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Crystallization of myosin subfragment 1

(contractility/x-ray diffraction/electron microscopy)

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ABSTRACT **Crystals of myosin subfragment 1 from avian skeletal muscle have been grown reproducibly. They diffract x-rays to at least 4.5-Å resolution. The subfragment 1 crystallizes in space group $P2_12_12_1$ where $a = 107$ Å, $b = 117$ Å, and $c = 278$ Å. The cell dimensions and intensity distribution on x-ray diffraction photographs are consistent with two molecules in the crystallographic asymmetric unit. Electrophoretic analysis shows that the myosin subfragment 1 present in the crystals contains a 95-kilodalton heavy chain fragment and both the essential and regulatory light chains.**

Myosin is a major cellular protein that has both structural and functional roles in motility (1). In muscle, the myosin rod forms the backbone of the thick filaments (2, 3), whereas the myosin heads form a substantial portion of the cross-bridges, which are responsible for force generation (4). Considerable effort has been concentrated on the functional activities and structure of the myosin heads. These include the interaction with actin and the kinetics of ATP hydrolysis (5–7), the function and location of the myosin light chains (8–10), the shape of the heads (11) and their structure in a rigor complex with thin filaments (12–14), the domain structure within the head (15, 16), and the contact surfaces between myosin and actin (17). However, the interpretation of these data has been restricted by the lack of detailed structural information. In this paper, we report the crystallization of the head portion of the myosin molecule (subfragment 1, S-1) in a form suitable for a high resolution structure determination.

MATERIALS AND METHODS

Myosin was prepared from adult White Rock chicken pectoralis muscle and chromatographed on DEAE cellulose (DE-52 Whatman) in 20 mM sodium pyrophosphate buffer (pH 7.5) (18). Myosin S-1 was prepared by limited proteolysis of a 1% suspension of myosin filaments in 0.2 M ammonium acetate, pH 7.0/2 mM $MgCl_2$ containing 15 μg of papain (20–25 units/mg, Millipore) per ml at 20°C for 5–10 min. (8). After the undigested myosin and myosin rod subfragment were pelleted (2 hr at 160,000 × *g*), the S-1 was dialyzed into 50 mM Tris·HCl, pH 7.9/2 mM $MgCl_2$/1 mM dithiothreitol and chromatographed on DEAE cellulose essentially as described (18). S-1 was concentrated from peak fractions by precipitation with ammonium sulfate at 60% of saturation (0°C), dialyzed into 10 mM potassium phosphate, pH 7.0/0.2 mM $MgCl_2$/1 mM dithiothreitol, and either used "fresh" for crystal trials or stored lyophilized. $NaDodSO_4$/polyacrylamide gel electrophoresis was done as described by Laemmli (19). Actin was prepared as described by Spudich and Watt (20); native thin filaments were obtained from pectoralis myofibrils (unpublished data).

F-actin or native thin filaments at 0.5 mg/ml were decorated in thin filament buffer (40 mM NaCl/5 mM NaH_2PO_4, pH 7.0/2 mM $MgCl_2$/1 mM EGTA/3 mM NaN_3) with a slight molar excess (1.2:1) of S-1. The decorated filaments were diluted 1:10 with thin filament buffer, suspended over holes on holey carbon film grids, and stained with 2% uranyl acetate essentially as described by Craig *et al.* (21). S-1 crystals were washed extensively with crystallizing solution to remove any associated mother liquor and redissolved in 10 mM NaH_2PO_4, pH 7.0/0.2 mM $MgCl_2$. The concentration of the S-1 released from the washed crystals was quite low, so the thin filaments were diluted about 1:10 into the S-1 solution and applied directly to the grids. Micrographs were recorded with a Philips EM301 electron microscope at 80 kV with a 30-μm objective aperature.

RESULTS AND DISCUSSION

Crystals of avian skeletal muscle myosin S-1 have been grown reproducibly by vapor diffusion at either room temperature or at 4°C from a solution of ammonium sulfate (pH 6.7). The crystals grow as thick birefringent plates (Fig. 1). Crystals of an identical morphology also have been obtained from solutions of sodium phosphate and of sodium or ammonium citrate. Details of the crystallization will be described elsewhere. Preliminary x-ray diffraction studies on small crystals (300 × 150 × 75μm) show reflections to at least 4.5-Å resolution on "still" photographs. The symmetry of small-angle precession photographs is consistent with space group $P2_12_12_1$ where $a = 107$ Å, $b = 117$ Å, and $c = 278$ Å (Fig. 2).

