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ISOLATED ENTAMOEBA HISTOLYTICA ACTIN DOES NOT INHIBIT DNAse-I ACTIVITY

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SUMMARY: Entamoeba histolytica 200-NIH contains a protein of 48,000 molecular weight, which constitutes 15-20% of the total amoeba protein. It was purified and shown to share homology with muscle actin by peptide mapping, activation of myosin ATPase and reversible cycles of salt-dependent polymerization and depolymerization.
It was, however, found to be singularly different from all known actin molecules in its inability to bind and inhibit DNAse-I. It displays one isoelectric point (pI=5.4) which is more acidic than α-actin.

Actin purified from various cells and tissue types has been found to be similar to actin from skeletal muscle in molecular size (1), peptide maps, amino-acid composition and sequence (2), in their ability to self-assemble into filaments (1-4), in their capacity to bind myosin and to activate rabbit muscle heavy meromyosin Mg^{2+}-ATPase (1,3,5) and in the inhibition of DNAse-I activity (6). However, a variety of studies have revealed that, although a highly conserved protein, actins from various sources are not identical (1). Differences were reported in migration rates on electrofocusing gels (1,7) and in the primary structure by amino-acid sequence analysis (1,2). In this paper we further extend the evidence of actin heterogeneity by presenting data which suggest that a 48,000-dalton polypeptide isolated from Entamoeba histolytica 200-NIH can be identified as actin. Actin isolated from this amoeba does not inhibit DNAse-I activity. It can be considered to be actin by the homology of peptide maps, by its capacity to be polymerized by adding 50 mM potassium chloride and 2 mM Mg^{2+} (final concentration) and by the finding that it activates the Mg^{2+}-ATPase activity of heavy meromyosin similarly to the activation by muscle actin.

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EXPERIMENTAL

Purification of amoeba actin: Actin was isolated from Entamoeba histolytica, essentially by the procedure described previously by Gordon et al. (5). Actin was isolated in 1 mg quantities from low ionic strength extracts of Entamoeba by chromatography on DEAE cellulose, followed by cycle of plymerization and depolymerization and gel filtration on Sephadex G-150.

DNAse-I inhibition activity assay was measured according to Lindberg (8).

ATPase activity assays were measured by the rate of release of (^{32}P)Pi from (γ^{32}P)ATP (9). Protein concentrations were estimated by the method of Lowry et al. (10), using bovine serum albumin as standard (Sigma). Dodecyl sulfate polyacrylamide slab gel electrophoresis was carried out according to Laemmli (11) and gels were stained with Coomassie blue (12).

Isoelectric focusing: The procedure of O'Farrell (13) was used. Gels were then soaked for 4 h in 50% methanol, changing the solution every hour to remove the ampholines, and stained according to the method of Fairbanks et al. (12).

Peptide mapping by limited proteolysis in dodecyl sulfate polyacrylamide gels was carried out essentially as described by Cleveland et al. (16). Amoeba actin and muscle actin were run on dodecyl sulfate polyacrylamide gel electrophoresis and the gels were briefly stained with Coomassie blue and rapidly destained. The regions containing actin band were cut out and the slices were placed immediately in the sample wells of a second gel, to which protease (Staphylococcus aureus V8, chymotrypsin, pepsin) had previously been added in amounts given in figure legends. Electrophoresis on the second gel was begun immediately.

RESULTS

Actin was purified from Entamoeba histolytica strain 200-NIH, essentially by the procedure described previously by Gordon et al. (5) Briefly, amoeba extract was chromatographed on DEAE-cellulose (Fig.1), the actin peak which was collected developed flow birefringence and increased viscosity indicative of F-actin when warmed to room temperature. Addition of 2 mM $MgCl_2$ accelerated the polymerization. The F-actin peak was purified through a cycle of polymerization and depolymerization and the depolymerized actin was chromatographed on Sephadex G-150. The highly purified Entamoeba actin-like molecule (Fig. 1, 1-5) has an apparent molecular weight of 48,000 daltons, and does not comigrate with rabbit skeletal muscle actin (Fig.2b, left) on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). On the isoelectric focusing gels Entamoeba actin displays one single band with a pI of approximately 5.4 (more acidic than α-actin) (Fig.2b, right).

