Acanthamoeba Actin. Composition of the Peptide That Contains 3-Methylhistidine and a Peptide That Contains N^{ϵ}-Methyllysine†

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ABSTRACT: Three peptides have been isolated from *Acanthamoeba* actin whose properties and amino acid compositions are very similar to peptides that had previously been isolated from muscle actin. The peptides were prepared by cleavage of ameba actin with cyanogen bromide and partially fractionated by chromatography on Sephadex G-50. One ameba peptide was then isolated by ion-exchange chromatography on phosphocellulose and on DEAE-cellulose. The composition of this peptide, including the presence of nearly 1 mole of 3-methylhistidine/mole of peptide, is very similar to the composition of peptide CB-10 from rabbit muscle actin. A second peptide isolated from ameba actin is distinguished by its insolubility in 1 mM Tris-chloride (pH 8) after elution from phosphocellulose. Its composition is very similar to that of peptide CB-16 from rabbit muscle actin, but unlike the muscle actin peptide it contains at least 0.42 mole of N^{ϵ}-methyllysine/mole of peptide. The third ameba actin peptide resembles peptide CB-17 from muscle actin in that it is eluted with 3 M NH_4OH from phosphocellulose and has a very high content of glutamic acid but no proline. The data are compatible with the view that muscle and ameba actins contain regions of similar amino acid sequence. Direct comparison of the molecular weights of *Acanthamoeba* actin and rabbit muscle actin by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, contrary to our earlier supposition, shows that the molecular weights of the two actins are identical (45,000–46,000).

Actin isolated from *Acanthamoeba* (Weihing and Korn, 1971) closely resembles muscle actin. Both proteins form double-helical filaments with identical half-pitches and widths (Pollard *et al.*, 1970). Both actins bind muscle heavy meromyosin to form arrowhead complexes (Huxley, 1963) which cannot be distinguished (Pollard *et al.*, 1970). Furthermore, the abilities of both ameba and muscle actin to activate heavy meromyosin adenosine triphosphatase (Weihing and Korn, 1971) and to interact with native tropomyosin from rabbit muscle are qualitatively similar (Eisenberg and Weihing, 1970).

The amino acid compositions of the two actins are also very similar and both proteins contain the unusual amino acid, 3-methylhistidine (Weihing and Korn, 1969). This suggested that muscle and ameba actin might have regions of similar amino acid sequences. We now report on the cleavage of ameba actin with cyanogen bromide and the isolation of the peptide containing 3-methylhistidine. The compositions of this ameba actin peptide and of the corresponding muscle actin peptide (previous isolated by Adelstein and Kuehl, 1970, and Elzinga, 1970) are similar but probably not identical.

Ameba actin contains two other unusual amino aicds, N^{ϵ}-dimethyllysine and smaller amounts of N^{ϵ}-monomethyllysine (Weihing and Korn, 1970, 1971), which are not present in muscle actin (Kuehl and Adelstein, 1969; Hardy *et al.*, 1970). We also report here that the composition of an ameba actin peptide which contains methylated lysine is very similar to that of peptide CB-16 from rabbit actin (Adelstein and Kuehl, 1970). A third peptide from ameba actin, which is remarkable for its high content of glutamic acid and for the absence of proline, has a composition close to that of peptide CB-17 from muscle actin (Adelstein and Kuehl, 1970). A preliminary report of this work has appeared (Weihing, 1971).

Previously we reported that the molecular weight of *Acanthamoeba* actin was 39,500 as determined by sedimentation equilibrium ultracentrifugation (Weihing and Korn, 1971). This is substantially lower than the molecular weight of muscle actin (45,000–46,000) (Rees and Young, 1967) and we remarked that this difference seemed larger than might be expected for a protein which otherwise resembled muscle actin so closely. In this paper we directly compare the molecular weights of *Acanthamoeba* actin and muscle actin by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The proteins migrate identically and we conclude that the molecular weights of the two proteins are, in fact, identical.

Methods

Isolation of Peptides. Acanthamoeba actin was purified as described previously (Weihing and Korn, 1971). The peptides produced by cleavage with cyanogen bromide were isolated using the procedure published for muscle actin (Adelstein and Kuehl, 1970; Elzinga, 1970). The procedure has been carried out with three separate preparations of ameba actin with essentially identical results. However, only the results for the preparation of actin which contained the highest content of 3-methylhistidine and methyllysine are presented because, on this basis, that preparation was deemed to be the purest.

