyosin (500 mg) was added to formic acid (4 ml, 90%) which was stirred until solution was complete. Hydrogen peroxide (0.45 ml, 30%) was added, the mixture allowed to stand for 30 minutes at 19°, transferred to the cold room and poured on to melting ice (10 ml). The solution was dialysed in a rapid dialyser against distilled water, followed by frequent changes of buffer (0.09 *M* KCl + 0.01 *N* HCl, pH 2.1). Osmotic measurements were made with the toluene osmometers and collodion membranes of ADAIR[13] in the above buffer at 0°. Viscosity measurements were made in two small Ostwald viscometers at 20° using the protein solutions from the osmometer experiments. The rate of shear lay within the limits 460–1130 sec^{-1} where kinetic energy correction is negligible (TSAO, BAILEY AND ADAIR[14]). Results of both osmotic and viscosity experiments are shown in Fig. 2.

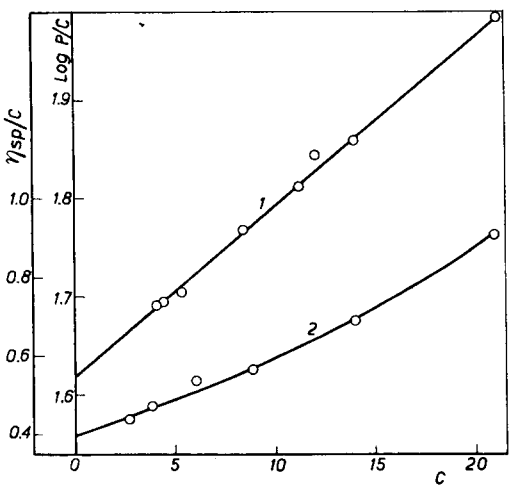

Fig. 2. Osmotic pressure and viscosity curves for oxidised tropomyosin. Curve 1. log P/C against C. (P = osmotic pressure in cm mercury, C = concentration in g/litre). Curve 2. η_{sp}/C against C (η_{sp} = specific viscosity).

A sample of the protein recovered from these experiments was reacted with FDNB and hydrolysed in a sealed tube with 6 *N* HCl for 24 hours at 103°. The ether-soluble fraction of the hydrolysate when run on buffered paper with tert-amyl alcohol buffer gave no DNP-amino acids. A sample of the oxidised protein itself was hydrolysed in the same way, evaporated down, dissolved and treated with Zeocarb-225 to remove salts and the bulk of the amino acids (cysteic acid is not absorbed). The supernatant was evaporated and chromatographed, giving a cysteic acid spot approximately equivalent to one mole per mole of tropomyosin.

RESULTS

The C-terminal group and sequence of tropomyosin

The result of treating tropomyosin with carboxypeptidase by the consecutive technique is shown in Table I. *Iso*leucine is clearly the most rapidly liberated amino acid, followed in order by serine, threonine, and methionine or valine. *Iso*leucine must therefore be a C-terminal group of tropomyosin. One of the difficulties of the carboxypeptidase method lies in deciding whether the second amino acid to appear is the penultimate residue of a single polypeptide chain or a second more slowly liberated end-group, particularly as SMITH[15] has shown a wide variation in the rates of liberation of different amino acids from the C-terminal position of a peptide. Serine, for example,

References p. 542.

is more slowly liberated than *iso*leucine. Since serine is present in the first chromatogram it is not clear whether it is also an end-group. Another difficulty is that the action of the enzyme may be blocked by a terminal or penultimate proline residue[16] or may be very slow in the case of a terminal glycine group[15].

TABLE I

THE LIBERATION OF AMINO ACIDS FROM TROPOMYOSIN BY CARBOXYPEPTIDASE

Incubation	*Time (min)*	*Amino acids**				
		isoleu	*ser*	*thr*	*met*	*ala*
1	15	30	4	1	1	1
2	30	6	10	2	1	1
3	30	2	12	10	1	1
4	60	1	8	10	4	1
5	60		10	10	6	1

* The numbers are arbitrary values showing the approximate relative intensities of the spots on the chromatogram as judged visually.

It seemed desirable to apply an independent method without these limitations. The hydrazine method, although having shortcomings of its own which are discussed below, does not fail for proline or glycine and liberates only C-terminal groups as free amino acids. The experimental results for tropomyosin are strongly positive for an *iso*-leucine end-group only, although the yield is only about a quarter of the theoretical for one end-group. The results of the two methods taken together show that *iso*leucine alone is C-terminal in tropomyosin.