Table I shows that the Entamoeba actin does not inhibit DNAse-I activity. It should be stressed that the same actin preparation which

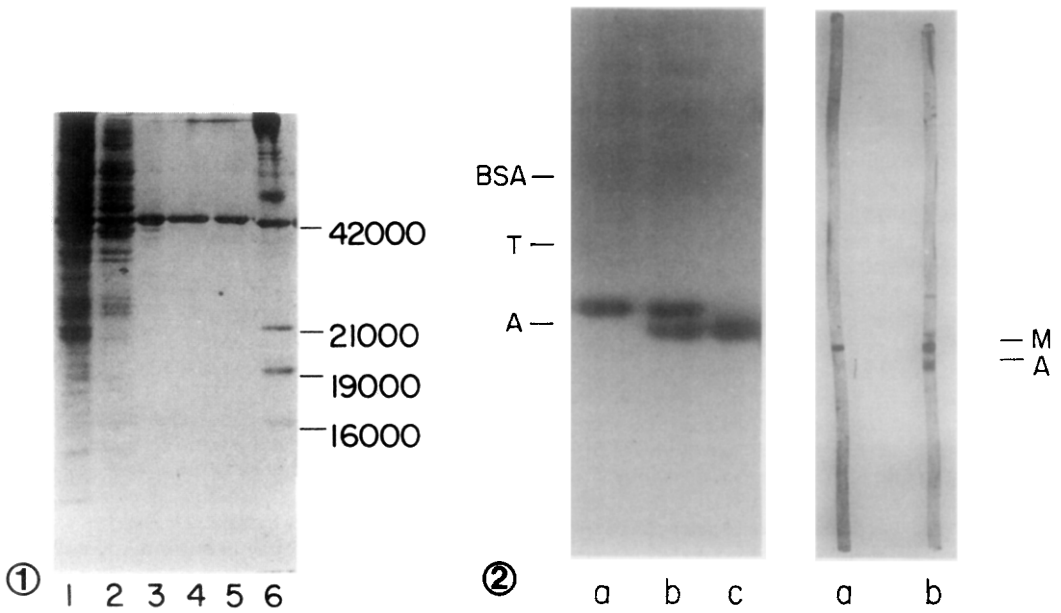

Fig.1 : Electrophoretic analysis of Entamoeba actin during the different purification steps. Aliquots of 35 µl each were applied to sodium dodecyl sulfate, 11.2% polyacrylamide slab gels, and electrophoresed according to Laemmli (10). 1 - Homogenate. 2 - Crude extract. 3 - DEAE cellulose fraction, actin peak, eluted with 175 mM KCl. 4 - Actin (DEAE cellulose peak) after one cycle of polymerization and depolymerization. 5 - G-actin peak which comes off Sephadex G-150.

Fig.2 : Comparison of the electrophoretic behavior between Entamoeba actin and muscle actin.
Left : Sodium dodecyl sulfate, 7.5% polyacrylamide slab gel electrophoresis.
a - Entamoeba actin. b - Comigration of muscle actin and Entamoeba actin. c - Muscle actin.
A - muscle actin - 42,000: T - tubulin - 55,000: BSA - 68,000.
Right: Isoelectric focusing gels of Entamoeba actin and muscle actin. a. Amoeba actin. b. Comigration of muscle actin and amoeba actin. M - muscle actin, A - Amoeba actin. The procedure of O'Farrell (13) was used. Gels were then soaked for 4 h in 50% methanol, changing the solution every hour, to remove the ampholines, and stained according to the method of Fairbanks et al. (12).

was used in the DNAse-I assay was able to polymerize upon addition of 2 mM $MgCl_2$ (final concentration) and to activate the Mg^{2+}-ATPase activity of heavy meromyosin (HMM) in a similar way to muscle actin.

Since this is the only case known, in which the purified actin has a polypeptide of 48,000 daltons and which does not inhibit DNAse-I activity, it is important to establish that the isolated 48,000 polypeptide

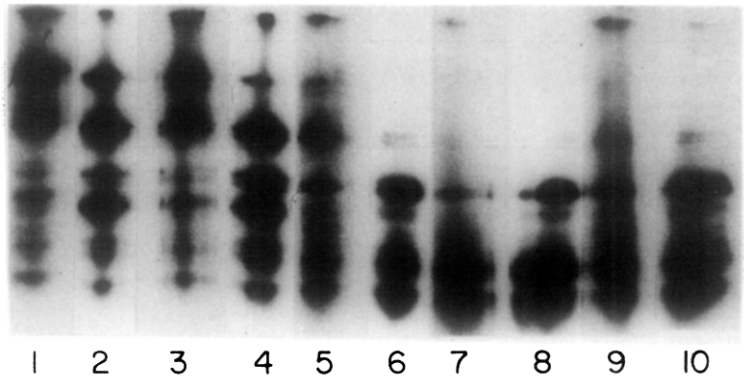

Fig.3 : Peptide maps of Entamoeba actin and skeletal muscle actin obtained by limited proteolysis. Peptide mapping by limited proteolysis in dodecyl sulfate polyacrylamide gels was carried out essentially as described by Cleaveland et al. (16). Peptide maps of muscle actin and Entamoeba actin were obtained by limited proteolysis with several enzymes. Proteins were radioiodinated, using Bolton and Hunter reagent. Approximately, 20,000 cpm were placed in the wells of 7.5% SDS-polyacrylamide gel and actin bands were cut out and placed in the sample wells of a second gel. The difference in the intensity of the label of two proteins was derived from the procedure of labeling via the DEAE cellulose peak of Entamoeba actin, and the purified actin was not labeled. The specific radioactivity of the Entamoeba actin band is lower compared to that of muscle actin band.

