Peptide Maps of the Myosin Isoenzymes of *Acanthamoeba castellanii*

Hana Gadasi, Hiroshi Maruta, Jimmy H. Collins, and Edward D. Korn

*From the Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205*

Extracts of *Acanthamoeba castellanii* contain four myosin-like ATPases (Maruta, H., Gadasi, H., Collins, J. H., and Korn, E. D. (1979) *J. Biol. Chem.* 254, 3624-3630): double-headed *Acanthamoeba* myosin II and single-headed *Acanthamoeba* myosins IA, IB, and IC, which have heavy chains of 170,000, 130,000, 125,000, and 130,000 daltons, respectively, as well as different light chains. In the accompanying paper, evidence is presented that suggests that *Acanthamoeba* myosin IC is the same molecule as *Acanthamoeba* myosin IA plus a regulatory 20,000-dalton peptide. This conclusion is confirmed by the identity of the peptide maps obtained by limited proteolysis of the heavy chains of *Acanthamoeba* myosins IA and IC by *Staphylococcus aureus* V8 protease. However, peptide maps of the heavy chains of *Acanthamoeba* myosins IA, IB, and IC obtained by limited proteolysis by the *Staphylococcus* protease and chymotrypsin and by chemical cleavage by cyanogen bromide and cyanolation have few, if any, peptides in common. From this evidence, and the enzymatic and subunit data in the accompanying paper, it is concluded that the three *Acanthamoeba* myosin isoenzymes, IA (IC), IB, and II, are products of different genes.

In the accompanying (1) and previous (2–7) papers, we have shown that extracts of *Acanthamoeba castellanii* contain four myosin-like ATPases. *Acanthamoeba* myosin II is a double-headed enzyme with a pair of heavy chains of about 170,000 daltons and two pairs of light chains of about 17,500 and 17,000 daltons (5, 8). Three single-headed enzymes are also present in *Acanthamoeba* (1). *Acanthamoeba* myosin IA contains a single heavy chain of about 130,000 daltons and single light chains of about 17,000 and 14,000 daltons; *Acanthamoeba* myosin IB contains a heavy chain of about 125,000 daltons and light chains of about 27,000 and 14,000 daltons; *Acanthamoeba* myosin IC contains a heavy chain of about 130,000 daltons and light chains of about 20,000, 17,000, and 14,000 daltons. In addition to its different native molecular weight and subunit composition, *Acanthamoeba* myosin II differs dramatically in its enzymatic properties from *Acanthamoeba* myosins IA, IB, and IC which are enzymatically rather similar to each other (1).

The question naturally arises whether any of these four enzymes have a common origin. We presented evidence in the previous paper (1) that *Acanthamoeba* myosin IC consists of *Acanthamoeba* IA plus a loosely bound 20,000-dalton regulatory peptide that inhibits some of the ATPase activities of the enzyme. On the basis of their subunit compositions and enzymatic properties, however, we speculated in the accompanying paper (1) that *Acanthamoeba* myosins IA(IC), IB, and II might have independent origins. This supposition can be directly tested in at least two ways. First, attempts can be made to convert *Acanthamoeba* myosin II, by controlled proteolytic digestion, to products resembling *Acanthamoeba* myosins IA, IB, and IC in subunit composition and enzymatic properties. Second, and without regard to the added complexity imposed by their different light chain compositions, the heavy chains of the several myosins can be compared by peptide mapping.

If, as we supposed (1), *Acanthamoeba* myosin IC is *Acanthamoeba* myosin IA plus a 20,000-dalton peptide, then peptide maps of the 130,000-dalton heavy chains of the two enzymes should be identical. If the 125,000-dalton heavy chain of *Acanthamoeba* myosin IB were derived from the 130,000-dalton heavy chain of IA, or if they have a common origin, then essentially all of the peptides in digests of the heavy chain of IB should also be present in digests of IA and approximately 95% of the peptides in digests of the heavy chain of IA should occur in digests of the heavy chain of IB. Similarly, if the heavy chains of *Acanthamoeba* myosins IA, IB, and IC are derived from the 170,000-dalton heavy chains of *Acanthamoeba* myosin II, almost all of the peptides in digests of the heavy chains of IA, IB, and IC should be present in digests of the heavy chain of II and about 75% of the peptides in digests of the heavy chain *Acanthamoeba* myosin II should occur in the digests of the heavy chains of *Acanthamoeba* myosins IA, IB, and IC.

