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Unshadowed myosin molecules: STEM mass-maps of myosin heads

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Myosin molecules were directly visualized without heavy metal shadowing by scanning transmission electron microscopy (STEM) under low dose conditions. The general appearance and dimensions of heavy metal-free molecules were similar to those of shadowed myosin, either after freeze-drying without or air-drying with glycerol. Two characteristic configurations of myosin head regions were found, a first type showing two pear-shaped heads with narrow necks and a second type showing two heads connected by an extra mass in the central regulatory domain where the light chains are located. The mass of the latter type (mol. wt. = 265 ± 39 kd) is in excellent accordance with biochemical data whereas the mass of the first type is somewhat lower (mol. wt. 219 ± 44 kd).

Key words: myosin molecules/STEM mass-maps

Introduction

Myosin, a hexameric polypeptide with a total mass mol. wt. = 470 kd, consists of two heavy chains (mol. wt. = 200 kd) and two pairs of different light chains (mol. wt. ~20 kd; Lowey and Risby, 1971; Weeds and Lowey, 1971; Weeds and Pope, 1971; Burridge and Bray, 1975; Kendrick-Jones *et al.*, 1976). The C-terminal halves of the heavy chains are wound around each other thus forming the extended, ~150 nm long double coiled α-helical rod portion of the molecule. At the head-tail junction the two N-terminal halves of the heavy chains (mol. wt. = 95 kd; Flicker *et al.*, 1983) separate to form the two condensed, globular head structures which in addition contain two different types of light chains each.

The morphology of this molecule has been studied extensively using the indirect heavy metal replication technique (Hall, 1956) and an ~20 nm long pear-shaped structure was attributed to the myosin head (Slayter and Lowey, 1967; Lowey *et al.*, 1969; Elliott *et al.*, 1976; Kendrick-Jones *et al.*, 1976; Elliott and Offer, 1978; Shotton *et al.*, 1979; Trinick and Elliott, 1979; Flicker *et al.*, 1983; Walzthöny *et al.*, 1982, 1983). However, when myosin is processed for electron microscopy by this technique the shape of the molecule is obscured by the heavy metal. So far, no information on the morphology and mass distribution of unshadowed or unstained myosin molecules has been available. In an attempt to overcome this lack of information, myosin molecules were sprayed and immobilized on carbon support films (instead of the commonly used mica) and were either air-dried or freeze-dried. Such samples were then directly visualized in the scanning transmission electron microscope (STEM) without shadowing (Walzthöny *et al.*, 1984), or were heavy metal shadowed for observation in the conventional transmission electron microscope (TEM) (Walzthöny *et al.*, 1982, 1983, and in preparation).

Two characteristic appearances of heads were consistently observed with and without shadowing: the first type showed two pear-shaped heads with a narrow neck region, while the second exhibited an additional central domain. Mass-maps (Engel *et al.*, 1982) obtained by averaging STEM dark field images of similar head conformations moreover confirmed that the first head type has a significantly smaller mass (mol. wt. = 219 ± 44 kd) than the second type (mol. wt. = 265 ± 39 kd). These new data were compared with existing models of head shapes (Lowey *et al.*, 1969; Moore *et al.*, 1970; Kretzschmar *et al.*, 1976, 1978; Elliott and Offer, 1978; Mendelson and Kretzschmar, 1979; Offer and Elliott, 1978) and suggest a conformational flexibility of the myosin neck region, in the regulatory domain (Winkelmann *et al.*, 1984), where both myosin light chains have been localized (Flicker *et al.*, 1981, 1983; Winkelmann *et al.*, 1983).

Results

Gel electrophoresis

Myosin prepared for STEM showed the full complement of light chains and was essentially free of contaminants as judged by polyacrylamide gel electrophoresis in the presence of SDS (Figure 1).

Morphology

Myosin molecules prepared for conventional or scanning transmission electron microscopy did not differ significantly in their overall morphology: in both cases they consisted of a rod portion (more or less straight, or kinked) and a head region exhibiting two pear-shaped heads with narrow necks or two heads connected by a central mass domain (Figures 2, 3 and 4). Similar to shadowed myosin molecules, a variety of myosin head configurations and angular arrangements of the heads, were also seen by STEM. They could best be classified into two types according to the presence or absence of mass in the neck region of the heads.

Type 1. The two extended heads included an angle between 120° and 180° and were of identical length and width. The mass of the heads was minimal at the proximal neck region and maximal near the distal end of the heads. These pear-shaped heads were frequently found in both shadowed and unshadowed myosin molecules (Figures 3a and 4a).

Type 2. Molecules of head type 2 exhibited a considerable thickness at the joining region of the two heads extending into the neck regions. Both heads were of identical length and included an angle between 80° and 180°. Thus depending on the angular distribution of the two heads, a triangular-like arrangement (Figures 3b and 4b) or an extended arrangement of the three domains of heads and neck could be seen (Figures 3c and 4c). Again, this head region type was frequently found in shadowed as well as unshadowed myosin molecules.