These crystals were grown from S-1 prepared by papain digestion of chicken pectoralis muscle myosin. This subfragment contains a 95-kilodalton heavy chain fragment and bands corresponding to the essential (LC1 and LC3) and regulatory (LC2) light chain classes (8) when analyzed by $NaDodSO_4$ gel electrophoresis (Fig. 3). Several crystals were isolated, washed extensively with crystallizing solution to remove any associated mother liquor, and redissolved in low ionic strength buffer for electrophoresis. The polypeptide composition of the crystals (Fig. 3*A*) is identical to the original S-1 (Fig. 3*B*), confirming that the crystals are a complex of the S-1 heavy chain with both classes of myosin light chains.

Electron micrographs of F-actin or thin filaments decorated with papain S-1 reveal characteristic "barbed" arrowheads (Fig. 4 *A* and *B*), consistent with the presence of both classes of light chain on this subfragment (21). S-1 released from washed and redissolved crystals retained its ability to decorate thin filaments (Fig. 4*C*), yielding images very similar to those obtained with S-1 prior to crystallization. This establishes that after a month at room temperature, the crystallized S-1 still binds to actin. Further biochemical studies are in progress to determine the enzymatic activity of the S-1 present in the crystals.

It is possible to suggest the number of molecules in the crystallographic asymmetric unit from the volume of the unit

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Abbreviations: S-1, subfragment 1; LC1, LC2, and LC3, light chains of myosin.

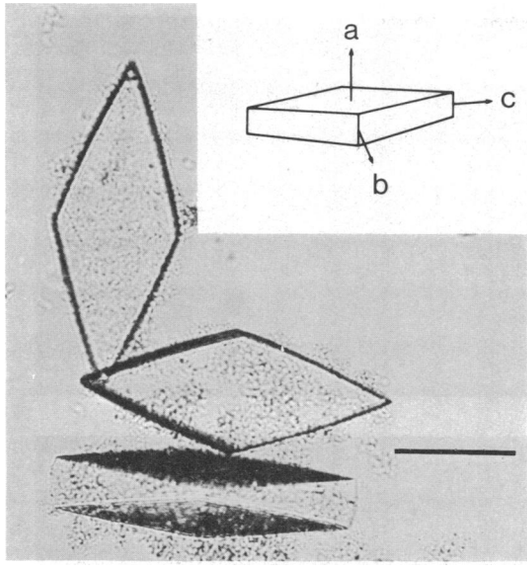


FIG. 1. Crystals of myosin S-1, which show prominent [100], [011], and [01$\bar{1}$] faces. Photomicrograph was taken with bright field optics. (Bar = 100 μm.)

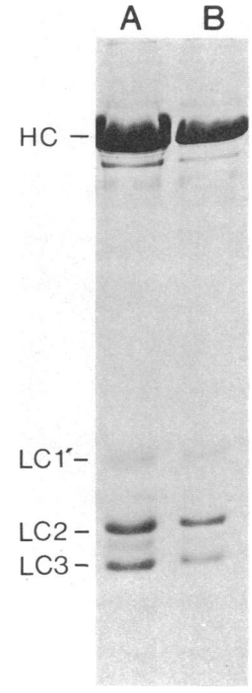
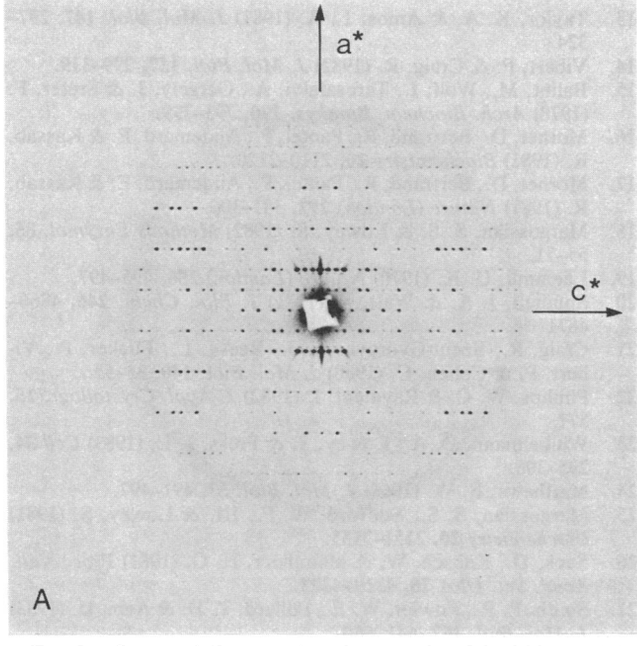