The results of the carboxypeptidase time-curve method are shown in Fig. 1. One mole of *iso*leucine is liberated very rapidly, followed by serine and after lag periods by threonine and methionine, each tending towards one mole. Alanine follows methionine at an increasing rate. This is good evidence for a single chain polypeptide structure with the order representing the C-terminal sequence. It will be seen, however, that the *iso*leucine curve continues above the one mole mark at a much lower gradient and indeed in longer term experiments extended above four moles, some of which, beyond two moles, is due to leucine. The gradient of the second phase of the *iso*leucine curve suggests that a second *iso*leucine group lies between methionine and alanine. The upward rising of the tail of the curve is undoubtedly due to a third *iso*leucine (or possibly leucine) group lying close behind alanine. Lysine, aspartic acid and asparagine also appear in smaller quantities.

It appears from these results that tropomyosin has a single C-terminal *iso*leucine residue and that the C-terminal sequence is *iso*leu–ser–thr–met–*iso*leu–ala– (*iso*leu or leu, lys, asp, aspine) the order of the bracketed amino acids being indeterminate.

The C-terminal group and sequence of actin

The liberation of amino acids from actin by the consecutive carboxypeptidase technique is shown in Table II. It can be seen that phenylalanine has a just detectable lead over *iso*leucine. Histidine is the next amino acid to appear in quantity. A single two hour incubation produced amounts of phenylalanine and *iso*leucine of the correct order for a single residue per molecule of weight 70,000. The hydrazine method showed only

References p. 542.

phenylalanine as a C-terminal group. The two methods are, therefore, in agreement with a single phenylalanine C-terminal group. The C-terminal sequence must be phe–*iso*leu–his (ser, val or met)—(ala, lys)– the order of the bracketed members being indeterminate.

TABLE II

THE LIBERATION OF AMINO ACIDS FROM ACTIN BY CARBOXYPEPTIDASE

Incubation	*Time (min)*	*Amino acids**							
		phe	*isoleu*	*his*	*ser*	*gly*	*ala*	*lys*	*val-met*
0	0				4	3	1	1	
1	2	1			1	t	t		
2	2	3	3		1	t			
3	2	3	3		1	t	t		t
4	4	6	6	t	2	1	t		t
5	20	20	20	20	4	1	2	1	4
6	40	25	25	2	8	1	8	6	12

* Numbers as in Table I; t = trace.

C-terminal groups of myosin

The amount of end-group present in myosin is of a lower order than found in actin and tropomyosin. Carboxypeptidase experiments show that *iso*leucine is C-terminal but only to the extent of about one mole in a particle weight of 300,000. Whether any of the amino acids, valine, alanine or leucine, which are liberated more slowly and in about half the above amount, are C-terminal is not clear and the results of the hydrazine method are too inconclusive to throw light on the question.

C-terminal group of ovalbumin

A sample of four times recrystallised ovalbumin prepared by Dr. K. BAILEY was not attacked by carboxypeptidase even on prolonged incubation under the conditions described for tropomyosin. STEINBERG[17, 18] reported the liberation of alanine by carboxypeptidase but later found this due to a DFP-sensitive, contaminating enzyme. TURNER AND SCHMERZLER[19], however, have found alanine to be C-terminal by the thiohydantoin method.

N-terminal group of actin

The normal procedure of SANGER has failed to reveal N-terminal groups. Hydrolyses under special conditions to minimise possible destruction of DNP-proline have yet to be carried out.

Observations on the hydrazine method

The originators of the method have made a brief report of its application to a number of proteins (AKABORI AND OHNO[20]). On heating the protein in anhydrous hydrazine peptide bonds are split with formation of amino acid hydrazides, while the C-terminal residues only are liberated as free amino acids. Hydrazides are removed by reaction with benzaldehyde to give insoluble dibenzal-derivatives and the remaining amino acids are identified by paper chromatography. The method appears simple in procedure and has the advantage of producing an amino acid as end-product. In the application of the

References p. 542.

method to the muscle structural proteins a number of difficulties have been experienced and the following observations and modifications have been made:

The recovery of microgram quantities of mixed amino acids heated in hydrazine has been investigated using quantitative paper chromatography. After heating for 8 hours at 100° arginine, cysteine, cystine and asparagine were completely destroyed. The other common amino acids were recoverable in amounts exceeding 85% with the exception of cysteic acid (55%), glutamic acid and tryptophan (40%) and aspartic acid (20%). Methionine was recovered almost entirely as sulphoxide. The decomposition products of arginine, cysteine and asparagine heated singly in hydrazine were found to be complex.

