Length of myosin rod and its proteolytic fragments determined by electron microscopy

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The mean length of rabbit myosin chymotryptic rod in electron micrographs of unidirectionally shadowed preparations was 154 nm. Tryptic light meromyosin prepared from this rod had a mean length of 86 nm whereas long and short S2 measured 62 and 43 nm, respectively. The combined length of light meromyosin and long S2 appeared to be less than that of rod, which indicated that possibly some material was lost on digestion. Molecular lengths agreed with sedimentation equilibrium M_r values but were lower than those indicated by gel electrophoresis.

Myosin *Myosin rod* *Light meromyosin* *Myosin subfragment-2* *Molecular length*

1. INTRODUCTION

Myosin is an important component of the contractile apparatus of muscle and many non-muscle cells. The molecules have two globular heads, which interact with actin to produce force, and long rod-like tails which interact with one another to form the aggregates needed for force transmission. The tail or 'rod' portion of myosin can be prepared by proteolysis and is constructed from 2 α-helical chains arranged in a coiled-coil [1]. Further digestion of rod [2] yields 3 well defined fragments: light meromyosin (LMM), long subfragment-2 (long S2) and short subfragment-2 (short S2). Fig.1 shows the general location of these fragments in the myosin molecule.

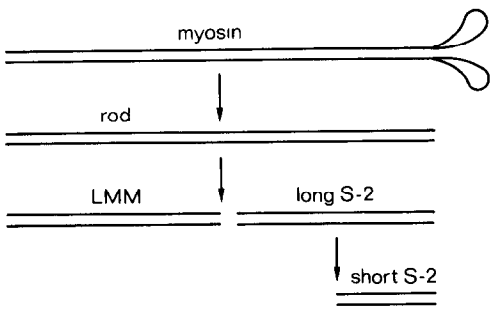

Fig.1. Digestion pattern of myosin [2].

A great deal of sequence and structural information is being obtained on myosin. However, to determine how the tail sections of the molecule interact and also to locate areas of flexibility in the rod, one must have accurate length information for the different rod fragments. Ideally, this could be derived from apparent M_r and taking, on the basis of the nematode sequence [1], the average residue weight as 116 and the axial translation per residue as 0.15 nm [3]. However, there is considerable variation in the published M_r values, with a range of 110000 [4] to 135000 [5] quoted for rod (corresponding to 140–165 nm); 60000–75000 [4,5] for LMM (75–94 nm); 50000–60000 [5] for long S2 (64–77 nm) and 34000 [6] to 40000 [5] for short S2 (44–52 nm). We have therefore measured the lengths of these fragments directly by electron microscopy and report here values for rabbit myosin chymotryptic rod, tryptic LMM, tryptic long S2 and tryptic short S2.

2. MATERIALS AND METHODS

Rabbit skeletal myosin rod, long S2 and short S2 were prepared as in [7]. Light meromyosin was obtained by dialysing trypsin-digested rod [6] to

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30 mM NaCl, 5 mM Na-phosphate (pH 6.5) and treating the precipitate with alcohol as in [2]. Preparations were checked by electrophoresis, using 10% polyacrylamide gels and a 0.1 M Tris–Bicine (pH 8.1), 0.1% SDS running buffer (fig.2).

Samples for electron microscopy were diluted to 0.1–0.05 g/l in 50% glycerol, 0.5 M NaCl, 10 mM Tris–HCl (pH 8) and sprayed onto freshly cleaved mica which was rapidly transferred to a vacuum evaporator and unidirectionally shadowed with platinum at an angle of approx. 6°, then coated with an approx. 10 nm layer of carbon. The film was floated from the mica and picked up on 400 mesh grids. These specimens were found to be more easily measured than material which had been rotary shadowed. Tropomyosin Mg-paracrystals (prepared as in [8]), negatively stained with uranyl acetate, were applied to the carbon side of the specimen for magnification calibration. The paracrystal axial repeat was taken as 40 nm [9]. Micrographs were recorded at magnifications near 33000, using Philips EM 301 and EM 400 electron microscopes operated at 80 kV, and contour lengths were measured from 3× prints using a computer-linked digitising tablet.

