SUBSTRUCTURE OF THE MYOSIN MOLECULE AS VISUALIZED BY ELECTRON MICROSCOPY*

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The general topography of the myosin molecule has been elucidated by electron microscopy of shadow-cast preparations.\(^1\)\(^-\)\(^3\) The model obtained from these data is that of a globular head, about 200 Å in diameter by 50 Å high, attached to a rodlike tail 1300 to 1400 Å long by 20 Å wide. This picture of the myosin molecule had been anticipated on the basis of physicochemical and X-ray diffraction studies on myosin and its proteolytic fragments.\(^4\)\(^-\)\(^5\) From light-scattering measurements of the length and mass of the highly \(\alpha\)-helical portions of myosin\(^6\) and X-ray diffraction investigations,\(^7\) it was concluded that the rod portion of myosin consists largely or entirely of a two-stranded coiled coil.\(^5\) However, other physicochemical studies of myosin have led to the proposal that myosin consists of three identical subunits.\(^8\)\(^-\)\(^10\) Some substructure has been seen in the globular head of the myosin molecule by electron microscopy,\(^2\)\(^,\)\(^11\) but these observations have been inadequate to permit the determination of the number of subunits.

In the present study, the use of improved techniques in specimen preparation has made it possible to detect two subunits in the globular region of myosin. Individual subunits of similar size have also been isolated from preparations of myosin which were exposed to low concentrations of insoluble papain. These findings suggest that the major portion of the myosin molecule consists of two polypeptide chains.

Protein Preparations.—Myosin was prepared as previously described.\(^5\) The proteolytic fragments of myosin, heavy meromyosin and heavy meromyosin subfragment 1, were isolated from myosin which had been digested with insoluble papain. The papain suspension was prepared by coupling the enzyme with diazotized p-amino benzyl cellulose.\(^12\) Details of this procedure will be described elsewhere. These protein preparations, at a concentration of about 1 to 2 mg/ml, were dialyzed exhaustively against 1 \(M\) ammonium acetate, pH 7.2, and then diluted for spraying onto mica.

Shadow Casting.—Preparations of myosin and subfragments in 1 \(M\) ammonium acetate, pH 7.2, at a concentration of 0.05 mg/ml, were sprayed through a high-pressure spray gun at freshly cleaved mica. Specimens were evacuated to a pressure in the \(10^{-7}\) Torr range and shadow-cast with platinum evaporated from a tungsten filament, at a shadow to height ratio of 10:1. Amounts of metal evaporated were minimized to permit optimal resolving power, consistent with adequate contrast enhancement. Larger amounts of evaporated metal led to increased grain size and resultant poorer resolution. The specimen was either held stationary or rotated through ten or more revolutions during the brief (10-sec) shadow-casting process. During evaporation the pressure rose only briefly to the middle of the \(10^{-6}\) Torr scale. A thin layer of carbon was evaporated on top of the platinum replica. The preparation was removed from the shadow-casting apparatus and
Fig. 1.—Myosin molecules rotary shadow-cast with platinum. Magnification 105,000×.

backed with 0.25 per cent collodion. Fragments of the dried replica films were mounted on 200-mesh copper grids.

*Electron Microscopy.*—Electron microscopy was carried out on a Siemens 1A
Fig. 2.—Myosin molecules shadow-cast unidirectionally with platinum. Magnification 105,000×.

electron microscope, at a magnification of 27,000×. Micrographs were recorded sufficiently close to focus that metal grain at the 20 Å level was clearly resolved on the original plates. Plates were enlarged four to six times photographically using
Fig. 3.—Top row: A composite of selected unidirectionally shadowed myosin. When shadows are perpendicular to the line connecting the two heads, the cleft can often be seen. At other angles the shadows can obscure structure. Second row: Rotary shadowed molecules with the axis of the doublet perpendicular to the rod. Third and fourth rows: Rotary shadowed molecules with the axis of the doublet parallel to the rod. Magnification 175,000x.

Results. Myosin substructure: When myosin was prepared for electron microscopy by the rotary shadowing technique, it was found that 60 per cent of the myosin molecules had bipartite heads, each lobe of which was approximately half the size of the single heads found in the remaining 40 per cent of the molecules.
Measurements of the large, single heads indicated a diameter of about 200 Å, in agreement with previously reported values.³ ¹¹ Obviously, if heads with two lobes are shadowed unidirectionally, the one lobe could easily overshadow the other, with the net result of a large percentage of apparently single heads (Fig. 2).

Occasionally, there are hints of a cleft between lobes even in the unidirectionally shadowed preparations, but the thicker, one-sided metal deposit tends to obscure such fine structure (Fig. 3, top row). The globular, bipartite head of myosin appears to possess considerable flexibility: Many of the molecules show the pair of lobes arranged parallel to the axis of the rod or, alternatively, perpendicular to the axis (Fig. 3). It seems as if each of the small lobes may be attached by a single strand to the more rigid rod portion of myosin, and by virtue of this structure can assume a wide variety of positions. Thus, the subunits can lie adjacent to each other or be separated by as much as 150 Å. Previous estimates of head size have probably been confused by the diversity of arrangements of the two subunits, which may lead to a range of lengths or widths depending on the shadowing direction. It is more meaningful to consider the size of the individual lobes, rather than to attempt to define a discrete size for the globular region of myosin.