Finally, consider the possibility that *Acanthamoeba* myosins IA, IB, IC, and II might all be degradation products of a common precursor molecule. The largest heavy chain found in a myosin from any source is about 225,000 daltons (9). Even if the 170,000-dalton chain of *Acanthamoeba* myosin II and the 130,000-dalton chain of *Acanthamoeba* myosin IA were derived from opposite ends of a 225,000-dalton precursor, the minimum overlap would be 75,000 daltons ((170,000 + 130,000) – 225,000). Therefore, about 60% of the peptides in digests of *Acanthamoeba* myosin I heavy chain would also be present in digests of *Acanthamoeba* myosin II heavy chain, while 44% of the peptides in digests of the heavy chain of II should occur in digests of the heavy chain of I. In fact, the overlapping sequence would almost certainly be greater than the arithmetic minimum because all the myosin heavy chains would have to include that portion of the precursor molecule that contained the ATP-binding and ATP hydrolytic sites and the actin-binding site (7).

The data in this paper show that tryptic digestion can convert *Acanthamoeba* myosin II to a single-headed molecule. However, the enzymatic activities of the tryptic digestion products are identical to those of the original enzyme and their subunit compositions also have no similarity to *Acanthamoeba* myosins IA, IB, or IC. Peptide maps of the heavy...
chairs of Acanthamoeba myosins IA, IB, IC, and II after digestion with either Staphylococcus aureus V8 protease, chymotrypsin, cyanogen bromide, or cyanation confirm the suspected identity of the heavy chains of Acanthamoeba myosins IA and IC, but, more importantly, show that they and the heavy chains of Acanthamoeba myosins IB and Acanthamoeba myosins II are the products of three different genes.

EXPERIMENTAL PROCEDURES

Materials—Acanthamoeba myosins IA, IB, and IC and muscle actin and myosin were prepared as described in the accompanying paper (1). Acanthamoeba myosin II was prepared by the procedure of Pollard et al. (8). Partially purified Acanthamoeba myosin IA heavy chain kinase was prepared as described previously (6). ATP, cyanogen bromide, imidazole, and [Tris] were purchased from Sigma Chemical Co.; dithiothreitol and 5,5'-dithiobis(2-nitrobenzoic acid) from Calbiochem Corp.; ultrapure guanidine from Schwarz/Mann; [14C]KCN and N-ethylmaleimide from Amerham Corp.; [32P]ATP from New England Nuclear; tosylphenylalanyl chloromethyl ketone-

FIG. 1. Dodecyl sulfate-polyacrylamide gel electrophoresis of the products of tryptic digestion of Acanthamoeba myosin II. Undigested myosin (T), the unfractionated tryptic digest (T), and fractions separated from the digest by agarose gel chromatography with molecular weights of about 310,000 (310), 275,000 (275), 125,000 (125), and 105,000 (105) were analyzed on 7.5% acrylamide gels. The sample of intact Acanthamoeba myosin II had partially broken down during prolonged storage in sodium dodecyl sulfate. The minor bands of less than 170,000 were not present in the material subjected to trypsin digestion. For details see “Experimental Procedures.”

RESULTS

Tryptic Digestion of Acanthamoeba Myosin II—There was almost no change in either Ca2+-ATPase activity (600 and 622 nmol-min−1 mg−1 before and after) or (K+EDTA)-ATPase activity (158 and 114 nmol-min−1 mg−1 before and after) when Acanthamoeba myosin II (400,000 daltons) was subjected to limited digestion with trypsin as described under “Experimental Procedures.” The two major reaction products (native molecular weights about 310,000 and 125,000) sepa-
Peptide Maps of Acanthamoeba Myosin Isoenzymes

