ASCARIS SUUM ACTIN: PROPERTIES AND SIMILARITIES TO RABBIT ACTIN*

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SUMMARY: Actin has been purified from the muscle of the parasitic roundworm Ascaris suum. The homogeneity of the preparation was tested by ultracentrifugation and SDS electrophoresis. Amino acid analysis of the purified actin revealed striking similarities between the actins from roundworm and rabbit muscles. A composition coefficient of 0.988 was calculated from the amino acid compositions which predicts a sequence similarity of 93 ± 2%.

The muscle of the parasitic roundworm, Ascaris suum, differs from vertebrate skeletal muscle in that it is obliquely striated (1). Further, there are 10-12 thin filaments surrounding the myosin thick filament instead of the usual hexagon arrangement in vertebrates (1). Myosin has recently been isolated from the muscle of Ascaris and was found to be similar to mammalian myosin (2). However, very little is known regarding the biochemical nature of actin from Ascaris muscle. Previous studies from this laboratory (3,4) have suggested that several glycolytic enzymes from Ascaris muscle are surprisingly similar to their mammalian muscle counterparts. Therefore, it was of extreme interest to purify and characterize the structural protein, actin, from the muscle of this

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mammalian parasite and compare it structurally with the well-characterized actin from rabbit muscle.

MATERIALS AND METHODS

All chemicals used were of reagent grade. Female ascarids were obtained from an abattoir and the muscle was dissected free from other tissues. Muscle powder was prepared according to the Straub procedure described by Szent-Gyorgyi (5) and Katz and Hall (6). The actin was extracted from the powder by the method of Rees and Young (7) and polymerized and depolymerized 3 times according to Spudich and Watt (8). Sodium azide (0.02%) was present in all buffers. Ultracentrifugation, SDS disc gel electrophoresis and amino acid analysis were carried out as described previously (9,3). Composition coefficients (3) were calculated from amino acid compositions using Fortran programs with an IBM-360-50 computer.

RESULTS AND DISCUSSION

The quantity of actin obtained from 200 g of fresh Ascaris muscle was usually 20-30 mg or about 10-15% of the yield obtained from rabbit muscle using the same procedure. A much higher yield of actin was obtained if the muscle powder was extracted for a longer period of time (e.g., 12-24 hrs instead of 30 min). However, with the longer extraction periods, it was necessary to carry out at least 5 polymerization-depolymerization cycles to rid the actin of the contaminating proteins. It is possible that the obliquely-striated characteristics of the muscle account for the low yield in short-term extractions.

The purified actin was judged to be homogeneous by several criteria. Sedimentation velocity ultracentrifugation of G-actin gave a single sedimenting boundary with a sedimentation coefficient of 3.15S (5.8 mg/ml). This value is similar to that of other actins (7). Polyacrylamide electrophoresis in the presence of 0.1% sodium dodecyl sulfate (Fig. 1A) showed that the dissociated protein

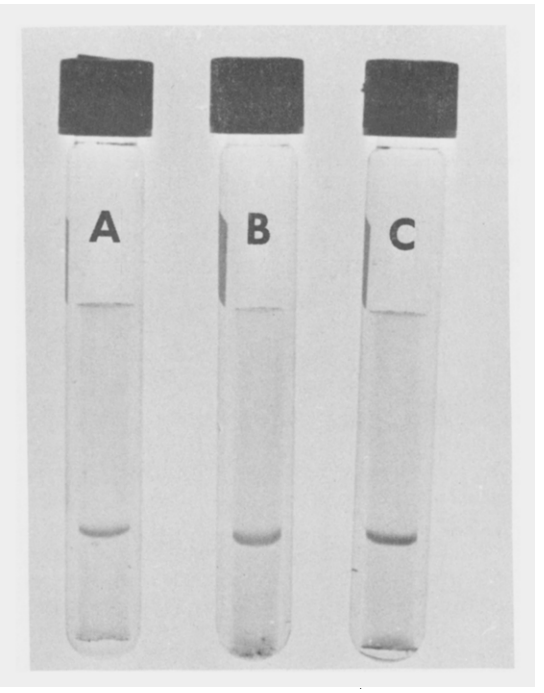

Figure 1. Sodium dodecyl sulfate (SDS) gel electrophoresis of Ascaris and rabbit actins. The purified proteins were dissociated in 1% SDS at 70° for 2 hours and subjected to electrophoresis (100 V at 3 ma per gel) for three hours in 5% gels. The gel columns (0.6 x 5 cm) and Tris-glycine buffer, pH 8.9, contained 0.1% SDS. (A) 12 μg Ascaris actin; (B) 10 ug rabbit actin; (C) Mixture of Ascaris (12 μg) and rabbit (10 μg) actins. The gels were stained with 0.25% (w/v) Coomassie Brilliant Blue in methanol: acetic acid: water (5:5:90) for one hour at 40°. The gels were destained by diffusion.

migrated as a single band. The molecular weight of the Ascaris G-actin thus appears to be essentially identical to that of the rabbit actin, since both proteins exhibited identical migrations in SDS gels (Fig. 1B), and co-electrophoresis of the two proteins also produced a single band (Fig. 1C). Rabbit muscle actin has recently been sequenced (10) and its molecular weight determined as 41,785. Thus, the SDS electrophoresis studies on Ascaris actin indicate

TABLE I

AMINO ACID COMPOSITION OF MUSCLE ACTINS

	(Residues per 374 Residues)	
Amino Acid	Ascaris	Rabbit[a]
Lys	20	19
His	7	8
N-Methyl His	1	1
Arg	18	18
Asp	33	34
Thr	28	27
Ser	21	22
Glu	42	39
Pro	17	19
Gly	28	28
Ala	32	29
Cys	5	5
Val	20	21
Met	16	16
Ile	27	30
Leu	28	26
Tyr	15	16
Phe	11	12
Try	5	4
	374	374
	CC = 0.988	

a. Data taken from Elzinga et al. (10)

that its molecular weight is 42,000 ± 2,000. Sedimentation equilibrium ultracentrifugation resulted in a linear plot (ln c vs. r^2) indicative of a monodisperse system. A value of 42,000 ± 2,000 was obtained for the weight-average molecular weight of the protein.

The similarity in the structure of the muscle actins is also evidenced from their amino acid compositions (Table I). Ascaris actin, like other actins, contains one equivalent of N-3-methyl histidine. The differences in all amino acids compared do not exceed three residues per mole and the calculated composition coefficient of the two muscle proteins is 0.988 (3). Therefore, the similarity between the two sequences is predicted to be 93 ± 2% (3). We have previously estimated that the sequence similarities of aldolases and glyceraldehyde 3-phosphate dehydrogenases from Ascaris and rabbit are 86 ± 2% (3). Therefore, the predicted actin similarities are significantly higher. There are several possibilities why actin might be expected to maintain such a conservative primary structure. First, it must interact with four adjacent G-actin monomers, forming a fiber with polarity. Second, actin must bind with the troponin complex, tropomyosin and with myosin cross bridges. Third, it must also maintain ATP and Ca^{2+} binding sites. Fourth, the extreme conservatism might also be necessitated in order to preserve regions for binding sites for aldolase, phosphofructokinase and other enzymes (11,12, Dedman and Harris, unpublished observation). Preliminary studies in our laboratory have shown that Ascaris aldolase (13) is capable of binding to its actin in a manner much like that of the rabbit aldolase-actin system (11). We are currently pursuing these studies.

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