In order to have a convenient, sensitive assay for the peptides during their isolation, actin was labelled with non-exchangeable tritium by the free radical interceptor method of White *et al.* (1969). Labeled actin (18 mg) was lyophilized repeatedly from water to remove exchangeable tritium, and the radioactive actin was mixed with about 26 mg of non-radioactive actin. The final specific activity was about 30,000 cpm/μmole of amino acid. Therefore, about 0.001 μmole of amino acid could be conveniently detected using the counting procedures described previously (Weihing and Korn, 1971).

The tritiated actin was reduced and alkylated with iodoacetamide as described previously (Weihing and Korn, 1971).

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TABLE I: Distribution of Methylamino Acids after Sephadex G-50 Chromatography of Cyanogen Bromide Peptides of Ameba Actin.[a]

Fraction[b]	Mole/Mole of Homoserine	
	3-Methylhistidine	N^{ϵ}-Methyllysine
A-3	0	0.04
A-4	0.1	0.11
A-5 + 6	0	0
Actin[c]	0.048	0.069

[a] The fractions were prepared and analyzed as described in Methods. [b] The eluate from the Sephadex G-50 column (Figure 1) was pooled as follows: A-1, 47–56 ml; A-2, 57–74 ml; A-3, 75–87 ml; A-4, 88–104 ml; A-(5 + 6), 106–124 ml; A-7, 125–155 ml. No data are shown for fractions A-1, A-2, and A-7 because insufficient material was available for quantitatively significant determinations of 3-methylhistidine and N^{ϵ}-methyllysine. [c] Calculated from the composition of ameba actin (Weihing and Korn, 1971) by dividing the moles of methylamino acid by the moles of methionine.

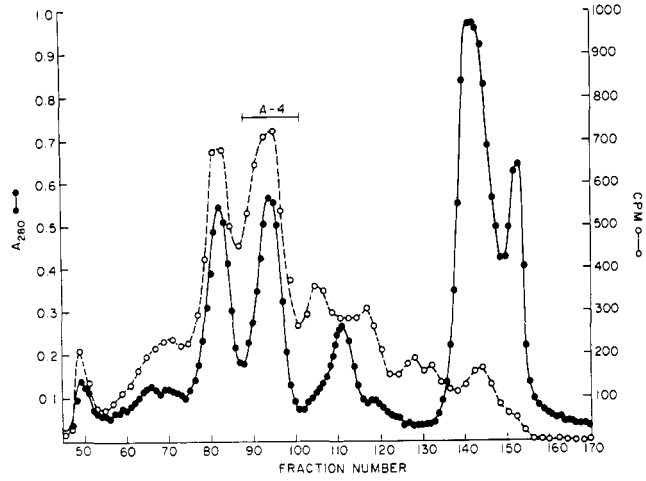

FIGURE 1: Fractionation of cyanogen bromide peptides of ameba actin on Sephadex G-50. Cyanogen bromide peptides of tritiated ameba actin were applied in a small volume of 70% formic acid to a column of Sephadex G-50 fine, about 1 × 200 cm, and eluted at room temperature with 20% formic acid containing 0.1% 2-mercaptoethanol. Fractions of 2.9 ml were collected, and aliquots of 0.01 ml were used for determination of radioactivity.

The actin was then subjected to reduction for 2 days at 37° by dialyzing it against a solution of 30% 2-mercaptoethanol–5 M guanidine hydrochloride–0.1 M NH_4HCO_3 as suggested by Adelstein and Kuehl (1970), to convert any methionine sulfoxide, which cannot react with cyanogen bromide, to methionine. After removal of solutes by dialysis against several changes of ice-cold water, the actin was lyophilized, dissolved in a small volume of 70% formic acid, and treated with cyanogen bromide (product of Eastman) as described by Adelstein and Kuehl (1970). The separation of the peptides formed by cyanogen bromide cleavage is described in the Discussion.