The author presents experiments using gel filtration and dodecyl sulfate-polyacrylamide gel electrophoresis to analyze the polypeptide components of Acanthamoeba myosin II. The results suggest that the larger product is a two-headed enzyme (analogous to muscle heavy meromyosin) and the smaller product a single-headed enzyme (analogous to subfragment-1), both with enzymatic properties similar to those of Acanthamoeba myosin II. Complete enzymatic activity was recovered after further tryptic digestion of the isolated 310,000-dalton product (Ca\(^{2+}\)-ATPase, 593 nmol·min\(^{-1}\)·mg\(^{-1}\); (K\(^{+}\),EDTA)-ATPase, 105 nmol·min\(^{-1}\)·mg\(^{-1}\)). The major products of this second digestion had apparent molecular weights of about 275,000 and 105,000, by agarose gel chromatography, and both major peptide components of about 70,000 daltons and 55,000 daltons (Fig. 1). None of the products of tryptic digestion was a substrate for Acanthamoeba myosin I heavy chain kinase (6). Thus, although tryptic digestion can convert Acanthamoeba myosin II into products with native and subunit molecular weights less than those of Acanthamoeba myosin IA, IB, or IC, the products retain the enzymatic properties characteristic of the Acanthamoeba myosin II from which they were derived. Acanthamoeba myosin II is also not converted into enzymes resembling Acanthamoeba myosin I when incubated with papain or with whole cell extracts (5, 8).

Protease Peptide Maps —The products formed by *Staphylococcus aureus* V8 protease digestion of the 170,000-dalton heavy chain of Acanthamoeba myosin II, the 130,000-dalton heavy chain of Acanthamoeba myosin IA, and the 100,000-dalton products of tryptic digestion of Acanthamoeba myosin II (see Fig. 1) are compared in Fig. 2 (left). There appear to be no common peptides in the maps derived from the heavy chains of Acanthamoeba myosins II and IA. As expected,

1. H. Gadasi, H. Maruta, J. H. Collins, and E. D. Korn, unpublished data.

![Peptide maps of Acanthamoeba myosins IA, IB, and II obtained by limited proteolysis with *Staphylococcus aureus* V8 protease. Left, samples are the heavy chains of Acanthamoeba myosin II and Acanthamoeba myosin IA and the 100,000-dalton peptide derived from Acanthamoeba myosin II by tryptic digestion (see Fig. 1) in 13% acrylamide gels. Right, a separate experiment in which the heavy chains of Acanthamoeba myosins IA, IB, and IC are compared in 13% gels. In both experiments, approximately 20 µg of heavy chain and 0.2 µg of protease were placed in the wells. The differences in the peptide maps of Acanthamoeba myosin IA in the left and right figures are almost certainly due to differences in the protease (the properties of which change upon storage) and the inability to reproduce exactly the experimental conditions (gel properties, digestion in the gel, etc.) in different experiments. When compared side by side, Acanthamoeba myosin IA has been consistently different from IB and from II in about 15 experiments.

![Peptide maps of Acanthamoeba myosins IA, IB, and II obtained by limited proteolysis by chymotrypsin. Approximately 20 µg of the heavy chains of Acanthamoeba myosins IA, IB, and II and 1 µg of chymotrypsin were placed in wells of a dodecyl sulfate-acrylamide gel (9 to 13% gradient).](http://www.jbc.org/)

This experimental approach provides valuable insights into the structural and functional properties of Acanthamoeba myosins, which are crucial for understanding the motile behavior of these amoeboid organisms.
however, almost every peptide in the map of the 100,000-dalton tryptic fragment of Acanthamoeba myosin II is also present in the map of the 170,000-dalton heavy chain of Acanthamoeba myosin II. This latter result serves as a useful control on the procedure. A similar comparison, in a separate experiment, of the heavy chains of Acanthamoeba myosins IA, IB, and IC (Fig. 2, right) shows that the peptide maps of the 130,000-dalton heavy chains of IA and IC are identical to each other but are almost totally dissimilar to the peptide map of the 125,000-dalton heavy chain of IB. Certainly, there are many fewer, if any, peptides of identical electrophoretic mobility in the maps of Acanthamoeba myosins IB and IA than would be expected if the 125,000-dalton chain of IB were derived from the 130,000-dalton chain of IA.