1,3,5,7,9 - Entamoeba actin
2,4,6,8,10 - Muscle actin
1 µg of Chymotrypsin was placed in wells 1 and 2.
5 µg of Chymotrypsin were placed in wells 3 and 4.
1 µg of Staphylococcus aureus V8 protease was placed in wells 5 and 6.
5 µg of Staphylococcus aureus V8 protease were placed in wells 7 and 8.
1 µg of Pepsin was placed in wells 9 and 10.

is indeed actin. The data presented below show that the isolated amoeba actin shares many properties with other actins that have been characterized. Thus, it retains the ability to undergo cycles of polymerization-depolymerization (Figs.1-4) and to activate rabbit heavy meromyosin ATPase (Table I). The peptide maps obtained by limited proteolysis of the 48,000-dalton polypeptide (by three different enzymes) and of the 42,000-dalton polypeptide of muscle actin are almost identical (Fig.3).

DISCUSSION

Our finding, that Entamoeba actin does not inhibit DNAse-I activity, requires further investigation, since the interaction between G-actin and pancreatic DNAse-I is now well documented. The only other

Table 1 : Characterization of Entamoeba actin

Source	Amount of F-actin added in µg	Addition	*Mg^{2+}-ATPase activity µmole Pi/min/mg HMM	†% of DNAse-I inhibition
Muscle	0	HMM	0.017	
	5	HMM	0.032	
	20	HMM	0.046	
Amoeba	8.75	HMM	0.019	
	17.5	HMM	0.038	
	35	HMM	0.073	
Muscle	0.48	DNAse-I		50
	0.98	DNAse-I		100
Amoeba	5	DNAse-I		0
	10	DNAse-I		5
	15	DNAse-I		5

*Mg^{2+}-ATPase activity was measured by the rate of release of ^{32}Pi from (γ-^{32}P) ATP (9). 40 µg of heavy memomyosin were placed in 0.5 ml assay medium containing 15 mM imidazole chloride, pH 7.5, 1 mM EGTA and 2 mM $MgCl_2$. Amoeba F-actin on muscle F-actin was added and the ATPase specific activity was reported as a function of F-actin concentration.

†DNAse inhibition activity was measured according to Lindberg (8). In the DNAse assay 3 ml of the DNA substrate are mixed with enzyme and the hydrolysis of DNA is followed by measuring the hyperchromicity at 260 nm. For determination of inhibitor (actin) activity a standardized amount of DNAse-I (0.75 µg) is mixed with 0.1-1.5 µg muscle actin. The standard amount of DNAse-I gave a slope over the linear part of the hyperchromicity curce of 0.055 OD units per min. The decrease in the activity is directly proportional to the amount of monomeric muscle actin added up to about 70% inhibition.

actin known to bind weakly to DNAse-I is that of actin isolated from Naegleria gruberi (14) which, interestingly, also has an amoeba stage of development. The potent DNAse-I inhibitor, originally crystallized from calf spleen, proves to be an actin-DNAse complex. If the reaction between actin and DNAse is biologically significant, it could regulate either DNAse function or actin polymerization in the cell. Since this is the first and only case in which the isolated G-actin does not inhibit DNAse-I activity, it may serve as a very convenient system to elucidate the biological role associated with the ability of DNAse-I to bind tightly

to G-actin and to depolymerize F-actin. It is important to isolate the factor(s) (if any) which might ingibit the interaction between Entamoeba actin and pancreatic DNAse-I. For example, it has already been shown that one can reverse the inhibition of actin on DNAse-I by 5'-nucleotidase (15).

The apparent polypeptide chain of Entamoeba actin is some 5000 daltons longer than that of muscle actin. The 12% longer chain might be related to its lack of DNAse-I interaction. It requires further investigation in order to answer the question whether the isolated actin is really a higher molecular weight protein or whether it has some irregular amino-acid residues at one end, or whether it was modified covalently by phosphorylation, for instance, which might be the reason for an apparent difference in the rate of migration of Entamoeba actin on SDS-PAGE gels.

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