Amino Acid Analysis. Conditions of hydrolysis and amino acid analysis were as described previously (Weihing and Korn, 1971) with the following exceptions. Hydrolysis was usually for 18–22 hr. The basic amino acids were separated on a 0.9 × 20 cm column of Beckman PA-35 resin eluted with 0.35 M citrate (pH 5.28) at 52° as used by Adelstein and Kuehl (1970), for analysis of muscle actin peptides. As previously noted, 3-methylhistidine is eluted about midway between histidine and ammonia. N^{ϵ}-Dimethyllysine and N^{ϵ}-monomethyllysine are coeluted midway between lysine and histidine. About 75% of the methylated lysine in ameba actin is N^{ϵ}-dimethyllysine and 25% is N^{ϵ}-monomethyllysine (Weihing and Korn, 1971). Because so many analyses were necessary and because this column was simpler and faster to use than the low-temperature, low flow rate systems used earlier to separate mono-, di-, and trimethyllysine (Weihing and Korn, 1971), we felt that this ambiguity in the composition of peptides containing methyllysine was tolerable. Most of the analyses were done on a Beckman 121 amino acid analyzer equipped with automatic sample injector, and an Infotronics integrator. Calculations were performed by computer using the integrator output. Tryptophan was estimated spectroscopically as described by Edelhoch (1967) except that peptides were left in the buffers with which they were eluted instead of being transferred to the phosphate-buffered guanidine solutions he described.

Electrophoresis. Electrophoresis was carried out as follows using polyacrylamide gels containing sodium dodecyl sulfate. Gels were polymerized from a mixture of 6.6% acrylamide, 0.35% bisacrylamide, 0.1 M Tris (pH 6.8) (prepared by 20-fold dilution of a stock buffer solution of 2 M Tris brought to pH 6.8 with concentrated phosphoric acid), 0.1% sodium dodecyl sulfate, 0.083% Temed, and 0.042% solid ammonium persulfate. The electrode buffer was 0.1% sodium dodecyl sulfate–0.1 M Tris (pH 6.8) (prepared by dilution of stock as above). Gels were subjected to preliminary electrophoresis without sample for 30–60 min at a constant voltage of 80 V which produced about 7 mA of current per gel. Samples, consisting of 10–20 μg of protein in 10–20 μl of 0.1 M Tris (pH 6.8) (prepared by dilution of stock as above), 1% sodium dodecyl sulfate, 10% sucrose, and Bromophenol Blue, were then subjected to electrophoresis for 1–1.5 hr under the same conditions. After the tracking dye had moved close to the bottom of the gel, the gels were removed from their tubes, and the position of the tracking dye was marked by injection of India ink with a Hamilton syringe (Fairbanks *et al.*, 1971). The gels were fixed and stained for 15 min in a mixture of 1% Amido Black, 7% acetic acid, and 40% ethanol. They were then washed with 7% acetic acid, 40% ethanol, and destained in the same solution with Bio-Rad resin AG1-X2 in the chloride form. After destaining, the gels were placed in 7% acetic acid, and after several days they were scanned at 600 nm using the gel scanning attachment of the Beckman Acta III spectrophotometer.

Results

Isolation of Peptides. The cyanogen bromide peptides of ameba actin, prepared as described under Methods, were partially fractionated on Sephadex G-50. The distribution of peptides (Figure 1) was similar to that reported by Adelstein and Kuehl (1970) in their Figure 1B.

One fraction of ameba actin peptides contained twice as much 3-methylhistidine relative to homoserine as did unfractionated actin (Table I). This fraction was eluted in the same position as that fraction of muscle actin peptides previously designated A-4 (Adelstein and Kuehl, 1970) which was also enriched in 3-methylhistidine relative to unfractionated actin. The A-4 fraction of ameba actin peptides also contained