These results are extended and confirmed by comparison of the peptide maps produced by limited proteolysis by chymotrypsin (Fig. 3). The heavy chains of Acanthamoeba myosins II, IA, and IB produce maps with essentially no common peptides.

Cyanylation Peptide Maps—Chemical cleavage at cysteine residues by the cyanylation reaction produced different peptide maps for Acanthamoeba myosins IA, IB, and II (Fig. 4). By Coomassie blue staining, essentially one peptide of about 85,000 daltons was obtained from Acanthamoeba myosin II, while cyanylation of both IA and IB produced many different peptides mostly of lower molecular weight. At least five of the six major peptides derived from Acanthamoeba myosin IB had different electrophoretic mobilities than any of the peptides derived from IA and there were no peptides derived from IB corresponding to the higher molecular weight peptides derived from IA. Almost all of the peptides derived from Acanthamoeba myosins IA and IB were larger than their light chains and, therefore, must have been derived from their heavy chains even though the intact molecule was used in the cyanylation reaction. Acanthamoeba myosin II contains about 36 cysteine residues (8). We assume that there were multiple cleavages in the cyanylation reaction and that many small peptides were removed by chromatography on Sephadex G-25 or were unresolved at the bottom of the electropho-

![Fig. 4. Comparison of the cyanylation products of Acanthamoeba myosins IA, IB, and II. The high molecular weight cyanylation cleavage products were recovered from Sephadex G-25 and analyzed on dodecyl sulfate-polyacrylamide gels (7.5%). Each of the three pairs of gels shows the intact myosin (left) and its cleavage products (right). The same results were obtained for cleavage times of 12 and 18 h in four experiments.](http://www.jbc.org/)

![Fig. 5. The cyanogen bromide peptides of Acanthamoeba myosins IA, IB, and II. Left (15% acrylamide), Acanthamoeba myosin IA (I), two samples of Acanthamoeba myosin II (II), the first reacted in 80% formic acid and the second in 2% sodium dodecyl sulfate, 7% formic acid. Right (17% acrylamide), a separate experiment in which Acanthamoeba myosins IA and IB were reacted in 80% formic acid. The molecular weight standards were run only with the gel on the left. The same results have been obtained for all three enzymes in four separate experiments with reaction times of 67 h. Cleavage of Acanthamoeba myosins IA and IB, but not of Acanthamoeba myosin II, was complete in 24 h.](http://www.jbc.org/)
Peptide Maps of Acanthamoeba Myosin Isoenzymes

retic gel. Cyanylation of the 100,000-dalton tryptic peptide derived from Acanthamoeba myosin II (Fig. 1) gave the same pattern (not shown) as obtained for the native enzyme.

The results with Acanthamoeba myosin IA are incompatible with the absence of cysteine residues in the amino acid analyses reported by Pollard and Korn (2). Primarily for that reason, we repeated the cyanation procedure using [14C]-KCN. Acanthamoeba myosins IA, IB, and II were all labeled with radioactivity and, therefore, each must have contained reactive cysteine residues. Between 48 and 56% of the radioactivity was recovered in the high molecular weight cleavage products recovered from each of the three myosins. Autoradiography of peptide maps obtained from these samples revealed labeled peptides corresponding to those shown in Fig. 4 except that the material at approximately 95,000 daltons derived from Acanthamoeba myosin II was resolved into two bands, presumably because of incomplete cleavage.

The presence of cysteine residues in Acanthamoeba myosin IA was also confirmed by reaction with N-ethyl[2,3,14C]maleimide under conditions that activate the Ca2+-ATPase activity and almost completely inhibit the (K+,EDTA)-ATPase activity of skeletal muscle myosin (12). Identical changes in enzymatic activities were obtained for Acanthamoeba myosin IA and dodecyl sulfate-polyacrylamide gel electrophoresis of the labeled enzyme showed the radioactivity to be localized almost entirely in the 130,000-dalton heavy chain.