The principal problem has been the complete removal of hydrazides and other ninhydrin-reacting material from the reaction mixture. This becomes more important with high molecular weight proteins. Thus in the case of insulin the alanine end-group could be readily detected in ca. 50% yield by one-dimensional chromatography of the evaporated hydrazine reaction mixture in phenol-ammonia, the hydrazides being faster-running. In the case of tropomyosin and particularly myosin, interference by residual hydrazides is more serious, especially as these tend to obscure the "leucine position" on chromatograms. Prolonged shaking with benzaldehyde was found to be inadequate, either with or without buffers. Trials were made of the absorption on the carboxylic resin IRC-50 of the hydrazides, which are more basic than most amino acids. The bulk of the hydrazides from a protein digest were absorbed on shaking a solution with the resin as described for tropomyosin, the supernatant (pH 6.3–6.4) containing mainly aspartic acid and glutamic acid hydrazides. It was found on the other hand that at pH 5–7 there is negligible absorption of most of the amino acids on to the ammonium resin from very dilute solution. The exceptions are arginine, histidine and lysine which are completely absorbed and tryptophan which is largely absorbed.

A final treatment of the resin-treated protein digest with benzaldehyde was then effective in removing the greater part of the residual non-amino acid material capable of reacting with ninhydrin. The pH tends to drop due to formation of benzoic acid but a final pH of ca. 6.3 can be achieved without introduction of inorganic buffer salts by adding a small amount of pyridine. In the case of tropomyosin and myosin some further fractionation of the product by paper chromatography was necessary for clear identification of the terminal amino acids.

In all experiments small amounts of other amino acids, particularly glutamic acid, serine and alanine, were visible in the chromatograms. In the case of tropomyosin and actin these spots were of a lower order of intensity than the end-group spot, but this was not so in the case of myosin. This "background" of spots sets a limit to the use of the method for high molecular weight proteins. It probably arises either from simple hydrolysis of peptide bonds due to the 1% water in the hydrazine solution, or from slight decomposition of hydrazides during the manipulations.

The rate of breakdown of proteins in hydrazine has not been studied in this work. AKABORI, OHNO AND NARITA[4] at first used 10 hours at 125° but later[20] considered 10 hours at 100° sufficient for complete breakdown. In view of the destruction of amino acids described above it would seem undesirable to use treatments more rigorous than this. Indeed it was found in an experiment where amino acids alone were heated in hydrazine for 10 hours at 108° that all the aspartic acid and most of the glutamic acid was destroyed.

References p. 542.

To summarize, the method will fail when the terminal residue is arginine, cysteine, cystine, asparagine or glutamine and may fail for a terminal aspartic acid group. If the resin treatment is used, lysine, histidine and probably tryptophan will also be lost.

The oxidation of tropomyosin with performic acid

In view of the presence of a C-terminal group and lack of N-terminal groups some independent evidence on number of peptide chains in tropomyosin seemed desirable. The α-type X-ray pattern of tropomyosin together with its particle weight of 53,000 and axial ratio (25% hydration) of 25 are in agreement with a double chain configuration (TSAO, BAILEY AND ADAIR[4]). The amino acid analysis of tropomyosin suggests either one or two cystine bridges in the molecule (BAILEY[6]). In the case of a cyclic structure or a single doubled-up chain, oxidation of cystine bridges would not affect the particle weight although axial ratio might be affected. In the case of two single peptide chains linked by cystine bridges a halving of the particle weight would be expected. SANGER[19] used the performic acid oxidation method to effect a clean fission of the chains of insulin. In the present experiment the same conditions have been used, but with double the reaction time considered necessary by SANGER for complete oxidation of insulin.

A series of osmotic pressure and viscosity measurements have been made on the oxidised tropomyosin using the acid depolymerising conditions earlier employed for tropomyosin. The results are shown in Fig. 2. The osmotic plot proved to be linear.

$$\lim (P/C)_{C \to 0} = 0.415$$

$$\text{Particle weight} = \frac{26{,}180}{0.415} = 63{,}100\ (\pm\ 1000)$$

$$\lim (\eta\ \text{spec}/C)_{C \to 0} = 0.0395$$

$$\text{Axial ratio (25\% hydration)} = 21\ \text{(Simha equation)}$$

The increase in particle weight of 10,000 over that found for tropomyosin is surprising. It is at least clear that the molecule is not split into two fragments by oxidation. The small decrease in axial ratio from 25 for tropomyosin to 21 for oxidised tropomyosin cannot be regarded as significant in view of the solution of the protein in formic acid. End-group assay of the oxidised protein by the DNP-method showed that no peptide bonds had been split, while acid hydrolysis showed that considerable oxidation of cystine links had been achieved.