We only rarely observed head configurations that showed

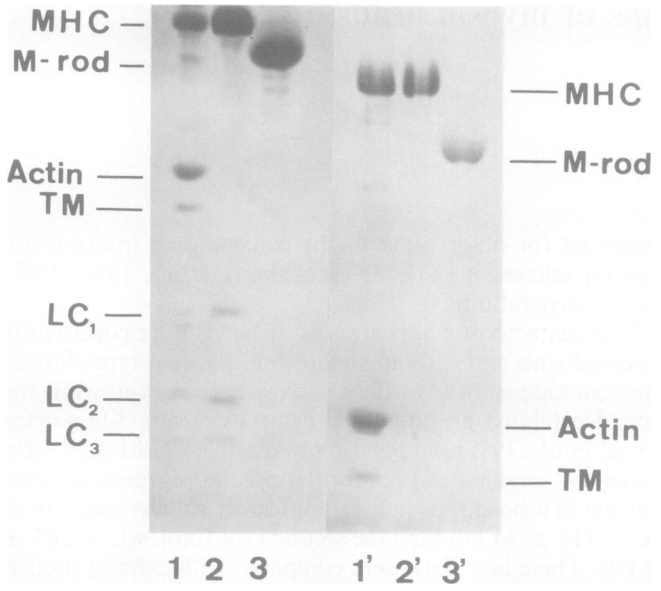

Fig. 1. Purity and light chain content of the myosin preparation used for STEM. **Left hand panel (1,2,3)**: 10% polyacrylamide gel run in the presence of SDS of purified chicken skeletal myosin (50 μg, **lane 2**); myosin rod (50 μg, **lane 3**) and myofibrillar proteins as mol wt standards (30 μg, **lane 1**); stained with acid Fast Green. Note the stoichiometry of LC_1 + LC_3 = LC_2. **Right hand panel (1',2',3')**: 5% polyacrylamide gel run in the presence of SDS of purified chicken skeletal myosin (50 μg, **lane 2'**); myosin rod (50 μg, **lane 3'**) and using myofibrillar protein as mol. wt. standards (**1'**); stained with acid Fast Green. Note the absence of significant contamination in the myosin preparation used for STEM. MHC: myosin heavy chain; M-rod: myosin rod; TM: tropomyosin; LC_1: alkali-1 light chain; LC_2: DTNB-light chain; LC_3: alkali-2 light chain.

heads with clearly curved axes either both pointing in the same direction (both heads clock-wise or counter clock-wise) or in opposite directions (pointing the curved ends towards each other). Heads within the same molecule that showed different lengths and/or widths as reflected by asymmetrical distribution of mass were also not frequently found.

Dimensions and mass-maps

Measurements of the lengths and widths of unshadowed myosin molecules yielded data which were comparable to those of shadowed molecules. The length of the rods measured from the end of the rod to the neck region of the heads was 144.7 ± 7.8 nm (n = 15), the rod width 2.9 ± 0.6 nm (n = 28), the head length 20.4 ± 2.4 nm and the head width at the widest point 6.9 ± 0.6 nm (n = 21).

The 74 mass values of myosin heads determined by electron scattering (see Materials and methods) exhibited a rather wide distribution, yielding an average of 255 kd, a standard deviation SD = 44 kd and an error of the mean EM = 5 kd (Figure 5a). Accumulating mass data from different head types in different histograms, a distinct mass difference between type 1 heads (Figure 5b) and type 2 heads (Figure 5c) was found: while type 1 heads lacking a neck domain had an average mass of 219 kd (SD = 44 kd, EM = 11 kd, n = 15), type 2 heads with a central mass domain in the neck region exhibited an average of 265 kd (SD = 39 kd, EM = 7 kd, n = 32). In spite of the large standard deviation which was probably due to the technique required for preparing individual myosin molecules, this difference was significant and indicated a loss of mass in the type 1 myosin head configurations.

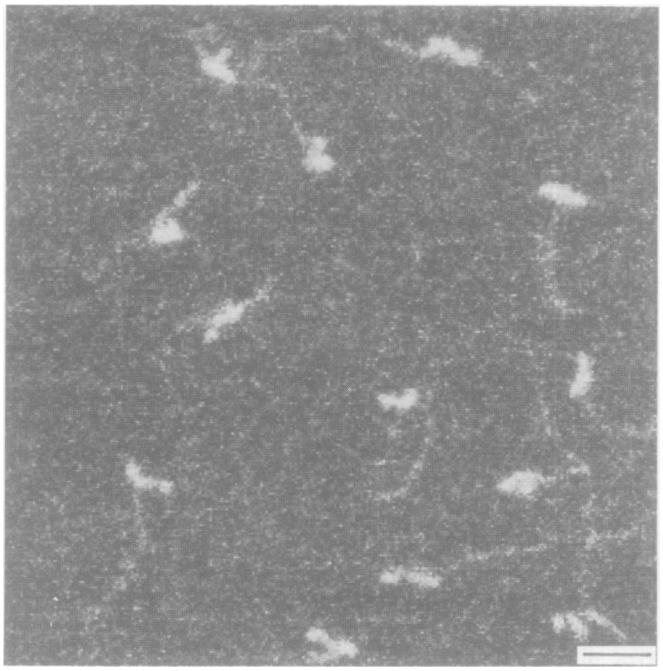

Fig. 2. 'Blind-shot' dark-field micrograph showing a field with kinked and extended myosin molecules as seen in the STEM. Magnification x 200 000; scale bar: 50 nm.