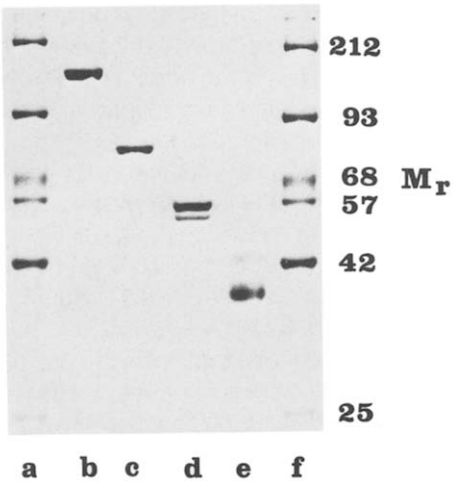

Fig.2. SDS–polyacrylamide gel electrophoresis of the myosin fragments examined. (a) M_r markers: myosin heavy chain (212000), phosphorylase (93000), BSA (68000), catalase (57000), actin (42000) and myosin light chain (25000); (b) rod; (c) LMM; (d) long S2; (e) short S2; (f) M_r standards.

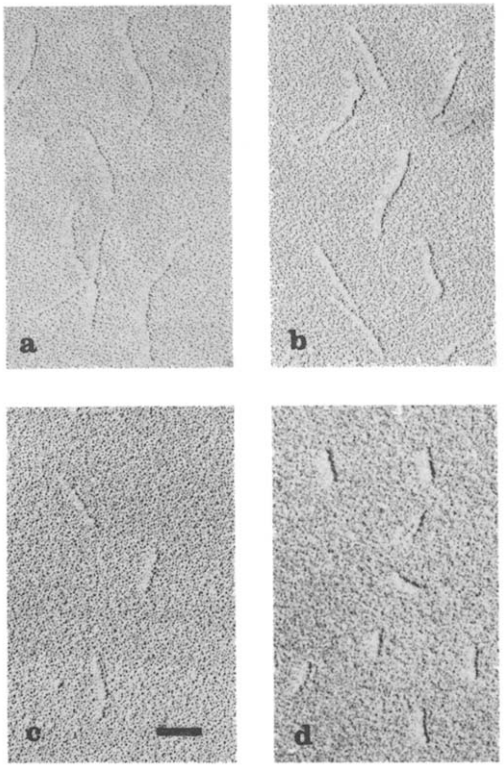

Fig.3. Examples of the micrographs of shadowed particles used for measurement. (a) Rod; (b) LMM; (c) long S2; (d) short S2. Bar is 50 nm.

3. RESULTS AND DISCUSSION

Examples of the specimens used are shown in fig.3 and table 1 summarises the measurements made on rabbit chymotryptic rod and the tryptic LMM, long and short S2 prepared from it. Fig.4 shows length histograms. However, errors need to be evaluated carefully before discussing the results in detail.

Table 1

Molecular lengths for myosin rod and its fragments

Sample	Length (nm)	SE (nm)	No. of observations
Rod	154	0.6	259
LMM	86	0.5	381
Long S2	62	0.3	527
Short S2	43	0.3	163

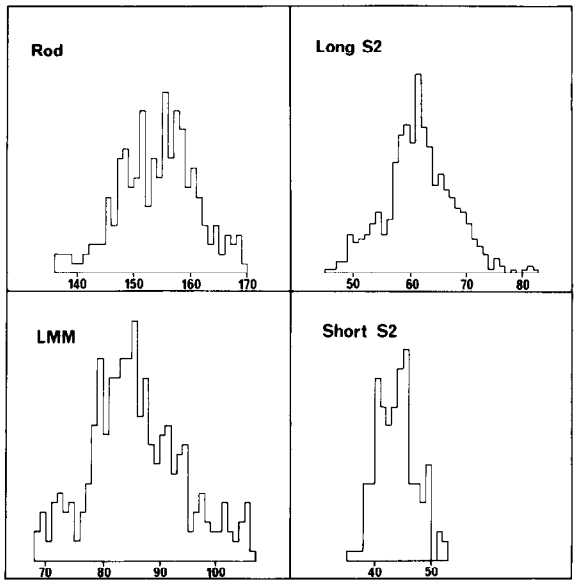

Fig.4. Length histograms for particles measured.

