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Actin, Its Amino Acid Composition and Its Reaction with Iodoacetate*

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Received July 30, 1962

G-Actin was reacted with iodoacetate under conditions that permitted complete reaction of the sulfhydryl groups. All the sulfur was accounted for by cysteine and methionine. Amino acid analyses indicated that the actin preparations were free of tropomyosin.

Since we were able to obtain actin preparations of high purity (Carsten and Mommaerts, 1963), the amino acid composition of G-actin was reinvestigated. Laki and Standaert (1960), in a discussion of a previous analysis of performic acid-oxidized actin by Kominz *et al.* (1954), estimated that their preparations contained about 10% tropomyosin. The amino acid analyses of actin reacted with iodoacetate presented in this paper confirm this estimate.

The reaction with iodoacetate also permitted an accurate analytical determination of the sulfhydryl groups by determining the *S*-carboxymethylcysteine in the hydrolysate. The results are also presented in this paper.

METHODS

Actin.—G-Actin was prepared according to the ultracentrifugal purification method (Mommaerts, 1952a, 1958) as described in the preceding paper (Carsten and Mommaerts, 1963). Protein concentrations were determined by the colorimetric method of Lowry *et al.* (1951), with lyophilized actin preparations used as standards.

Reaction with Iodoacetate.—This was carried out according to Moore *et al.* (1958). Iodoacetic acid, recrystallized from deionized water, was dissolved in 8 M deionized urea, containing 0.02% Versene. About 40 mg of freshly prepared actin in 4 ml of solution at *p*H 8.5 was reacted with 20 mg of iodoacetic acid in 12 ml of urea solution. After mixing, the *p*H was readjusted to 8.5 by addition of a few drops of 1 N NaOH; the final *p*H adjustment was made with a *p*H stat (TTT 1 b, Radiometer, Copenhagen), the *p*H adjustment taking not more than 2 minutes (timed). The reaction was allowed to proceed for 20 minutes at 25°. The mixture was then filtered through a 3 × 40 cm column of Sephadex G-25 which had been equilibrated with 0.05 M ammonium acetate, *p*H 8.0, and was eluted with this same ammonium acetate solution The *S*-carboxymethylactin was recovered in the effluent well in advance of the urea and was lyophilized.

Hydrolysis and Analysis.—The protein was hydrolyzed according to the method of Hirs *et al.* (1954), with 1 ml of constant-boiling HCl (three-times redistilled in glass) in evacuated sealed tubes. Hydrolysis was conducted at 110° for 22 hours, and also for 70 hours in order to correct for losses. After the termination of hydrolysis, the solutions were evaporated to dryness at 40° in a stream of nitrogen.

Amino acid analyses on the hydrolysates were carried out with the Spinco automatic amino acid analyzer (Spackman *et al.*, 1958). The analyses were corrected for 4.9 to 6.4% moisture.

Tryptophan was determined spectrophotometrically in the unhydrolyzed protein according to Beaven and Holiday (1952).

RESULTS

The actin preparations used in this work were ultracentrifugally homogeneous according to the criteria available in this case (Mommaerts, 1952a). Their behavior on zone electrophoresis and on fractionation with ammonium sulfate have been described in the preceding paper (Carsten and Mommaerts, 1963) and indicate the complete absence of tropomyosin.

Reaction with Iodoacetate.—Under the conditions used, cysteine was the only constituent which reacted with iodoacetate, since chomatographic analysis revealed no derivatives originating from other amino acids. The analyses presented in Table I show that after correction for losses during hydrolysis there were 6.7 moles of *S*-carboxymethylcysteine per mole (60,000 g) of actin. The correction for decomposition was derived from the difference between the 22 and the 70 hour hydrolysates and, assuming linearity with time, was estimated to be 7% after 22 hours of hydrolysis. The chromatogram showed no indication of any cysteine or cystine that had not reacted with the iodoacetate.

Amino Acid Analysis.—Four different preparations of *S*-carboxymethylactin were analyzed, one of them in duplicate, with an average recovery of 95% of the dry weight. Corrections for decomposition of threonine

* This investigation was supported by Research Grant H-3067 of the National Heart Institute, National Institutes of Health, Bethesda, Md., and a grant from the Muscular Dystrophy Associations of America, Inc.