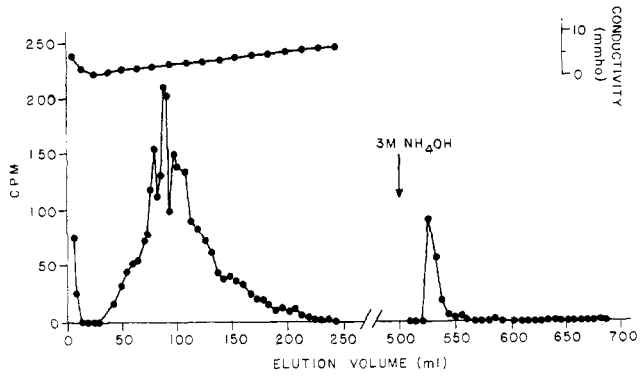

FIGURE 2: Fractionation of peptide fraction A-4 on phosphocellulose. Fraction A-4 was dried under reduced pressure, dissolved in a small volume of 20% acetic acid at 55°, and applied to a 0.6 × 20 cm column of phosphocellulose which had previously been equilibrated with 1% acetic acid. The column was then eluted at 55° with a linear gradient formed with 70 ml of 1% acetic acid in the mixing chamber and 70 ml of 0.2 M pyridine brought to pH 3.1 with glacial acetic acid in the reservoir chamber of a Büchler gradient mixer. After 100 ml of this mixture had been pumped through the column, the fluid in the mixing chamber was replaced with 200 ml of 0.15 M pyridine brought to pH 3.1 with glacial acetic acid, and that in the reservoir was replaced with 200 ml of 0.7 M pyridine brought to pH 3.1 with glacial acetic acid. Fractions of 3 ml were collected and aliquots of 0.01 ml were used for determination of radioactivity. Conductivity was monitored with a Radiometer conductivity meter.

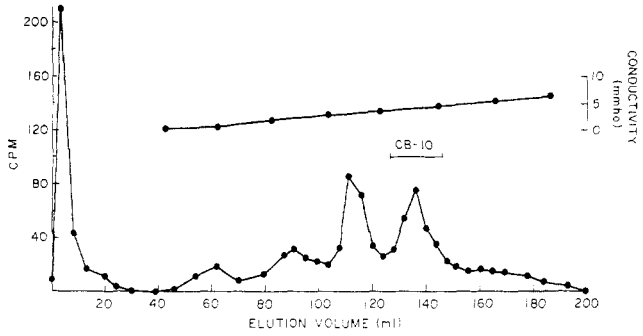

FIGURE 3: Further fractionation of peptide mixture A-4 on DEAE-cellulose. The mixture of peptides eluted from phosphocellulose between 30 and 220 ml were pooled and dried under vacuum and then heated in a small volume of 1 mM Tris-chloride (pH 8) at 60°. The soluble peptides were applied to a 0.6 × 24 cm column of DEAE-cellulose previously equilibrated with 1 mM Tris-chloride (pH 8) at 25°. The column was eluted with a linear gradient formed with 200 ml of 1 mM Tris-chloride (pH 8) in the mixing chamber and 200 ml of 1 mM Tris-chloride (pH 8) containing 300 mM NaCl in the reservoir of the Büchler mixer (Elzinga, 1970). Fractions of 4.1 ml were collected and aliquots of 0.01 ml were used for determination of radioactivity.

twice as much methyllysine relative to homoserine as did unfractionated actin (Table I). Therefore only this fraction was used for further fractionations. However, a significant fraction of methylysine (but not 3-methylhistidine) was also present in Sephadex fraction A-3.

Initially we attempted to isolate ameba actin peptide CB-10 (the 3-methylhistidine peptide) from Sephadex fraction A-4 by chromatography on phosphocellulose using pyridine–acetate gradients (Adelstein and Kuehl, 1970). 3-Methylhistidine (as well as methylysine) was found in three overlapping peaks which were eluted between 30 and 220 ml (Figure 2). These fractions were pooled and dried under reduced pressure.

The pooled peptides were heated at 60° in a small volume of 1 mM Tris-chloride (pH 8). After several cycles of heating and cooling about 70% of the peptides were solubilized. The soluble peptides were fractionated on DEAE-cellulose (Figure 3). 3-Methylhistidine was found only in the fraction eluted at a conductivity of 4 mmhos, which was close to the position at which Elzinga (1970) found peptide CB-10 of muscle actin. The composition of this fraction corresponded to that of peptide CB-10 from muscle actin (see below).

As already stated about 30% of the peptides eluted from phosphocellulose with pyridine–acetate did not dissolve in 1 mM Tris-chloride. Amino acid analysis of this insoluble fraction showed that it was highly enriched in methylysine and that its composition was close to that of peptide CB-16 from rabbit actin (see below).

After the mixture of peptides which contained 3-methylhistidine and methyllysine had been eluted from phosphocellulose with pyridine–acetate gradients, the column was stripped with 3 M NH_4OH (Figure 3). The amino acid composition of this fraction was close to that of peptide CB-17 from rabbit actin (see below).