Three characteristic head configurations were selected and their respective average projections calculated (see Materials and methods) in order to visualize more clearly the domains of the myosin head region (Figure 4). An attempt was made to classify pear-shaped heads with narrow necks (Figure 4a), 'trinodular-like' heads with a triangular configuration (Figure 4b) and those with the three domains arranged in line (Figure 4c). From a pool of 60 distinct myosin heads ~15 particles were found for each conformation that yielded a correlation coefficient ≥0.6 with respect to one of the characteristic references, marked by asterisks in Figure 4. These mass distributions in turn were averaged to yield the low resolution maps displayed in Figure 4a, b and c.

The first map (Figure 4a) confirmed the pear shape of myosin heads reported previously. A relatively wide but rather flat mass distribution of the neck indicated that the angle included by the two heads must have varied by ±15° among the head regions selected and that for some particles a significant mass domain was present in the neck region. This was further illustrated by those eight individual heads that yielded the best correlation coefficient with respect to the reference.

The two other maps demonstrated the presence of a central mass domain in the neck region which appeared to be similar to the two distal mass domains of the heads (Figure 4b and c). These maps also illustrated that myosin heads can assume a wide range of conformations by a rotation of the S-1 domains around the swivel joint of the neck. Here we have selected simply two extremes: in the first case the two heads could not get much closer presumably due to steric hindrance, in the other they were fully extended. The average mass of triangular myosin head regions was 269 kd (SD = 40 kd, EM = 10 kd, n = 16) and that of the extended head regions was 261 kd (SD = 39 kd, EM = 10 kd, n = 16). Therefore, mass values of trinodular heads were pooled in the same histogram