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TABLE I
AMINO ACID COMPOSITION OF HYDROLYSATES OF S-CARBOXYMETHYL-ACTIN[a]

No. of amino acid residues for m. w. 60,000	
Lysine	25.2 ± 0.5[b]
Histidine	10.1 ± 0.2
Ammonia	(44.4 ± 5.1)
Arginine	24.4 ± 0.2
S-Carboxymethylcysteine[c]	6.7 ± 0.2
Aspartic acid	46.9 ± 0.8
Threonine[c]	37.8 ± 0.9
Serine[c]	32.3 ± 1.5
Glutamic acid	54.7 ± 1.3
Proline	25.2 ± 1.0
Glycine	37.4 ± 1.5
Alanine	40.5 ± 0.3
Half-cystine	0
Valine	24.9 ± 0.6
Methionine	21.8 ± 0.2
Isoleucine[d]	36.7 ± 1.6
Leucine	34.5 ± 0.7
Tyrosine[e]	21.5 ± 0.2
Phenylalanine	15.7 ± 0.3
Tryptophan[e]	5.1 ± 0.1
Total	501.4

[a] Corrected for moisture. [b] The variation is expressed as the average of the deviations from the mean. [c] Corrected for losses during hydrolysis. [d] Corrected for incomplete hydrolysis. [e] The concentration of tryptophan was calculated from the ultraviolet spectra (Beaven and Holiday, 1952). Tyrosine calculated from the spectra gave a value of 22.1 ± 0.7 residues per mole.

and of serine, as described above for S-carboxymethylcysteine, amounted to 7.7 and 12% respectively. For isoleucine, the value after 70 hours of hydrolysis was 9% higher than that after the shorter period, and this value was accepted. The other amino acids did not change significantly during hydrolysis.

The tryptophan contents were derived from the absorption spectrum (Fig. 1). Tyrosine values were likewise derived from the spectrum, and these agreed well with those found analytically.

An analysis for total sulfur[1] gave a value of 1.55% in the dry protein, somewhat above the previously reported figure (Kominz *et al.*, 1954; Mommaerts 1951). In Table I, the sum of the S-carboxymethylcysteine sulfur and the methionine sulfur was 1.51%; the same calculation for the sample on which the sulfur analysis was made gave exactly 1.55% S.

DISCUSSION

The results of this investigation are of interest in two respects. First, they can be related to the question of the purity of actin; it was shown that the preparations were free of tropomyosin according to the ammonium sulfate precipitation test and also by starch gel electrophoresis (Carsten and Mommaerts, 1963). The results of amino acid analyses are in good agreement with those of Laki's laboratory (Kominz *et al.*, 1954; Laki and Standaert, 1960), after correction of the latters' results for the estimated 10% tropomyosin contamination. Tropomyosin stands out for its high glutamic acid and lysine contents, which are more than twice those of actin. The actin preparations of Kominz *et al.* (1954) contained 31.2 lysine and 61 glutamate, residues per 60,000 g of protein, of which 6.6 and 13, respectively, can be ascribed to tropomyosin, leaving 24.6 and 48 for actin itself. Our direct analytical values were 25 and

[1] Elek Micro Analytical Laboratories, Los Angeles 16, Calif.

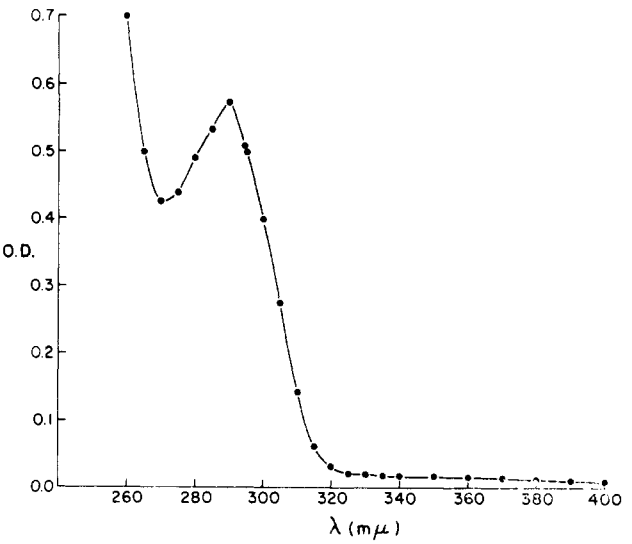

FIG. 1.—Absorption spectrum of S-carboxymethylactin (0.5 mg/ml) in 0.1 N NaOH.

55, respectively, in good agreement with the values of 27 and 53 calculated from the data of Kominz *et al.* for 60,000 g of actin. Similar calculations can be made for alanine.

Secondly, the sulfur and cysteine contents deserve comment. After complete substitution of the SH groups with iodoacetate, 6.7 ± 0.2 moles of S-carboxymethylcysteine were obtained per 60,000 g, in excellent agreement with the results of Kominz *et al.* (1954) on the analysis of performic acid-oxidized actin. That this is not an integral number should not cause concern at this time, in view of the uncertainty of various data particularly of the molecular weight determinations. Taken at its face value, the result would be compatible with 7 SH groups for a molecular weight of approximately 61–62,000. Stoichiometric equivalent weight determinations have been fluctuating around 60,000 (57,000 was given by Mommaerts, 1952b, and 62,000 by Ulbrecht *et al.*, 1960), the emphasis recently being on the higher molecular weight. In a direct analytical study of the SH groups with *n*-ethylmaleimide, mercurial, and silver reagents, Katz and Mommaerts (in press), found 5.8–6.7 groups per 60,000, and Tonomura and Yoshimura (1962), also using *p*-chloromercuribenzoate, found 6.1–6.8 SH groups for 61,000 g of actin.