Peptide CB-10. This ameba actin peptide, like the corresponding peptide from muscle actin, was isolated in good yield from Sephadex fraction A-4 (Adelstein and Kuehl, 1970) and was then purified by a combination of procedures previously used for the muscle actin peptide—phosphocellulose chromatography (Adelstein and Kuehl, 1970) followed by elution from DEAE-cellulose with an NaCl gradient (Elzinga, 1970). The ameba actin peptide contains at least 0.75 mole of 3-methylhistidine/mole of homoserine (Table II) which is close to the value 0.8 reported for CB-10 by Adelstein and Kuehl (1970), but which is somewhat smaller than the value 1.05 reported by Elzinga (1970). The remainder of the composition of the ameba actin peptide fraction is also very close to that of peptide CB-10 from muscle actin. We conclude that regions of very similar amino acid sequence and which contain 3-methylhistidine are present in both muscle and ameba actin.

The presence of less than 1 mole of 3-methylhistidine/mole of homoserine in the ameba actin peptide CB-10 suggests incomplete methylation of that histidine residue. This possibility is consistent with our earlier observation that the purified ameba actin contains an average of 0.78 mole of 3-methylhistidine/45,000 g of protein[1] (Weihing and Korn, 1971) instead of 1 mole/45,000 g as in muscle actin (Adelstein and Kuehl, 1970; Elzinga, 1970). However, since we have not isolated a nonmethylated peptide, we cannot rule out the possibility that the protein and the peptide were incompletely purified.

While the composition of this ameba actin peptide is nearly identical with that of muscle actin peptide CB-10 (Table II), a few differences are apparent. The ameba actin peptide appears to contain two lysines, but the corresponding muscle peptide contains three. This difference was observed in the two other independent analyses that are not shown which suggests that the difference is real. In those other two analyses, the contents of 3-methylhistidine and lysine were lower than in the muscle peptide but in the same ratio relative to each other.

[1] As discussed elsewhere in the text, we now believe that the correct molecular weight of ameba actin is 45,000. Therefore, the content of a given amino acid relative to 1 mole of protein is presented relative to 45,000g of protein rather than 39,500g as was used previously (Weihing and Korn, 1971).

TABLE II: Comparison of Amino Acid Composition of Certain Cyanogen Bromide Peptides of Ameba Actin to Corresponding Peptides from Muscle Actin.[a]

	CB-10		CB-16		CB-17	
Residue	Ameba	Muscle[b]	Ameba	Muscle[b]	Ameba	Muscle[b]
HSer	1	1	1	1	1	1
Lys	2.46 (2)[c]	3	1.77 (2)	2	3.06 (3)	3
$(Me)_2$Lys[d]	0 (0)	0	0.42[e]	0	0 (0)	0
His	0.16 (0)	0	0.08 (0)	0	0 (0)	0
3-Me-His	0.75 (1)	1	0 (0)	0	0 (0)	0
Arg	0.94 (1)	1	1.16 (1)	1	3.35 (3)	3
CMCys	0 (0)	0	0 (0)	0	0.39 (1)[f]	1
Asp	4.61 (5)	5	1.58 (2)[c]	1	2.54 (3)[c]	4
Thr	2.01 (2)	2	1.59 (2)	2	4.58 (5)[c]	3
Ser	2.34 (2)	2	3.05 (4)	4	1.28 (1)	1
Glu	4.36 (4)	4	4.18 (4)	4	8.83 (9)[c]	7
Pro	1.16 (1)	1	1.98 (2)	2	0.29 (0)	0
Gly	3.85 (4)	4	2.72 (3)	3	1.70 (2)[c]	1
Ala	1.66 (2)[c]	1	2.64 (3)	3	2.61 (3)	3
Val	1.39 (1)	1	1.13 (2)[g]	2	1.62 (2)[c]	3
Ile	1.97 (2)[h]	4	2.98 (3)[c]	4	2.21 (2)[c]	3
Leu	2.12 (2)	2	2.53 (3)	3	3.40 (3)[c]	4
Tyr	1.67 (2)	2	1.19 (1)	1	2.15 (2)	2
Phe	0.40 (0)	0	1.21 (1)	1	2.30 (2)	2
Trp	2.32 (2)[c]	1	1.21 (1)	1		0
Total	34	35	35	35	40	41
Yield[i] (%)	42		54		13	