DISCUSSION

On finding no N-terminal groups in myosin or tropomyosin, BAILEY[1] suggested cyclopeptide structures. The present failure to find an N-terminal group in actin is in line with his result. The presence of a single C-terminal group in tropomyosin and actin however, does not support the cyclopeptide idea, although the C-terminal group could be on the side-chain of a cyclopeptide having a branch point at a β-aspartyl or γ-glutamyl link. It may well be that an N-terminal group exists but that coupling with FDNB is prevented by steric hindrance or by substitution of the group with some simple radical not yet detected or destroyed in protein hydrolysates. Until some further evidence on the question can be obtained it seems unwise to pursue the cyclopeptide theory. It may

References p. 542.

be noted, however, that ovalbumin appears to be a protein devoid of either N- or C-terminal groups.

Although both myosin and tropomyosin possess C-terminal *iso*leucine the pattern of liberation of amino acids is quite distinct. The small amount of C-terminal group found in myosin cannot be due to contaminating actin or tropomyosin. Whether this small amount is of significance for the larger sub-units of myosin of whether it belongs to the smaller fragments found by TSAO[5] after urea treatment is at present under investigation.

The methods used for determining the C-terminal sequences should be capable of wide application to proteins and peptides.

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SUMMARY

1. The results of carboxypeptidase and hydrazine methods show tropomyosin to have a single isoleucine C-terminal group. The C-terminal sequence has been found to be isoleucine–serine–threonine–methionine–isoleucine–alanine.

2. Similarly actin has been shown to have a single phenylalanine C-terminal group and a C-terminal sequence phenylalanine–isoleucine–histidine.

3. Myosin has been shown by the use of carboxypeptidase to have at least one isoleucine C-terminal group in a particle weight of about 300,000. The possibility that some other amino acids may be C-terminal is not excluded.

4. Ovalbumin is not attacked by carboxypeptidase.

5. The SANGER technique gave negative results for N-terminal groups in actin.

6. The scope of the hydrazine method of AKABORI, OHNO AND NARITA is discussed and some modifications of the method have been made.

7. An attempt to effect oxidative fission of tropomyosin with performic acid was unsuccessful.

RÉSUMÉ

1. La carboxypeptidase et la méthode à l'hydrazine révèlent, dans la tropomyosine, un seul groupe C-terminal appartenant à l'isoleucine. La séquence C-terminale est: isoleucine–sérine–thréonine–méthionine–isoleucine–alanine.

2. De même l'actine présente une seule phénylalanine C-terminale, la séquence C-terminale étant: phénylalanine–isoleucine–histidine.

3. La carboxypeptidase révèle dans la myosine au moins une isoleucine C-terminale par particule de poids 300,000 environ. Il se peut que d'autres aminoacides occupent des positions C-terminales.

4. L'ovalbumine n'est pas attaquée par la carboxypeptidase.

5. La technique de SANGER ne détecte aucun groupe N-terminal dans l'actine.

6. La valeur de la méthode d'AKABORI, OHNO ET NARITA est discutée et quelques modifications y ont été apportées.

7. Il n'a pas été possible de scinder la tropomyosine par oxydation performique.

References p. 542.

ZUSAMMENFASSUNG

1. Die mit der Carboxypeptidase und der Hydrazinmethode gewonnenen Ergebnisse zeigen, dass Tropomyosin eine einzige Isoleucin C-Endgruppe besitzt. Die Reihenfolge der Aminosäuren am C-Ende ist: Isoleucin–Serin–Threonin–Methionin–Isoleucin–Alanin.

2. Auf ähnliche Weise wurde gezeigt, dass Aktin eine Phenylalanin-Endgruppe am C-Ende besitzt und die Reihenfolge der Aminosäuren am C-Ende Phenylalanin–Isoleucin–Histidin ist.

3. Am Myosin wurde mit Hilfe der Carboxypeptidase gezeigt, dass auf ein Teilchengewicht von 300,000 zumindest eine Isoleucin-C-Endgruppe entfällt. Das Vorhandensein einiger anderer C-endständiger Aminosäuren konnte nicht ausgeschlossen werden.

4. Ovalbumin wird durch die Carboxypeptidase nicht angegriffen.

5. Am Aktin gibt die SANGERsche Methode für die N-endständigen Aminosäuren kein Ergebnis.

6. Die Bedeutung der Hydrazinmethode von AKABORI, OHNO UND NARITA wird diskutiert und einige Modifikationen der Methode angegeben.

7. Ein Versuch Tropomyosin mit Perameisensäure oxydativ aufzuspalten, war ohne Erfolg.

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