AMEBA ACTIN: THE PRESENCE OF 3-METHYLHISTIDINE

Robert R. Weihing and Edward D. Korn

Laboratory of Biochemistry, National Heart Institute

National Institutes of Health, Bethesda, Md. 20014

Received May 15, 1969

Summary:

Actin has been isolated from the ameba, Acanthamoeba castellanii (Neff) by procedures similar to those used previously for the isolation of actin from slime mold and skeletal muscle. The three actins are very similar in their amino acid composition, and ameba actin, like skeletal muscle actin, contains one mole of 3-methylhistidine per mole of protein. Ameba actin resembles the other two actins in its molecular weight, its precipitation by added myosin at low ionic strength, its increase in viscosity upon the addition of myosin at high ionic strength which is reversed by ATP, and its ability to undergo a reversible G-F transition.

Actin and actomyosin-like proteins have been identified in several kinds of cells which presumably lack the highly organized contractile system of muscle (1,2,3). The most carefully studied of these proteins, the actin-like protein of the slime mold, Physarum polycephalum, closely resembles skeletal muscle actin in many respects including its amino acid composition (1). It is not known, however, whether slime mold actin also contains the unusual amino acid 3-methylhistidine which occurs in skeletal muscle actin in the ratio of 1 mole of 3-methylhistidine per 1 mole of protein (4,5). We now report the isolation from the soil ameba, Acanthamoeba castellanii (Neff), of "ameba actin", a protein which closely resembles skeletal muscle and slime mold actins in its physical properties and amino acid composition, and which contains about 1 mole of 3-methylhistidine per mole of protein.

Extraction of Amebas and Demonstration of Actin-like Activity. Mass cultures of amebas were grown at 22-24° in proteose-peptone medium in 15 l carboys under continual oxygenation with pure O_2 at about 85 ml/min. Cells were harvested by low-speed centrifugation and either stored at -15° for up to 2 weeks or extracted immediately. All subsequent procedures were carried

out at 2-4°. Packed cells were mixed with 2 volumes of 1.4M KCl (final concentration, 0.93M). Cells extracted immediately after collection were disrupted by homogenization with 3-4 strokes of a tight-fitting Dounce homogenizer. Frozen-thawed cells were nearly completely lysed and were extracted without additional disruption. The extracting mixture was brought from pH 6.6-6.8 to pH 7.4-7.5 with very small volumes of 1M NaOH and the mixture was incubated for 1 hour at 0° and then centrifuged for 15 min at 9000 X g.

Actin-like activity was identified in the supernatant fluid in the following way. Addition to the extract of a small amount of highly purified skeletal muscle myosin led to a large increase in viscosity which was reversed upon addition of ATP (Table I). Only small viscosity changes occurred when ATP was added to the extract in the absence of myosin. Addition of myosin to 0.93 M KCl produced only a small increase of viscosity which was not affected by ATP.

Purification of Ameba Actin. Modifications of procedures developed for the isolation of slime mold (1) and muscle actin (6,7,8) were used. The 9000 X g supernatant of the ameba extract was mixed with muscle myosin (1 g myosin/10 g extract protein) and was dialyzed overnight against 0.05M KCl. The resultant precipitate was collected and washed as described by Hatano and Oosawa (1) for slime mold actin. An acetone powder of the washed precipitate was then prepared using 9-10 volumes of acetone maintained at -21°. The acetone powder was extracted 1 hour with 60 ml of $5X10^{-4}M$ ATP-$3X10^{-3}M$ cysteine, pH 8.2, per g of powder. Actin was reprecipitated from the soluble extract by addition of 0.1 volumes of 1M acetic acid-1M sodium acetate. The precipitate was dissolved by dialysis against $5X10^{-4}M$ ATP-$3X10^{-3}M$ cysteine, pH 8.2.

At this stage of purification, actin could be made to undergo the G-F transformation in 0.1M KCl as judged by the appearance of flow birefringence and by the conversion of a 3S ultracentrifugal component into a 30S component.

Table I

Identification of an Actin-like Activity in a KCl extract of Acanthamoeba

Addition to viscometer	Time[a] (min.)	Relative Viscosity
9000 x g supernatant fluid	0	2.27
	10	2.42
+ATP (6.6×10^{-4}M)	15	2.37
	42	2.47
+Myosin	56	2.94
	104	3.99
	157[b]	7.42
+ATP (3.0×10^{-4}M)	170	3.29
	290	8.39

The relative viscosity of 3 ml. of a 9000 x g supernatant fraction of amebas containing 24.2 mg/ml of protein was measured at room temperature (about 25°) in an Ostwald-type viscometer with an outflow time for 3 ml. of 0.93M KCl of 90.8 seconds. 4.65 mg. of rabbit skeletal muscle myosin (gift of Dr. W.W. Kielley) was added in 0.3 ml. of 0.5M KCl. The relative viscosity of an identical aliquot of muscle myosin added to 3 ml. of 0.93M KCl did not vary from 1.50 in the presence and absence of 3.0×10^{-4}M ATP. Enough 0.1M ATP was added to produce the final concentrations noted in the table.