[a] Values are expressed as moles of amino acid per mole of homoserine.Values in parentheses have been rounded off to the nearest whole integer, with the exceptions discussed below. [b] Values taken from Adelstein and Kuehl (1970). The values presented by Elzinga (1970) for CB-10 from muscle actin are identical. [c] As discussed in the text, the difference between this value and the value for the muscle peptide appears significant. [d] Identification as N^{ϵ}-dimethyllysine is tentative (see text). [e] This value has not been rounded off to zero because, as discussed in the text, it is clear that methyllysine is present in this peptide. It has not been rounded off to one because it is not known whether the peptide is completely methylated. [f] This value has been rounded off to one instead of zero because the corresponding muscle actin peptide contains one CMCys and because the yield of CMCys in the ameba actin was low (only 3.8 moles/45,000 g instead of the expected 5.6 moles/45,000 g) (Weihing and Korn, 1971).[19] [g] The result of a prolonged hydrolysis was considered in assigning this value. [h] As discussed in the text, the difference between this value and the value for the muscle peptide appears questionable. [i] The yield was calculated for the steps following Sephadex fractionation as: (moles of amino acid in purified peptide × 100)/(fraction of total actin amino acids expected in peptide × moles of total amino acids in all fractions from Sephadex G-50).

It is possible, therefore, that the ameba actin peptide actually contains three lysines instead of two, if the ameba peptide actually contains 1 mole of 3-methylhistidine instead of 0.75. However, since we do not know if contaminating peptides caused the variations in content of 3-methylhistidine in the three analyses, and since the lowest measured value for lysine (1.88 moles/mole of homoserine) still rounds off to 2, we must accept the difference in lysine as real.

Ameba actin peptide CB-10 appears to contain two alanines, but the muscle peptide contains only one. This difference was also found in the other two analyses which are not shown.

Ameba actin peptide CB-10 appears to contain two isoleucines instead of the four present in the muscle peptide. Since some of the isoleucine in the peptide from muscle actin is released only after prolonged hydrolysis (personal communication from M. Elzinga, cited by Adelstein and Kuehl, 1970), however, this difference may not be significant.

The ameba actin peptide appears to contain two tryptophans, one more than the single tryptophan present in the peptide from muscle actin. This difference was observed in the two analyses in which tryptophan was estimated, making it likely that this difference is real.

Peptide CB-16. Like the corresponding muscle actin peptide, peptide CB-16 was isolated from Sephadex fraction A-4 (Adelstein and Kuehl, 1970). The ameba actin peptide was eluted from phosphocellulose with pyridine acetate gradients at a lower concentration of pyridine than reported for the peptide from muscle actin (Adelstein and Kuehl, 1970). A new finding is that after elution from phosphocellulose the peptide can be isolated as that fraction which is insoluble in 1 mM Tris-chloride (pH 8.1). These properties agree in general with those of CB-16 from muscle actin, and the composition of the ameba actin peptide fraction is so close to peptide CB-16 from muscle actin (Table II) that we have concluded that similar peptide sequences, designated CB-16, occur in both muscle and ameba actin.

Ameba actin peptide CB-16 contains at least 0.42 mole of methyllysine/mole of homoserine (Table II). The ameba actin peptide therefore differs in this respect from peptide CB-16 from muscle actin which does not contain methyllysine