Cyanogen Bromide Peptide Maps—Similarly, the peptide maps of Acanthamoeba myosins IA, IB, and II obtained after chemical cleavage at methionine residues by cyanogen bromide show very few, if any, common bands (Fig. 5) and the maps, as a whole, are very different. Cleavage of Acanthamoeba myosin II heavy chain was clearly incomplete since the sum of the molecular weights of the products (Fig. 5) exceeds the molecular weight of the heavy chain (170,000). Reaction in 2% dodecyl sulfate, 7% formic acid, conditions which others (18) have found to give more complete cleavage of muscle myosin than occurs in 80% formic acid, did not result in greater cleavage of Acanthamoeba myosin II (Fig. 5). There were too many peptides of too low molecular weight generated from Acanthamoeba myosins IA and IB to assess whether their cleavage was complete.

DISCUSSION

The identity of the peptide maps of the 130,000-dalton heavy chains of Acanthamoeba myosins IA and IC obtained by limited proteolysis by Staphylococcus aureus V8 protease confirms the conclusion tentatively reached in the accompanying paper from enzymatic data and subunit composition: Acanthamoeba myosin IC is Acanthamoeba myosin IA plus a 20,000-dalton regulatory peptide. In contrast, limited cleavage of the heavy chains of Acanthamoeba myosins IA, IB, and II by proteases, cyanation, and cyanogen bromide produced sets of peptide maps that have very few, if any, peptides in common. The results are certainly incompatible with the predicted 95% identity of peptides if Acanthamoeba myosin IB were derived from IA and almost 75% identity if either were derived from Acanthamoeba myosin II. Although any one procedure might have given a misleading result, the fact that four different procedures gave the same answer strongly indicates that the amino acid sequences of the heavy chains of Acanthamoeba myosins IA(IC), IB, and II are different and, therefore, that they are the products of different genes. The apparent light chains (1) and several other properties (2-7) of these enzymes are also different, adding further strength to the conclusion that they are true isoenzymes. However, the relatively low molecular weights of these myosins, and especially the fact that Acanthamoeba myosins IA, IB, and IC are single-headed enzymes, makes it necessary to consider whether the isolated enzymes are degradation products of unidentified native molecules. No experimental evidence in support of this possibility has been found (1, 4, 5, 8). Moreover, whether or not the isolated Acanthamoeba myosins are the functional forms in vivo, there still must be at least three myosin isoenzymes in this organism because the peptide maps of the heavy chains of the isolated enzymes are too different for them to have been derived from a common precursor.

The ratio of Acanthamoeba myosin I to Acanthamoeba myosin II has remained the same in cultures maintained in this laboratory for almost 10 years (2) and Acanthamoeba myosins IA, IB, and IC occur in the same proportions in cultures that have been maintained separately for 5 years in different laboratories. We have recently found that the myosin isoenzymes also occur in the same ratio in a strain of Acanthamoeba (I-D-4) cloned by Dr. R. J. Neff, Vanderbilt University, in 1976 and selected for its encystment efficiency. This strain also has different nutritional requirements than that which we used in most of our experiments. We believe it is unlikely that all these cultures would still contain the same mixed population of cells but absolute proof that the myosin isoenzymes are in a single cell will require cloning the amoeba2 or reacting them with specific myosin antibodies.

Myosin isoenzymes have been shown to occur in rabbit skeletal muscle (19-23), nematode muscle (24), and chicken tissues (25), but not yet in any other non-muscle cell. Possibly, the complexity of motile activities in free living, exponentially growing amoeba requires the presence of myosins not required, at least in substantial amounts, in differentiated cells. Processes such as ameboid motility, cell division, salivary motion, endocytosis, and exocytosis, although all dependent on actomyosin, may utilize significantly different molecular mechanisms involving different myosin isoenzymes. It is generally assumed that motile events in non-muscle cells involve a sliding filament mechanism analogous to that of muscle, with the actin filaments attached to the structures to be moved and the myosin organized into bipolar filaments that pull the actin filaments. Acanthamoeba myosin II (8), but not Acanthamoeba myosin I (2), has been shown to make bipolar filaments in vitro. More speculatively, actin filaments might be made to slide relative to one another by mechanisms requiring myosin molecules but not myosin filaments, for example, in the way that the tubulin-dynein system seems to function in ciliary axonemes (26, 27). Moreover, movement of intracellular organelles has been envisaged as possibly occurring by the interaction of actin filaments with myosin attached to the organelles (28, 29) and such a mechanism also might not require filaments. Therefore, although we do not rule out the possibility that the single-headed Acanthamoeba myosins I may be degradation artifacts of two-headed myosins or, alternatively, that they may be able to form bipolar filaments, these myosins might function in Acanthamoeba by mechanisms that do not require myosin filaments.