FIG. 3. Polypeptide composition of myosin S-1 as analyzed by $NaDodSO_4$ gel electrophoresis. (*A*) S-1 released from washed crystals contains a 95-kilodalton heavy chain (HC) fragment and bands corresponding to myosin light chains (LC1–3). (*B*) Papain S-1 prior to crystallization. Note that LC1 is modified by papain, resulting in a triplet of diffuse bands (LC1'). The LC2 band was also identified immunochemically (23).

cell and molecular weight of myosin S-1. The volume-per-unit molecular weight in the unit cell for most crystalline globular proteins (defined as $V_m = V$ of unit cell per nM, where M is the molecular weight of the protein and n is the number of particles in the cell) lies within the range 1.68–3.53 Å^3/dalton (24). There are four equivalent positions in the space group $P2_12_12_1$; hence, there should be $4n$ copies of the myosin S-1 in the unit cell. By using a molecular weight of 130,000 for this subfragment (25), the calculated V_m of the unit cell for 4, 8, or 12 copies of S-1 are 6.7, 3.4, or 2.2. Since $V_m = 6.7$ falls outside the normal range for proteins, it is unlikely that there is only one copy of S-1 in the crystallographic asymmetric unit. On the basis of V_m alone, it is difficult to decide whether there are two or three molecules in the asymmetric unit, since both values (3.4 and 2.2) lie within the range 1.68–3.53 Å^3/dalton observed for most crystalline globular proteins. However, the intensity distribution on the *hk0* zone (Fig. 2*B*) strongly suggests that there are two copies in the asymmetric unit.

The *hk0* zone (Fig. 2*B*) shows a pronounced nonsystematic absence of reflections of the type $h = 2n + 1$, indicating that in projection the unit cell is halved along a. This can



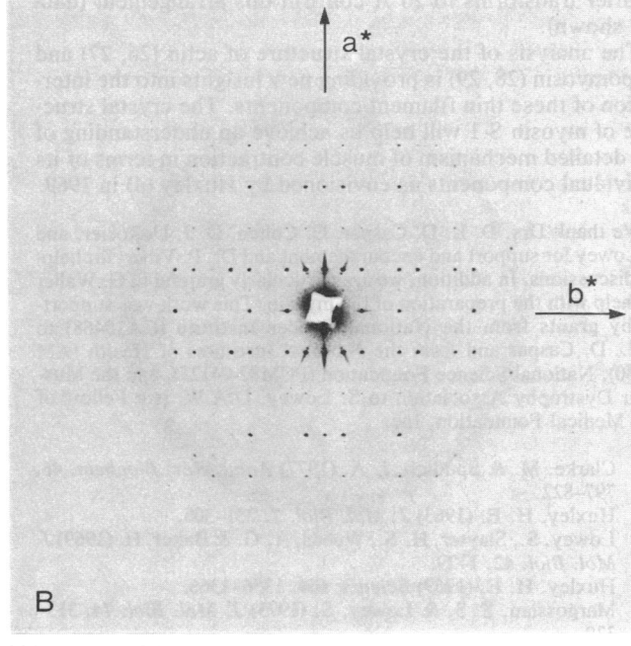


FIG. 2. Screened 4° precession photographs of the *h0ℓ* zone (*A*) and *hk0* zone (*B*) of the reciprocal lattice. The systematic absences along a^*, b^*, and c^* indicate the presence of three 2-fold screw axes. The diffraction photograph was recorded on an Elliott GX20 rotating anode x-ray generator operated at 30 kV and 28 mA with a 100-μm focal cup and double focusing mirrors (22). The exposure time was 24 hr at a crystal-to-film distance of 100 mm.