Some of the scatter may represent heterogeneity in the preparations, although gel electrophoresis (fig.2) indicated that this was unlikely to be a major problem. Random errors in tracing the path of the molecule would be expected to increase with the length measured and were probably the major cause for the increase in scatter observed with the longer particles. The major source of random error in the shorter particles probably occurred in defining molecular ends. The effect of random errors decreases with increasing number of measurements and so the large number of measurements made ensured that the difference attributable to random errors between the true length and that measured was small. If one takes 3 standard errors as 95% bounds, the random error associated with the means in table 1 was of the order of ± 1.5 nm.

Systematic errors associated with magnification and enlargement were minimised by including calibration standards in all fields measured. Potential sources of systematic error associated with tracing the contour of the molecule were reduced by only measuring particles which were well separated from their neighbours and which were not obviously crumpled. Maximum contrast was helpful and so only molecules aligned near to perpendicular to the shadowing direction were measured. No correction was made for metal deposition at the ends of molecules, because measurement of fibrous molecules of known length indicated that this was unnecessary. Tropomyosin, for example, gave a length of 40.8 nm (SE = 0.4; n = 91) which was in excellent agreement with the value of 41.2 nm obtained by X-ray diffraction [9]. An indication that systematic errors were probably small was the good correspondence between (a) the length of rod determined here as 154 nm and that of 156 nm obtained with whole myosin [10] and (b) the LMM length of 86 nm and the estimate of just under 88 nm obtained with paracrystals of similarly digested material [11].

As is usually observed [5,7], the long S2 preparation contained a small quantity of lower M_r material, which presumably derived from further proteolysis. This could have slightly reduced the average length. Gel electrophoresis indicated that the contaminant had 93% of the M_r of the major band and that it comprised about 10% of the sample. This would have resulted in the average length

Table 2

Comparison of length data with M_r values for myosin rod and its fragments

Sample	M_r			
	Calculated from length	Sedimentation equilibrium	SDS gel electrophoresis	
			Here	Others
Rod	120000	110000 [4]	128000	135000 [5]
LMM	66000	60000 [4]	74000	75000 [4,5]
Long S2	49000	50000 [5]	55000	60000 [5]
Short S2	33000	34000 [6]	35500	40000 [5]

being underestimated by 1%. Therefore it would seem prudent to assign the mean length of long S2 to a range of 62–63 nm.

Table 2 compares the length data obtained here with M_r estimates for the fragments. The M_r values derived from molecular lengths, particularly those for LMM and long S2, were lower than those obtained by SDS gel electrophoresis which was, perhaps, not unexpected since gel electrophoresis often gives unreliable M_r values for fibrous proteins [5]. There was excellent agreement between the length data and sedimentation equilibrium M_r for the long and short S2 fragments. However, both LMM and rod were longer than indicated by ultracentrifugation, which may be a result of the rather involved corrections for self-association which had to be applied to the sedimentation equilibrium data in the case of these fragments [4]. The difference in length measured here between long and short S2 was 18 nm, which was less than previous estimates of about 23 nm and this may be important when evaluating contraction models based on the melting of this fragment [5]. Certainly the difference measured here was substantially greater than the approx. 25 residues (4 nm) in this section of the rod which show lower stability by NMR [7].

It is interesting to speculate whether the difference between the sum of the lengths of long S2 and LMM (148–149 nm) and that of the myosin rod (154 nm) was significant. This difference represented more than 7 standard errors and so would normally be considered as highly significant. However, it is difficult to exclude unequivocally systematic errors of this sort of magnitude and so the result should probably be viewed as suggestive of a difference rather than demonstrating it. If there were a significant difference it would indicate that some extra material had been removed by proteolysis. Long S2 and rod have the same N-terminus [12] and so this extra material would have to derive from the junction between long S2 and LMM or the LMM C-terminus (or both). This would be consistent with the production of a slightly longer (90 nm or M_r 76000) LMM by very limited tryptic digestion of myosin [13], which one could identify with material from which a small extra peptide had not been removed, and also with recent work [14] which indicated that there is a tryptic cleavage site near the rod C-terminus. Extra material removed between long S2 and LMM might represent a region of local flexibility.

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