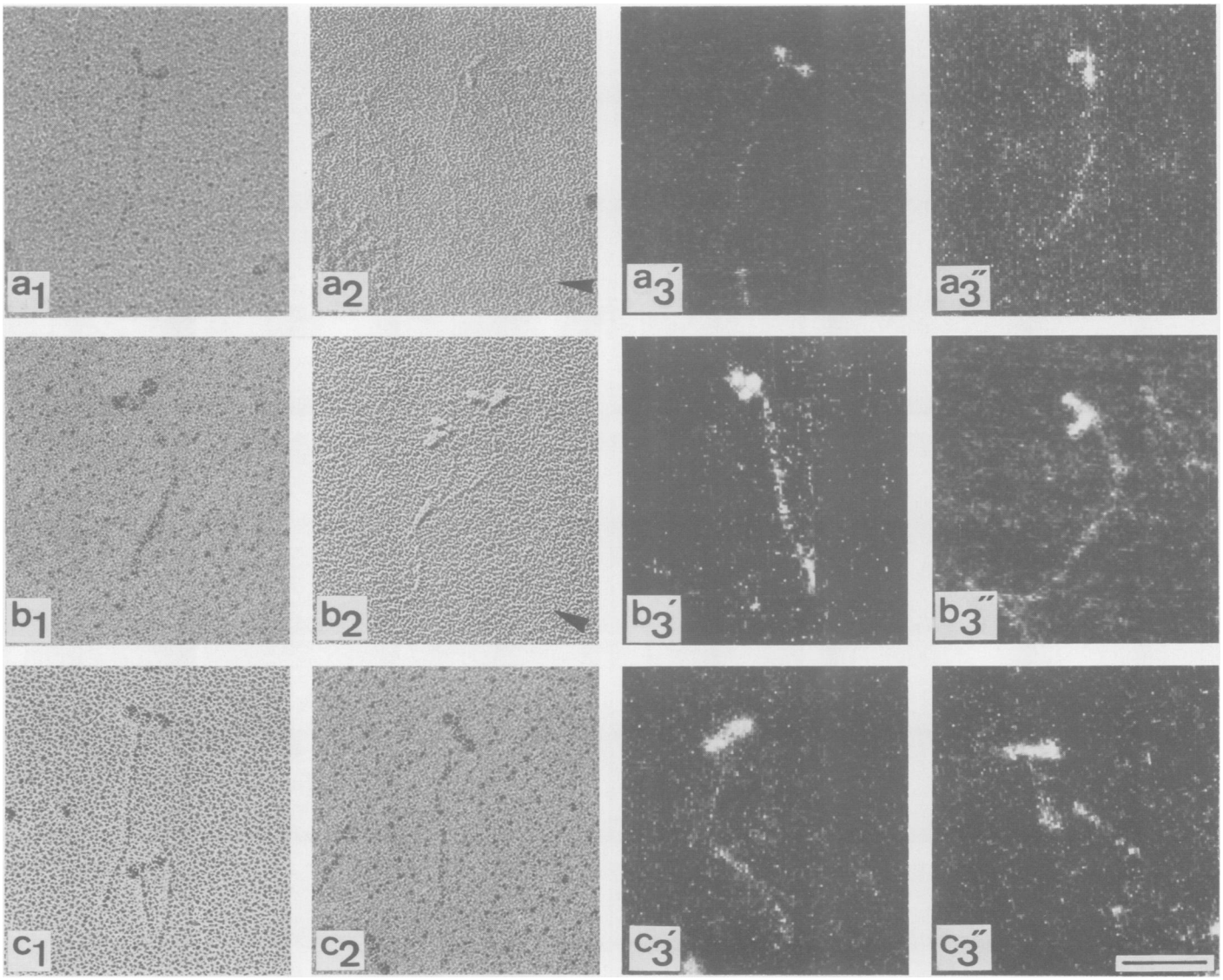

Fig. 3. Myosin molecules with different conformations of the head regions. Type 1 showing two pear-shaped heads with narrow necks (**a**), and type 2 exhibiting a distinctly trinodular structure with two heads and an extra mass in the central regulatory domain (**b,c**); either with a triangular arrangement of the three mass domains (**b**); or with an angle of 180° between the head domains (**c**); air-dried from a solution containing glycerol ($\mathbf{a_1,a_3,b_1,b_3,c_1,c_3}$); and freeze-dried from a solution without glycerol ($\mathbf{a_2,b_2,c_2}$); shadowed ($\mathbf{a_1,a_2,b_1,b_2,c_1,c_2}$); and unshadowed, observed in the STEM dark field mode under low dose conditions ($\mathbf{a_3,b_3,c_3}$). Arrow: shadowing direction; scale bar: 50 nm. Magnification x 250 000.

(Figure 5c). Again, those individual heads that exhibited the highest correlation coefficient with respect to the reference were displayed for both trinodular maps (Figure 4b and c).

Discussion

An important feature of striated muscle is the cross-bridges which consist of myosin heads protruding from the thick filament backbone. Cyclic attachment and detachment of cross-bridges in conjunction with structural change within the myosin molecule are thought to produce the force necessary to slide the interdigitating thick and thin filaments passed each other which then leads to contraction (Huxley and Hanson, 1954; Huxley *et al.*, 1980). Therefore, a better knowledge of the structure of the myosin head is of great importance. Any additional structural information derived by other means than shadowing techniques (Lowey *et al.*, 1969; Elliott and Offer, 1978; Flicker *et al.*, 1983; Walzthöny *et al.*, 1983) will be of help to obtain more subtle details of the myosin molecule, and especially the myosin heads. Due to the fact that decoration and self-shadowing phenomena of heavy metals during evaporation can influence the final positions of heavy metal grains on a given molecule (Walzthöny *et al.*, in preparation), the shape of the myosin head may be obscured or portrayed in an incorrect way, e.g., accumulation of heavy metal grains may simulate molecular mass or structural protrusions which do not exist. Therefore, the observation of unshadowed, unstained myosin molecules in the STEM under low dose conditions is likely to give a more realistic picture of the myosin molecule, since, by this method, the mass distribution of molecules can be directly visualized. In addition, such samples are prerequisites for localizing myosin subunits carrying heavy metal markers.

The overall measurements of lengths and widths of unstained myosin molecules as visualized in the STEM were consistent with the values reported earlier from shadowed molecules (Elliott and Offer, 1978; Walzthöny *et al.*, 1983). While banana-shaped (Moore *et al.*, 1970) and cylindrical