[a] Time refers to the start of the viscosity measurement.

[b] The flow time at t = 157 min. was 11 min. 14 sec. ATP was added and mixed as quickly as possible after flow had stopped and the flow time measured at t = 170 min.

Further purification was achieved by making the solution 0.1M in KCl and sedimenting the resulting F-actin by preparative ultracentrifugation at 105,000 X g for 3 hours. The F-actin was depolymerized by dialysis against ATP-cysteine for 2-3 days. Final purification was achieved by gel filtration on Sephadex G-200. The purified G-actin was found at Kav (the partition coefficient between the liquid phase and the gel phase (9)) close to 0.5, which is similar to the behavior reported for muscle actin by Adelstein et al. (8). About 0.1% of the protein of the 9000 X g supernatant fraction was recovered as purified actin. Two to three carboys of amebas are necessary for a yield of 5-10 milligrams of protein.

Control Experiments. The low yield of actin was not unexpected but it made necessary control experiments to prove that the isolated actin was not derived from a contaminant of the skeletal muscle myosin that was used as a specific precipitating reagent in the first step of purification. Several unsuccessful attempts were made to isolate actin from that amount of myosin used in the precipitation step.

As a positive control, amebas were grown in the presence of ^{3}H-histidine and actin was isolated in the usual way. The specific radioactivity of the mixed proteins of the 9000 X g supernatant was 99,000 cpm/mg. The specific radioactivity of the purified actin eluted from Sephadex G-200 was 91,000 cpm/mg. The isolation from amebas grown in the presence of ^{3}H-histidine of radioactive actin with a specific radioactivity identical to that of the total ameba proteins proves that the actin was synthesized by the amebas.

Physical Properties of Ameba Actin. Purified actin in 5M guanidine HCl was analyzed by short column equilibrium centrifugation at three speeds. A single component was present as judged by the linearity of plots of ln c vs. X^2 prepared from ultraviolet scanner traces (292 mμ). The molecular weight, calculated from the slope of the ln c vs. X^2 plots, appears to lie in the range 40,000-50,000, but cannot be specified more closely until the partial specific volume is either measured directly or can be calculated from the complete amino acid composition. (Tryptophan and amide N have not been measured). The molecular weight is probably not very different from that of skeletal muscle or slime mold actin.

The purified G-actin became birefringent in 0.1M KCl and the F-actin was revealed by negative staining electron microscopy as long fibers with a substructure compatible with a helix.

Amino Acid Analyses. In Table II we compare the amino acid composition of ameba actin to published values for actin from the slime mold, Physarum polycephalum (1), and rabbit skeletal muscle (4). Ameba actin is strikingly similar to muscle actin in its content of about 1 mole of 3-methylhistidine

Table II

Comparison of the Amino Acid Composition of Ameba Actin with Other Actins

Residue	Acanthamoeba	Physarum	Rabbit Skeletal Muscle
Lysine	43.4	44.3	49.5
Histidine	16.4	18.4	18.5
3-Methyl Histidine	1.8	---	2.4
Arginine	44.2	43.2	43.6
Asparatic Acid	76.1	90.9	82.6
Threonine	68.7	65.9	63.1
Serine	61.3	60.5	53.9
Glutamic Acid	102	123	104
Proline	45.0	65.9	45.2
Glycine	94.9	80.0	68.5
Alanine	82.6	73.5	72.8
1/2 Cystine	13.1[a]	11.9	12.7[c]
Valine	48.2	34.6	49.2
Methionine	29.4[b]	30.3	37.5
Isoleucine	56.4	36.7	69.9
Leucine	81.8	65.9	64.2
Tyrosine	31.1	34.6	36.9
Phenylalanine	30.3	31.3	28.7

[a]Determined as cysteic acid
[b]Determined as methionine sulfone
[c]Determined by Johnson et al.(15) as S-carboxymethyl cysteine.