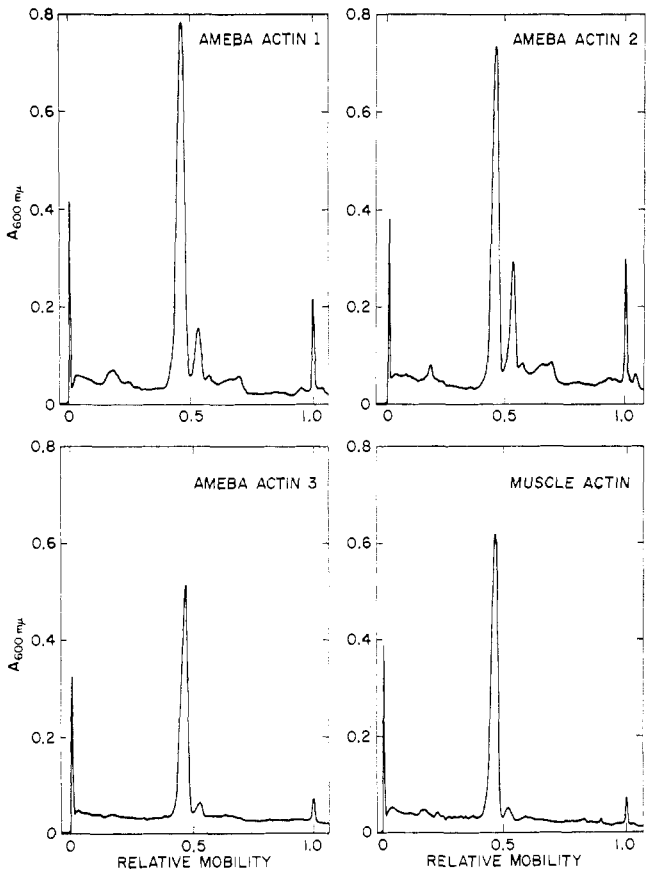

FIGURE 4: Polyacrylamide gel electrophoresis of ameba and muscle actin in the presence of sodium dodecyl sulfate. Electrophoresis was performed and the gels were scanned as described in Methods using muscle and ameba actin prepared and reduced and alkylated as described previously (Weihing and Korn, 1971). Relative mobility of zero is the top of the gel; relative mobility of one is the position of the tracking dye marked by injection of India ink.

(Adelstein and Kuehl, 1969; Hardy *et al.*, 1970). Nevertheless, the compositions of this ameba peptide and muscle peptide CB-16 are so close that we have concluded that the ameba peptide is properly identified as CB-16 and that it contains methyllysine.

The ameba actin peptide CB-16 appears to contain two aspartic acids, but the corresponding muscle actin peptide contains only one. This difference was also observed in the two analyses not shown and the difference, therefore, is probably real.

The ameba actin peptide appears to contain three isoleucines but the muscle actin peptide contains four. The lower value was also observed in a prolonged hydrolysis, which is not shown, and the difference, therefore, seems to be real.

As discussed under Methods, the chromatographic system used for the separation of the basic amino acids does not separate N^{ϵ}-dimethyllysine from N^{ϵ}-monomethyllysine. Therefore it cannot be ascertained from the present data whether ameba peptide CB-16 contains only N^{ϵ}-dimethyllysine or a mixture of N^{ϵ}-di- and N^{ϵ}-monomethyllysine. The peptide cannot contain only N^{ϵ}-monomethyllysine because an average of only 0.27 mole of this residue is present per mole of protein[1] (Weihing and Korn, 1971), and the peptide contains at least 0.42 mole of methyllysine/mole of homoserine.

The isolation of less than 1 mole of methyllysine/mole of homoserine suggests that the peptide is incompletely methylated. However, as with peptide CB-10, we have not isolated the nonmethylated peptide and we cannot rule out completely the possibility that the peptide was incompletely purified.

TABLE III: Parameters of Polyacrylamide Gel Electrophoresis of Ameba and Muscle Actin in the Presence of Sodium Dodecyl Sulfate.[a]

Source of Actin[c]	Relative Mobility[b]		% Actin[d]
	Actin	X	
Muscle	0.46	0.51	96
Ameba-1	0.45	0.51	86
Ameba-2	0.45	0.52	74
Ameba-3	0.47	0.51	93

[a] Electrophoresis was performed as described in Methods. [b] The relative mobility was calculated by dividing the distance the protein had moved from the top of the gel by the distance the tracking dye had moved from the top of the gel. [c] Muscle and ameba actin were prepared and reduced and alkylated as described previously (Weihing and Korn, 1971). [d] The percent actin was calculated from the relative areas under the peaks in Figure 4 by triangulation.

Peptide CB-17. The ameba actin peptides also yield a fraction whose properties and composition correspond with those of peptide CB-17 from muscle actin. Like the corresponding muscle actin peptide (Adelstein and Kuehl, 1970), it is isolated in relatively low yield from Sephadex fraction A-4 by elution with ammonia from phosphocellulose following pyridine–acetate elution of other peptides. The remarkably high content of glutamic acid combined with the absence of proline, which is unusual for actin peptides of this size (Adelstein and Kuehl, 1970), completely distinguish CB-17 from other actin peptides.