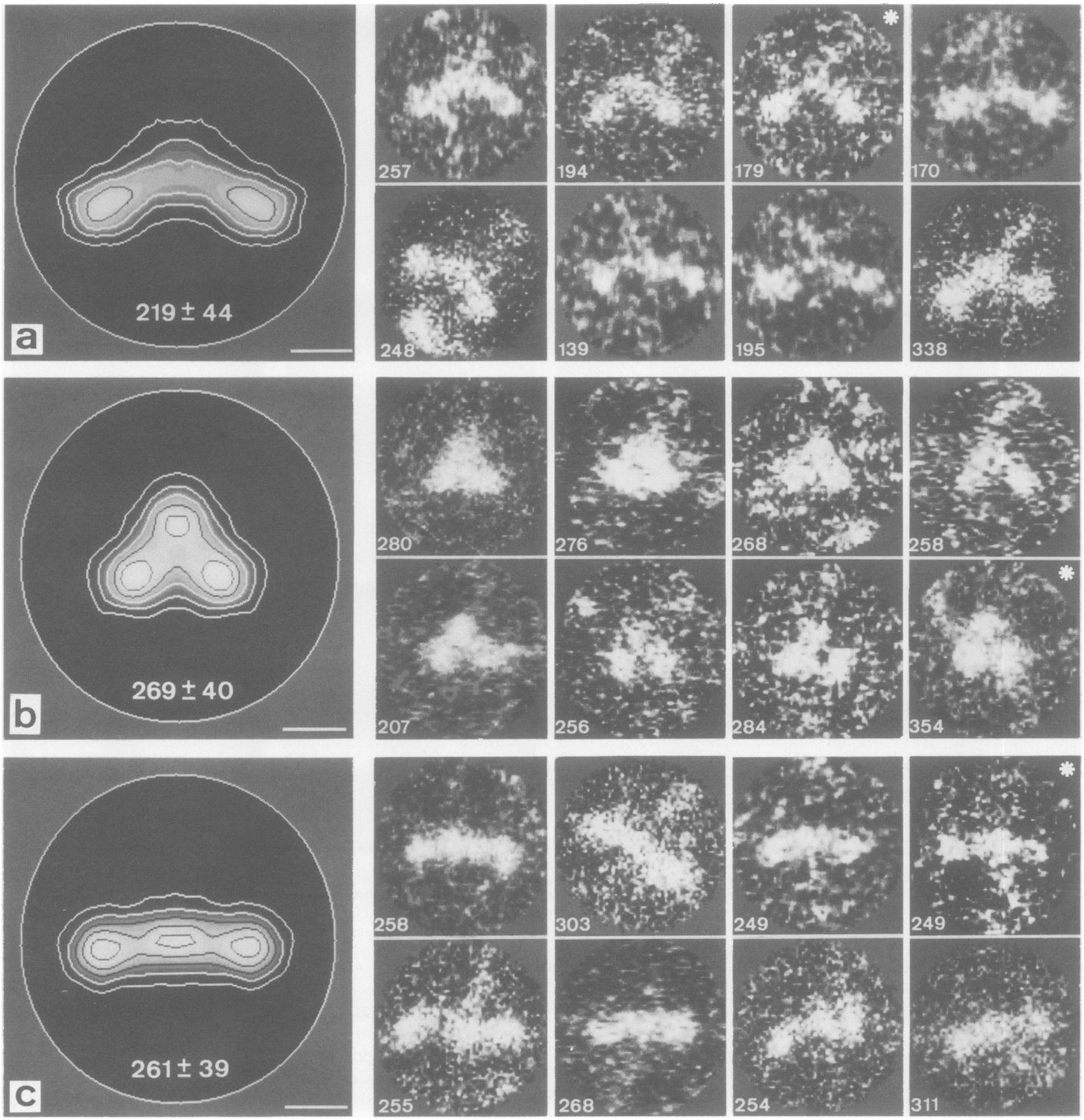

Fig. 4. Maps of myosin heads derived from low dose STEM dark field micrographs of unstained molecules. Pear-shaped heads (**a**), triangular heads (**b**) and extended heads (**c**). The maps were calculated from 15 ± 1 head regions exhibiting correlation coefficients larger than 0.6 with respect to the corresponding references (marked with an asterisk). To the right of the maps those eight individual myosin head pairs are displayed that yielded the best correlation coefficients. Mass values of individual particles are given in kd, and the averages from those particles contributing to the respective mass-maps and the corresponding standard deviations are indicated on the left. Scale bar: 10 nm.

heads (Kretzschmar *et al.*, 1976, 1978) were only very rarely seen, pear-shaped heads (Lowey *et al.*, 1969; Elliott and Offer, 1978) with a very narrow neck region were found frequently (~40%). This form (type 1) has been widely accepted as the standard myosin head conformation as seen in the electron microscope. However, myosin molecules with a thick neck region, i.e., with a central domain (type 2) were found even more frequently (~60%). Such head structures (2 heads and a central mass domain in the neck region) were observed as well on micrographs of shadowed myosin molecules prepared under a variety of conditions (Walzthöny *et al.*, unpublished data). In fact, upon close examination of published micrographs (Elliott and Offer, 1978; Margossian *et al.*, 1983) type 2 head regions can also be identified frequently,