Ameba actin, purified through gel filtration was dialyzed vs. water for 2 days, lyophilized, and hydrolyzed in 6N HCl for 22 hrs. at 105° in an evacuated, sealed tube. The neutral and acidic amino acids were determined by the accelerated procedure of Spackman (13) using a Beckman 120B automatic amino acid analyzer operated at a combined flow rate of buffer and ninhydrin of 102 ml/hr. Basic amino acids were separated in two ways: (a) on a 0.9 x 10 cm. column of Beckman resin PA-35 eluted with 0.35M Na citrate, pH 5.28, at 51°C or (b) on a 0.9 x 20 cm column of PA-35 resin, eluted with 0.38M Na citrate, pH 4.26, operated at 37°C for 122 minutes and at 64° C thereafter. Buffer flow rates were likewise 102 ml/hr. In method

(a) epsilon-N-methyl lysine, histidine, and 3-methylhistidine were eluted 8 minutes, 19 minutes and 26.5 minutes after lysine respectively. In method (b) epsilon-N-methyl lysine, histidine and 3-methylhistidine were eluted 6 minutes, 24 minutes, and 36 minutes after lysine respectively. The analysis shown is for ameba actin subjected to performic acid oxidation by the method of Hirs (14), and the values listed under 1/2cystine and methionine therefore refer to cysteic acid and methionine sulfone, respectively. The value shown for tyrosine, which was completely destroyed by the oxidation, has been taken from the analysis of a separate, unoxidized preparation of actin whose analysis was otherwise in close agreement with the present analysis. The analyses shown for slime mold actin and rabbit skeletal muscle actin have been recalculated from the data presented in references (1) and (15). All values are expressed as moles amino acid per 10^5 gms recovered amino acid.

---

per mole of protein. The published analysis of slime mold actin does not include a value for 3-methylhistidine. Comparison of the other amino acids reveals significant similarities and differences among the three actins. The contents of the basic amino acids, of half-cystine, and of phenylalanine are very similar in all preparations. On the other hand, the contents of aspartic acid, glycine, and isoleucine are different in all three preparations.

An unidentified component, which is eluted at the same position as epsilon-N-methyllysine, has consistently appeared in the analyses of the most highly purified preparations of ameba actin in the approximate ratio of 1 mole per mole of 3-methylhistidine.

Discussion. The amino acid compositions of the ameba, slime mold and rabbit actins resemble one another as closely as do the compositions of homologous cytochromes c (11). In addition, 3-methylhistidine is present in actins isolated from such widely divergent forms as protozoa (this report), fish, birds, and mammals (4,5). These observations are consistent with the view that each molecule is the evolutionary descendent of a primordial actin molecule.

In view of its extensive physical and chemical similarities to skeletal muscle actin, it will not be surprising if ameba actin (and other actins of non-muscular origin) is found to participate in cell motility, shape changes, and perhaps in phagocytosis. We also believe it likely that ameba actin is

a component of the 50Å fibrils which comprise a major component of the hyaline cytoplasm adjacent to the plasma membrane and which are the central core of the microprojections that extend from the surface of Acanthamoeba (12).

Acknowledgements. We thank Drs. Robert Adelstein and Michael Kuehl for help with amino acid analyses and useful discussions, Drs. W.W. Kielley and E. Mihalyi for advice in the ultracentrifugal analyses, Dr. Emma Shelton for the electron microscopy. Miss Paddy L. Wright provided technical assistance at certain phases of this research.

References

1. Hatano, S. and Oosawa, F., Biochim. Biophys. Acta, 127, 488, (1966).
2. Hoffman-Berling, H., Biochim. Biophys. Acta, 19, 453, (1956).
3. Bettex-Galland, M. and Luscher, E.F., Nature, 184, 276, (1959).
4. Johnson, P., Harris, C.I. and Perry, S.V., Biochem. J., 105, 361, (1967).
5. Asatoor, A.M. and Armstrong, M.D., Biochem. Biophys. Res. Comm. 26, 168, (1967).
6. Szent-Gyorgyi, A., Chemistry of Muscular Contraction, Academic Press, New York (1947).
7. Mommaerts, W.F.H.M., J. Biol. Chem. 198, 445 (1952).
8. Adelstein, R.S., Godfrey, J.E. and Kielley, W.W., Biochem. Biophys. Res. Comm., 12, 34,(1963).
9. Sephadex-Gel Filtration in Theory and Practice, Pharmacia Fine Chemicals, Uppsala, (1966).
10. Rees, M.K. and Young, M., J. Biol. Chem. 242, 4449, (1967).
11. Nolan, C. and Margoliash, E., Ann. Rev. Biochem., 37, 727, (1968).
12. Bowers, B. and Korn, E.D., J. Cell. Biol. 39, 95, (1968).
13. Spackman, D.H. in Serum proteins and the Dysproteinemias ed. F.W. Sunderman and F.W. Sunderman, Jr., Lippincott, Philadelphia (1964).
14. Hirs, C.H.W., Meth. Enz. 11, 197, (1967).
15. Johnson, P. and Perry, S.V., Biochem. J. 110, 207 (1968).