It is noteworthy that when the rod portion of myosin is shadow-cast by the rotary method, small kinks and bends are more apparent than when it is shadowed unidirectionally. Thus, there are obvious advantages to the rotary method for the measurement of contour length in myosin.

Papain fragments of myosin: The use of insoluble papain in the digestion of myosin has provided a simple means for obtaining relatively undegraded fragments from myosin.¹² When myosin is digested briefly with extremely low concentrations of papain (0.002 mg/ml), the water-soluble fraction of the digest shows two peaks in the ultracentrifuge: The leading peak has an intrinsic sedimentation constant of 7.2S, which is the value determined for heavy meromyosin (HMM). (Refer to Fig. 7 for a pictorial explanation of the nomenclature used in this section.) The slower peak has a value of 5.9S, which is the value commonly accepted for heavy meromyosin subfragment 1 (HMM S-1), the enzymic portion of HMM.¹³ ¹⁴ (Fig. 4, top). Electron micrographs of this preparation show that about one third of the particles have heads similar in appearance to those seen in myosin. The tails of many of these HMM molecules are not observed, possibly due to breakage during preparation for electron microscopy. The remaining two thirds of the particles,
Fig. 5.—Top row: Early digest with papain showing HMM circled and HMM S-1. Bottom row: Advanced digest with papain showing HMM S-1. Magnification 150,000X.
Fig. 6.—Distributions of widths of HMM S-1 and half heads of myosin. (A) Distribution of HMM S-1 particles present in the early papain digest, excluding HMM particles (see Fig. 5, top). (B) Distribution of HMM S-1 particles in advanced digest (see Fig. 5, bottom). Virtually all the particles in the fields measured are included in the histogram. (C) Distribution of single lobes as measured in native myosin molecules.

identified as HMM S-1, have the appearance of a single lobe of the pair characteristic of HMM and myosin molecules (Fig. 5, top row). Distribution of diameters for the HMM S-1 particles and, for comparison, the individual lobes of bipartite heads of myosin molecules are shown in Figure 6A and C. The skewing seen in these histograms may be due to asymmetry of the lobes, or may simply be ascribed to limitations in the assessment of particle size.

If more papain is added to the early digest, the HMM is completely converted to the smaller fragment, HMM S-1 (Fig. 4, bottom). (The trace of slow-sedimenting protein in the ultracentrifuge pattern probably represents the tail of HMM, or HMM S-2.) The corresponding electron micrographs show only single lobes (Fig. 5, bottom row). These particles are more uniform in size than those from shorter digests, as shown by the histogram in Figure 6B.

Conclusions.—By rotary shadowing myosin molecules, it has been possible to detect two subunits in their globular regions. Each of these subunits has approximately the same diameter as that found for the isolated HMM S-1 molecule. There can be little doubt that the small, nearly spherical HMM S-1 fragments containing both the ATPase and actin combining sites of myosin are located in the head of the myosin molecule.11, 13, 14 The question of whether these two HMM S-1 subunits are identical remains moot. On the basis of the electron micrographs presented here, as well as the earlier hydrodynamic and X-ray diffraction studies,
the myosin molecule can be depicted as consisting of two polypeptide chains. The two supereooled \(\alpha\)-helical segments of the molecule stabilize each other by strong side-chain interactions along most of their length, while each chain terminates in a separate globular conformation. Such a model is illustrated schematically in Figure 7. This drawing is designed to identify the proteolytic fragments, and to show how these fragments are related to the native myosin molecule. The molecular weights given should only be considered as approximate values, since the size is dependent on the conditions of the proteolytic degradation.\(^\text{12}\) The mass of the globular lobe seen in electron micrographs may be calculated from the most frequent diameter of about 90 \(\text{Å}\), assuming that a correction for 20 \(\text{Å}\) of shadowing metal is applicable. The value of about 150,000 so obtained is consistent with the molecular weight of 120,000 which has been reported for HMM S-1.\(^\text{10}\)

X-ray diffraction studies of living frog muscle\(^\text{15}\) as well as X-ray diffraction and electron-microscope studies of glycerinated insect flight muscle\(^\text{16}\) have shown that the cross bridges between actin and myosin filaments undergo movement on contraction or in the transition from a relaxed state to rigor. These observations on whole muscle imply that the portion of the myosin molecule involved in cross bridges must be capable of movement. In this study, we have demonstrated that the two globular subunits of myosin have the potential for a high degree of flexibility. Rotary shadowing also serves to emphasize the bending in the rod region of myosin. Whether this apparent flexibility has relevance for the interaction between myosin and actin remains to be elucidated.

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