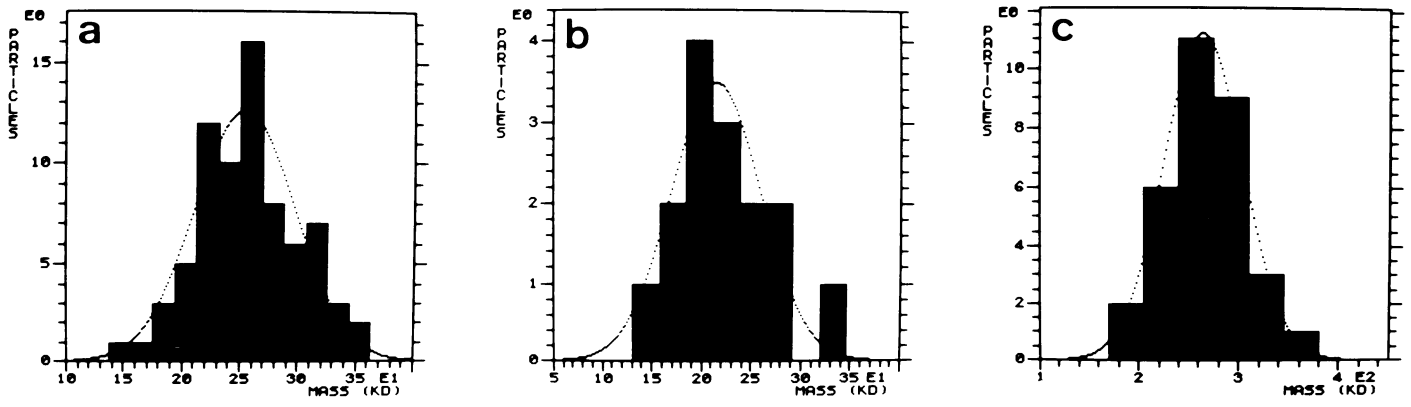

Fig. 5. Mass values of myosin head regions. **(a)** Pooled data of all head types (average = 255 kd; standard deviation (SD) = 44 kd; error of the mean (EM) = 5 kd; n = 74); **(b)** pear-shaped heads with narrow necks (average = 219 kd; SD = 44 kd; EM = 11 kd; n = 15); **(c)** trinodular heads with extra mass in the neck region (average = 265 kd; SD = 35 kd; EM = 7 kd; n = 32). The distributions were fitted by a Gaussian peak using a Marquardt algorithm.

even though this head structure with thick necks was seldom referred to. As far as the different angular positions of the myosin heads respective to the rod axis are concerned, there seems to exist a great flexibility in the head-tail swivel-junction (Elliott and Offer, 1978; Flicker *et al.*, 1983). Kinks in the hinge region of myosin rods (Elliott and Offer, 1978; Flicker *et al.*, 1983) were also well discernable.

The value for the average mass of myosin head regions, irrespective of morphological subtypes, was determined by STEM to be 255 ± 44 kd (Figure 5a) which is in excellent agreement with biochemical data of chicken myosin isoenzymes (Lowey and Risby, 1971; Lowey *et al.*, 1983; 2 S-1 heavy chains + 2 A_1 light chains + 2 DTNB light chains; 2 S-1 heavy chains + 1 A_1 + 1 A_2 + 2 DTNB light chains; and 2 S-1 heavy chains + 2 A_2 + 2 DNTB light chains corresponding to molecular weights of 268, 263, and 258 kd, respectively). However, the distribution of individual mass values shows a considerably larger width than expected from a statistical error analysis (Engel, 1982). This heterogeneity of individual measurements clearly indicates the limitations introduced by the preparation conditions that are required for a satisfactory deposition of myosin molecules on the supporting films. Glycerol as well as the high concentration of volatile buffer may bind to the molecules or increase the background resulting in a mass-increase and mass-decrease, respectively.

From 60 apparently well preserved myosin head regions three characteristic reference molecules were selected representative for the three head configurations displayed in Figure 4. The correlation coefficients calculated for all myosins with respect to all references provided the basis for an unbiased classification of the head regions. For all three configurations correlation coefficients ≥0.6 were found for at least 15 myosins, from which the mass-maps shown in Figure 4 were evaluated and the average mass values were calculated. In spite of the heterogeneity of mass data, it is interesting to note that the two conformations with the central mass between the heads yielded mass averages that were identical within the error limits (EM = 10 kd, mass difference MD = 8 kd) while the pear-shaped conformation with narrow necks exhibited a significantly lower average mass (~40 kd) than the head regions with thick necks. This apparent difference may be related to the light chains which recently have been localized near the head-tail junction (Flicker *et al.*, 1983; Winkelmann *et al.*, 1983, 1984) and which are thought to contribute significantly to the mass of the myosin S-1 neck (Vibert and Craig, 1982). This is also the location of the additional domain observed in type 2 head regions (Figures 3b,c and 4b,c) and the mass of which would approximately correspond to that of two light chains. SDS-PAGE electrophoresis of myosin used in this study confirmed the presence of a full complement of light chains prior to specimen preparation for STEM. Furthermore, the conditions used for mounting the myosin sample on the grids (0.3 M ammonium acetate with 50% glycerol, adjusted to pH 7.0 with 0.3 M ammonium carbaminate) were shown not to affect adversely the light chain content of scallop myosin (Flicker *et al.*, 1983). However, it is conceivable that some of the light chains (presumably the DTNB light chains) might be removed, after adsorption of the myosin molecules, by shearing forces (Engel and Meyer, 1980) during dehydration, resulting in a loss of mass within the regulatory domain leading to myosin heads with narrow necks. An alternative explanation may be a significant redistribution or relative movement of light chains within the regulatory domain which has recently been shown to take place in scallop myosin (Hardwicke *et al.*, 1983). However, the mass-maps presented here seem to be in fairly good agreement with the tadpole-like structure of S-1 which has very recently been crystallized and shown to contain a distinct proximal mass in the neck region of the heads (Rayment and Winkelmann, 1984).

It will be interesting to further investigate conditions which reduce during freeze-drying the shearing forces on the myosin molecules, to study molecules with and without DTNB light chains and to investigate the detailed conformation of myosin head regions under rigor and activation conditions.