REFERENCES

1. Maruta, H., Gadasi, H., Collins, J. H., and Korn, E. D. (1979) J. Biol. Chem. 254, 3624-3630
2. Pollard, T. D., and Korn, E. D. (1973) J. Biol. Chem. 248, 4682-4690
3. Pollard, T. D., and Korn, E. D. (1973) J. Biol. Chem. 248, 4691-4697
4. Maruta, H., and Korn, E. D. (1977) J. Biol. Chem. 252, 399-402
5. Maruta, H., and Korn, E. D. (1977) J. Biol. Chem. 252, 6501-6508

*The original culture has now been cloned. Cells derived from a single amoeba contain Acanthamoeba myosins IA(IC), IB, and II in the same ratio as the parent culture (1).
6. Maruta, H., and Korn, E. D. (1977). J. Biol. Chem. 252, 8329–8332
7. Maruta, H., Gadasi, H., Collins, J. H., and Korn, E. D. (1978) J. Biol. Chem. 253, 6297–6300
8. Pollard, T. D., Stafford, W. P., III, and Porter, M. E. (1978) J. Biol. Chem. 253, 4789–4808
9. Korn, E. D. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 588–599
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
11. Pettit, F. H., Hamilton, L., Munk, F., Namihira, G., Eley, M. H., Willms, C. R., and Reed, L. J. (1973) J. Biol. Chem. 248, 5282–5290
12. Reisler, E., Burke, M., and Harrington, W. F. (1974) Biochemistry 13, 2014–2022
13. Anker, A. S. (1970) FEBS Lett. 7, 293
14. Laemmli, U. K. (1970) Nature 227, 680–685
15. Fairbanks, G., Steck, T. L., and Wallach, D. F. (1971) Biochemistry 10, 2606–2617
16. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102–1106
17. MacLeod, A. R., Waterston, R. H., Fishpool, R. M., and Brenner, S. (1977) J. Mol. Biol. 114, 133–140
18. Bálint, M., Srèter, F. A., Wolf, I., Nágy, B., and Gergely, J. (1975) J. Biol. Chem. 250, 6168–6177
19. Starr, R., and Offer, G. W. (1973) J. Mol. Biol. 81, 17–31
20. Pope, B. J., Wagner, P. D., and Weeds, A. G. (1977) J. Mol. Biol. 109, 470–473
21. Holt, J. C., and Lowey, S. (1977) Biochemistry 16, 4398–4402
22. Wagner, P. D., and Weeds, A. G. (1977) J. Mol. Biol. 109, 455–470
23. Hob, J. F. Y. (1978) FEBS Lett. 90, 297–300
24. Schachat, F. H., Harris, H. E., and Epstein, H. F. (1977) Cell 10, 721–728
25. Burridge, K., and Bray, D. (1975). J. Mol. Biol. 99, 1–14
26. Summers, K. E., and Gibbons, I. R. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 3092–3096
27. Sale, W. S., and Suzuki, P. (1976) J. Cell Biol. 71, 589–605
28. Berli, S., Puskin, W., and Nicklas, J. (1973) Science 179, 441–446
29. Allen, N. S., and Allen, R. D. (1978) Annu. Rev. Biochem. 47, 497–526
Peptide maps of the myosin isoenzymes of Acanthamoeba castellanii.
H Gadasi, H Maruta, J H Collins and E D Korn

J. Biol. Chem. 1979, 254:3631-3636.

Access the most updated version of this article at http://www.jbc.org/content/254/9/3631

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/254/9/3631.full.html#ref-list-1