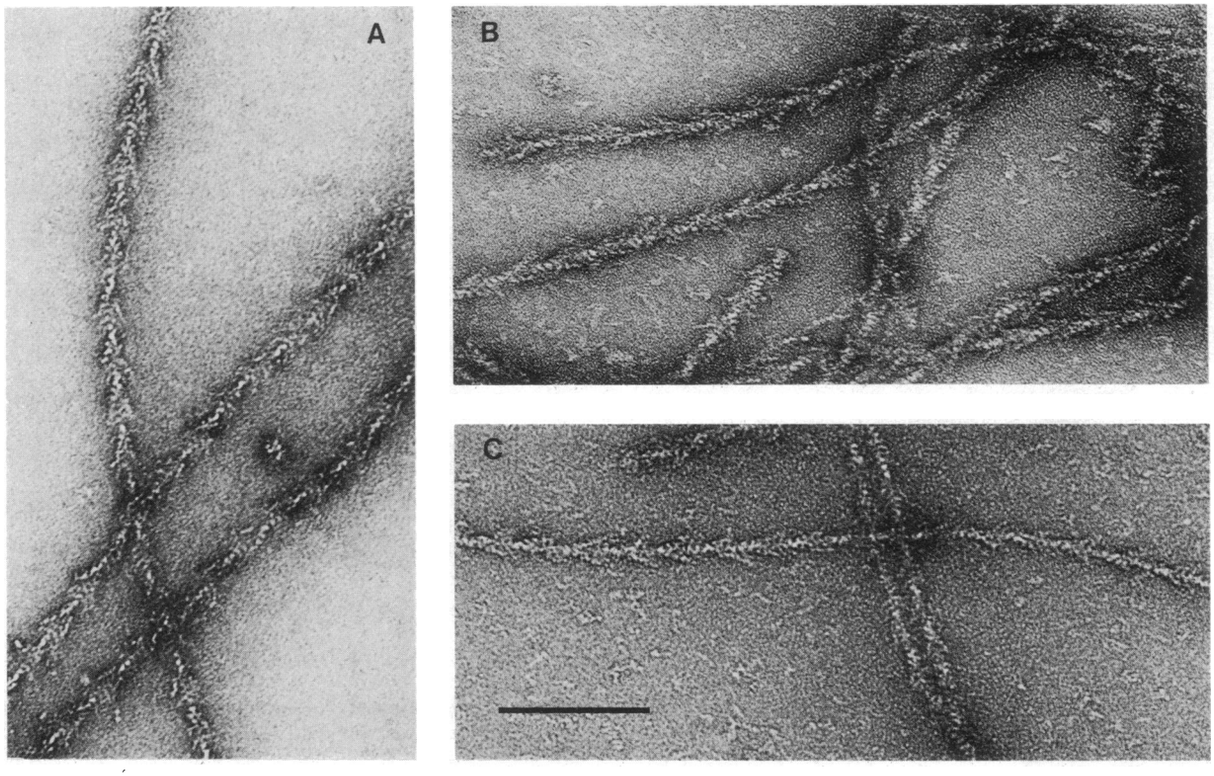


FIG. 4. Electron micrographs showing decoration of F-actin (*A*) and thin filaments (*B*) with papain S-1 prior to crystallization. (*C*) Decoration of thin filaments with S-1 released from washed and redissolved crystals. (Bar = 100 nm.)

only occur if there is additional noncrystallographic symmetry present in the asymmetric unit. In this case, the absences are consistent with a noncrystallographic 2-fold axis parallel to *a* in the *a*,*c* plane. The appearance of the *h0ℓ* zone (Fig. 2*A*) indicates that the noncrystallographic 2-fold axis is not coincident with the crystallographic 2-fold screw axis parallel to *c*. Electron micrographs of thin sections from embedded and oriented crystals that show diffraction maxima on Fourier transforms to 30 Å confirm this arrangement (data not shown).

The analysis of the crystal structure of actin (26, 27) and tropomyosin (28, 29) is providing new insights into the interaction of these thin filament components. The crystal structure of myosin S-1 will help us achieve an understanding of the detailed mechanism of muscle contraction in terms of its individual components as envisioned by Huxley (4) in 1969.

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