Materials and methods

Isolation and preparation of myosin

Myosin was isolated from chicken breast muscle by high ionic strength extraction, low salt precipitation and ammonium sulfate fractionation followed by chromatography on DEAE-Sephadex A-50 (Offer *et al.*, 1973; Starr and Offer, 1982). Myosin was pooled, low salt precipitated and dialysed against 40% ammonium sulfate, 5 mM P_i, 3 mM $MgCl_2$, 0.1 mM EGTA, 0.1–0.5 mM β-mercaptoethanol, 1–2.5 mM ATP (Wallimann and Szent-Györgyi, 1981) in which it was stored at 4°C at a concentration of 5 mg/ml (Flicker *et al.*, 1983). Before use, myosin was dialysed overnight against 40 mM NaCl, 5 mM P_i, pH 6.8, centrifuged and resuspended in 0.7 M ammonium acetate, pH 7.0. Immediately before spraying, the myosin was diluted to 10 μg/ml in 0.3 M ammonium acetate at 0°C, adjusted to pH 7.0 with 0.3 M ammonium carbaminate and used without further dilution for freeze-drying experiments. For air-drying experiments myosin was diluted to

10 μg/ml in 0.3 M ammonium acetate adjusted to pH 7.0 with 0.3 M ammonium carbaminate, containing 50% glycerol. Aliquots of a suspension containing polystyrene spheres (diameter 91 ± 3 nm, Balzers-Union, Balzers, Liechtenstein) were added to the spray solution as markers in order to find the myosin molecules in the STEM at 10^4 x magnification.

Air-drying experiments

Droplets of the myosin spray solution containing glycerol were sprayed onto grids with a 3 nm thin carbon film supported by a fenestrated thick carbon layer (Engel and Meyer, 1980) by a spray gun device (Desaga spray gun, Desaga, Heidelberg, FRG), transferred into the STEM, dried for ~1 h at $p = 10^{-8}$ mbar and observed without heavy metal shadowing at room temperature.

Myosin molecules sprayed with glycerol onto carbon-coated grids, air-dried and shadowed were processed for standard electron microscopy as described by Walzthöny *et al.* (1983).

Freeze-drying experiments

Myosin molecules sprayed without glycerol onto carbon-coated grids, frozen, freeze-dried and shadowed were processed for standard electron microscopy as described by Walzthöny *et al.* (1983).

Scanning transmission electron microscopy and mass-mapping

Co-prepared latex spheres were recognized in TV mode at a magnification of 10 000 x, allowing the grids to be scanned quickly for areas containing myosin molecules. Correction of astigmatism was facilitated by small gold particles which were evaporated onto the fenestrated carbon film and stabilized by an additional carbon layer. Myosin molecules were recorded at 200 000 x magnification and doses between 5 and 15 80 kV electrons/A^2 in digital format without preirradiation thereby preventing excessive mass loss (Engel, 1979). Each dark-field micrograph contained 512 x 512 picture elements (pixels) in which up to 255 single electron counts could be accumulated (Engel *et al.*, 1981). Scans containing well preserved myosins were transferred to tape for mass evaluation. All parameters pertinent to the quantitative analysis (magnification, probe current, scan speed, image size) were recorded automatically at the acquisition time and saved in the headers of the digitized micrographs. Mass data were extracted as described (Engel, 1979) determining the contours of myosin head regions from low-pass filtered images (Driedonks *et al.*, 1981). TMV particles were used to check the calibration of the mass-scale. Mass values were accumulated in histograms from which average and standard variation of selected peaks could be calculated. A Marquardt algorithm (Bevington, 1969) was used to fit a Gaussian peak to the mass data.

Well preserved myosin heads of different types were selected by eye, oriented angularly and their common origin determined using their cross-correlation function with an appropriate reference. Particles exhibiting a correlation coefficient of 0.6 or better were then selected and averaged after further refinement of their orientation and translation with respect to the reference. The resulting mass-maps included 14–16 particles, and were represented by contour plots after two-fold symmetrisation taking the same contour interval for all maps. Mass-maps were calculated using the SEMPER image processing system (Saxton *et al.*, 1979).