It was assumed by Laki and Standaert (1960) that there is one disulfide bond per mole of actin, since methylmercury nitrate titration yielded only 4–5 SH groups (Kominz *et al.*, 1954). *This is not true for our actin preparations.* As iodoacetate under the experimental conditions reacts only with free SH groups, any SS groups as well as non-reacted SH groups should have appeared in the chromatogram as cystine. *No cystine could be detected.* Within the limits of error of the analytical methods there appear to be 22 moles of methionine and 7 moles of cysteine. Twenty-nine moles of sulfur for a molecular weight of 60,000 account precisely for a sulfur content of 1.55%.

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Physical Properties and Polymerization Reactions of Native and Inactivated G-Actin

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Received August 23, 1962

Actin has been prepared which is tropomyosin-free, and some of its physical properties have been studied in low ionic strength as well as in 0.6 M KI solutions. Native G-actin in 0.5 mM ATP, 2 mM Tris buffer, *p*H 8, has an intrinsic viscosity of about 0.10 dl/g, $s^{\circ}_{20,w}$ of 3.25 Svedberg units, and molecular weight of 57,200. G-actin which has been inactivated by EDTA treatment has an intrinsic viscosity of about 0.075 dl/g, $s^{\circ}_{20,w}$ of 6.6 Svedberg units, and molecular weight of 125,000; when the ionic strength is raised to 0.1, inactivated actin undergoes a transformation into high-molecular-weight aggregates. Native G-actin in 0.6 M KI is gradually inactivated to form high-molecular-weight aggregates, indicating that an ATP solution is to be preferred for performing physical measurements on native G-actin. It is suggested that the large drop in effective volume of the native G-actin molecule which occurs upon its inactivation may be due to the collapse of a flexible, solvent-penetrable domain within the molecule after removal of the Ca-ATP prosthetic group.

The discovery that usual preparations of actin may contain appreciable amounts of tropomyosin (Laki and Cairns, 1959; Laki and Standaert, 1960) casts doubt on the physical measurements made on such preparations and on the interpretations which have been drawn from them. The ability to prepare actin which is free of tropomyosin (Martonosi, 1962a,b; Laki *et al.*, 1962) offers an opportunity to study pure G-actin and its transformation products.

It has long been recognized that G-actin containing bound ATP polymerizes into F-actin upon addition of salt, and that removal of the bound ATP results in a complete loss of this polymerizability (Laki *et al.*, 1950; Straub and Feuer, 1950). G-actin which has been treated in any manner allowing release of bound ATP and loss of ability to form F-actin may be termed inactivated G-actin. Viscosimetry (Martonosi and Gouvea, 1961; Barany *et al.*, 1961; Grubhofer and Weber, 1961; Strohman and Samorodin, 1962) and flow birefringence (Asakura, 1961) have shown that no linear polymerization occurs when inactivated G-actin is treated with salt, but this does not rule out the possibility that other types of aggregates are formed. We shall describe here the use of ultracentrifugal techniques to demonstrate the formation of random aggregates from inactivated G-actin in the presence of salt.

Material and Methods

Actin was extracted from rabbit muscle by the method of Straub (1943) and purified according to Mommaerts (1952a). It was then subjected to a procedure, described by Martonosi (1962b) and by Laki *et al.* (1962), which removes contaminating tropomyosin. The tropomyosin-free F-actin pellet was depolymerized by dialysis against 0.5 mM ATP in 2 mM Tris buffer at *p*H 8.0 for 2–3 days at 3–4°. The G-actin was then clarified by centrifugation for 1 hour at 30,000 rpm.

G-actin was inactivated by ethylenediaminetetraacetate (EDTA) treatment as reported by Maruyama and Martonosi (1961); EDTA releases the bound Ca^{++} and ATP concomitantly from G-actin (Tonomura and Yoshimura, 1961; Maruyama and Gergely, 1961), leading to loss of its polymerizability (Martonosi and Gouvea, 1961; Barany *et al.*, 1961; Grubhofer and Weber, 1961; Strohman and Samorodin, 1962). After removal of free ATP by treatment with Dowex-1 (Asakura, 1961), actin was incubated for 10 minutes at room temperature in the presence of 10 moles EDTA per mole actin in 2 mM Tris buffer *p*H 8.0. The KCl concentration of the inactivated G-actin solution was made 0.1 M by the addition of 3 M KCl.

Viscosity was measured in an Ostwald-type viscosimeter with a water flow-time of 82.5 seconds at 25.0 ± 0.01°. Sedimentation rates were measured at 25° in a Spinco Model E ultracentrifuge equipped with a rotor temperature indicator and control unit.

The molecular weights were determined in a second Spinco Model E ultracentrifuge equipped with the rotor temperature indicator and control unit and with the Rayleigh interference optical system. With the exception of the actin treated with KI for 3 days, which was examined at 27.0°, all determinations were carried out at 4.0°. The sedimentation equilibrium experiments utilized the short-column technique with interference

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