The frequencies of occurrence in ameba actin CB-17 of aspartic acid, threonine, glutamic acid, and glycine differ by one or two residues from the muscle peptide (Table II). These differences were also observed in the other available analyses of the ameba peptide and, therefore, are probably real.

The content of valine, isoleucine, and leucine appears to be lower by one residue each in ameba actin CB-17 than in muscle actin CB-17. Although peptide bonds involving these residues often resist hydrolysis, identical periods of hydrolysis (18–22 hr) were used for the ameba actin peptides and for the muscle actin peptides (Adelstein and Kuehl, 1970), making it likely that the differences observed between the two peptides are significant.

Polyacrylamide Gel Electrophoresis. Electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate was used as a rapid assay for the purity of the actin used for isolation of peptides. In addition, we wished to compare directly the molecular weights of the ameba actin and muscle actin because we previously reported that the molecular weight of ameba actin was about 10% less than the molecular weight of muscle actin (Weihing and Korn, 1971).

As much as 93% of the stainable protein of ameba actin migrated with the same electrophoretic mobility as 96% of the protein of muscle actin (Figure 4 and Table III). Therefore, the molecular weight of homogeneous ameba actin must be the same as the molecular weight of homogeneous muscle actin, *i.e.*, 45,000–46,000 (Rees and Young, 1967).

The electrophoretic pattern of the ameba actin used for preparing the peptides whose composition is reported in this paper is labeled "ameba actin 1" in Figure 4. Of the stained protein in that preparation, 86% is found in a single band of molecular weight 45,000 (Table III). Therefore the ameba actin used for this study was of sufficient purity for the isolation of peptides whose composition was to be compared to peptides of a protein known to be similar.

The nature of the lower molecular weight components which can be identified in all the preparations of actin shown in Figure 4 is unknown. It is of interest that the major contaminant band is of identical molecular weight in the four gels shown here, and that a contaminant with an identical molecular weight has been identified in two other preparations of ameba actin and one of muscle actin (not shown). It seems unlikely that the same contaminant protein would be present in actin isolated from muscle and amebas. Another possibility is that, during the long periods required to isolate the actin and to carry out reduction and alkylation, bacterial proteases cause partial and rather specific digestion of both muscle and ameba actin. Further experiments will be necessary, however, to explain the origin of the lower molecular weight contaminants.

Discussion

We previously reported several similarities between muscle and ameba actins which made it reasonable to suppose that the two actins contain regions of similar or identical amino acid sequence (Weihing and Korn, 1971). The present results, which show that ameba actin yields cyanogen bromide peptides whose compositions are very similar (but probably not identical) to those produced from muscle actin, are entirely consistent with our previous view. Of course, only the determination of the complete amino acid sequence of both proteins will establish the extent of identity.

We previously reported that the molecular weight of ameba actin, as determined by sedimentation equilibrium centrifugation, was 39,500 which is about 10% less than the most recent molecular weight of 45,000–46,000 published for muscle actin (Rees and Young, 1967). We have now directly compared the molecular weight of ameba actin with muscle actin using polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The electrophoretic mobility of the two proteins is identical in this system which indicates that their molecular weights are in fact identical.

The reason for our earlier observation of a lower molecular weight for ameba actin is unknown. We are certain, however, that the low molecular weight reported earlier cannot be the result of using actin consisting mostly of the low molecular weight contaminant observed by gel electrophoresis of many actin preparations. One of the two molecular weight measurements reported previously was made on the nearly homogeneous ameba actin which gave the electrophoretic pattern "ameba-3" (Figure 4). Other possible sources of error, such as the use of an incorrect partial specific volume or incorrect calibration of the centrifuge, cannot be checked without further extensive experiments, and the source of the error must remain unknown.

Close structural and functional resemblances of proteins in organisms which are phylogenetically as distant as mammals and protozoa are frequently thought to signify that the present day molecules have evolved from a primordial precursor molecule. Another mechanism which is consistent with such similarity is transduction of the structural gene (Anderson, 1970) from one organism to another *via* viruses (Merril *et al.*, 1971).[2] Our data do not exclude either of these possibilities.

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[2] Professor T. E. Thompson suggested this mechanism to us.