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References

Bevington,P.R. (1969) *Data Reduction and Error Analysis for the Physical Sciences,* published by McGraw Hill, pp. 235-237.
Burridge,K. and Bray,D. (1975) *J. Mol. Biol.,* **99**, 1-14.
Driedonks,R.A. Engel,A., TenHeggeler,B. and Van Driel,R. (1981) *J. Mol. Biol.,* **152**, 641-662.
Elliott,A., Offer,G. and Burridge,K. (1976) *Proc. R. Soc. Ser. B,* **193**, 43-53.
Elliott,A. and Offer,G. (1978) *J. Mol. Biol.,* **123**, 505-519.
Engel,A. (1979) *Ultramicroscopy,* **3**, 273-281.
Engel,A. (1982) *Micron,* **13**, 425-436.
Engel,A. and Meyer,J. (1980) *J. Ultrastruct. Res.,* **72**, 212-222.
Engel,A., Christen,F. and Michel,B. (1981) *Ultramicroscopy,* **7**, 45-54.
Engel,A., Baumeister,W.K. and Saxton,W.O. (1982) *Proc. Natl. Acad. Sci. USA,* **79**, 4050-4054.
Flicker,P.F., Wallimann,T. and Vibert,P. (1981) *Biophys. J.,* **33**, 279a.
Flicker,P.F., Wallimann,T. and Vibert,P. (1983) *J. Mol. Biol.,* **169**, 723-741.
Hall,C.E. (1956) *J. Biophys. Biochem. Cytol.,* **2**, 625-628.
Hardwicke,P.M.D., Wallimann,T. and Szent-Györgyi,A.G. (1983) *Nature,* **301**, 478-482.
Huxley,H.E. and Hanson,J. (1954) *Nature,* **173**, 973-976.
Huxley,H.E., Faruqi,A.R., Berdas,J., Koch,M.H.J. and Milch,J.R. (1980) *Nature,* **284**, 140-143.
Kendrick-Jones,J., Szentkiralyi,E.M. and Szent-Györgyi,A.G. (1976) *J. Mol. Biol.,* **104**, 747-775.
Kretzschmar,K.M., Mendelson,R.A. and Morales,M.F. (1976) *Biophys. J.,* **16**, 126a.
Kretzschmar,K.M., Mendelson,R.A. and Morales,M.F. (1978) *Biochemistry,* **17**, 2314-2318.
Lowey,S. and Risby,D. (1971) *Nature,* **234**, 81-85.
Lowey,S., Benfield,P.A., LeBlanc,D.D. and Waller,G.S. (1983) *J. Muscle Res. Cell Motility,* **4**, 695-716.
Lowey,S., Slayter,H.S., Weeds,A. and Baker,H. (1969) *J. Mol. Biol.,* **42**, 1-22.
Margossian,S.S., Bhan,A.K. and Slayter,H.S. (1983) *J. Biol. Chem.,* **258**, 13359-13369.
Mendelson,R.A. and Kretzschmar,K.M. (1979) *Biophys. J.,* **25**, 20a.
Moore,P.B., Huxley,H.E. and DeRosier,D.J. (1970) *J. Mol. Biol.,* **50**, 279-292.
Offer,G. and Elliott,A. (1978) *Nature,* **271**, 325-329.
Offer,G., Moos,C. and Starr,R. (1973) *J. Mol. Biol.,* **74**, 653-676.
Rayment,I. and Winkelmann,D.A. (1984) *Proc. Natl. Acad. Sci. USA,* **81**, 4378-4380
Saxton,W.O., Pitt,T.S. and Horner,M. (1979) *Ultramicroscopy,* **4**, 343-354.
Shotton,D.M., Burke,B.E. and Branton,D. (1979) *J. Mol. Biol.,* **131**, 303-329.
Slayter,H.S. and Lowey,S. (1967) *Proc. Natl. Acad. Sci. USA,* **58**, 1611-1615.
Starr,R. and Offer,G. (1982) *Methods Enzymol.,* **85**, 130-138.
Trinick,J. and Elliott,A. (1979) *J. Mol. Biol.,* **131**, 133-136.
Vibert,P. and Craig,R. (1982) *J. Mol. Biol.,* **157**, 299-319.
Walliman,T. and Szent-Györgyi,A.G. (1981) *Biochemistry,* **20**, 1176-1187.
Walzthöny,D., Bähler,M., Wallimann,T., Gross,H., Eppenberger,H.M. and Moor,H. (1982) in B.Le Poole *et al.* (eds.), *10th Int. Congr. Electron Microsc., Hamburg,* pp. 87-88.
Walzthöny,D., Bähler,M., Wallimann,T., Eppenberger,H.M. and Moor, H. (1983) *Eur. J. Cell Biol.,* **30**, 177-181.
Walzthöny,D., Bähler,M., Eppenberger,H.M., Wallimann,T. and Engel, A. (1984) in A.Csanády *et al.* (eds.), *8th Europ. Congr. Electron Microsc., Budapest,* pp. 1505-1506.
Weeds,A.G. and Lowey,S. (1971) *J. Mol. Biol.,* **61**, 701-725.
Weeds,A.G. and Pope,B. (1971) *Nature,* **234**, 85-88.
Winkelmann,D.A., Lowey,S. and Press,J.L. (1983) *Cell,* **34**, 295-306.
Winkelmann,D.A., Almeda,S., Vibert,P. and Cohen,C. (1984) *Nature,* **307**, 758-760.

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Note added in proof

Very recently, considerable variability of myosin head shapes has also been described by Knight and Trinick (*J. Mol. Biol.,* **177**, 461-482, 1984) after negative staining of native thick filaments spread out onto hydrophilic carbon grids. In a gallery of 20 myosin head configurations visualized by negative contrast (Figure 3, Knight and Trinick, 1984) 12 molecules also show considerable mass in the central regulatory domain often leading to a 'trinodular-like' appearance. These results fully confirm those presented here.