EFFECTS OF C-PROTEIN ON SYNTHETIC MYOSIN FILAMENT STRUCTURE

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ABSTRACT In the absence of C-protein, synthetic filaments prepared from column-purified myosin exhibit the following features: individual filament diameters are uniform over a long length, but a wide distribution of diameters is apparent over the population; ~25% of the filaments have a frayed appearance and take up stain poorly, whereas the remaining 75% are well-stained; optical diffraction of well-stained filaments reveals a 14.3-nm subunit period and a 43-nm axial period (Koretz, 1978; Koretz, 1979). Addition of C-protein to myosin before filament formation affects all of these features in a manner related to C-protein concentration. At the physiological ratio of C-protein to myosin in the banded region of the natural thick filament, synthetic aggregates are uniform in diameter over the population and show <10% frays. Whereas the subunit period remains unchanged, the axial period has increased to 114.4 nm, or eight times the subunit repeat. Above and below the physiological ratio, disorder of a specific nature is apparent. Addition of C-protein after filament formation appears to coat the aggregates so that elements of backbone ultrastructure are obscured, and some evidence of axial period change is visible in diffraction patterns. A model is presented for the binding of C-protein to myosin, and its observed effects on filament structure are explained in terms of this model.

INTRODUCTION

Identification and preliminary molecular weight characterization of nonmyosin muscle proteins in myosin preparations were first performed by Starr and Offer in 1971. They found a number of nonmyosin proteins, but by far the largest in quantity was a 140,000-mol wt protein, named C-protein by them. Hydrodynamic and spectroscopic studies by Offer et al. (1973) indicate that C-protein is a long rod-shaped molecule with little or no alpha helix content. Perfusion of antiserum to C-protein through intact rabbit skeletal muscle and subsequent longitudinal sectioning (Pepe and Drucker, 1975; Craig and Offer, 1976) demonstrate that C-protein is located in the outer 7 of the 11 bands, originally identified as 10 bands by negative staining of isolated A-segments (Hanson et al., 1971), which are located on either side of the bare zone at ~43-nm intervals.

Studies of the binding of C-protein to myosin and its fragments demonstrate its strong affinity to the rod portion of the myosin molecule. Moos et al. (1975) have shown that C-protein will disrupt synthetic filament structure at molar ratios of approximately one C-protein per myosin molecule, and will also bind to rod preparations, disrupting aggregate

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Some of the results discussed here were presented in abbreviated form at the 22nd Annual Meeting of the Biophysical Society in Washington, D.C., 27–30 March 1978 (Koretz, 1978).

formation. Binding of C-protein to light meromyosin paracrystals results in the visualization of stripes across the paracrystal surface at ~40-nm intervals. Later studies of C-protein binding to light meromyosin paracrystals where the two proteins were mixed before paracrystal formation (Chowrashi and Pepe, 1977), simultaneously demonstrate the related C-protein and underlying light meromyosin periodicity. Recent ultracentrifugation studies (Starr and Offer, 1978) show that C-protein has an affinity for the subfragment-2 portion of the myosin rod; the authors interpret these and the earlier light meromyosin results in terms of specific binding sites for C-protein located in each of these regions.

The function of C-protein in thick filaments is not clear. The binding studies suggest that C-protein location in vivo may be determined by underlying myosin periodicity. This implies a relatively passive role for C-protein, for example as a clamp or stabilizer of thick filament structure during muscle contraction in response to forces generated by shortening. Starr and Offer (1978), however, suggest the possibility of control of crossbridge movement because of C-protein's ability to bind to subfragment-2. The 44-nm^{-1} x-ray reflection observed by Rome et al. (1973) in intact muscle through which antibodies to C-protein had been perfused, if not attributable to an interference fringe generated by bare zone spacing, could indicate that C-protein does not have the underlying myosin periodicity and might thus act as a vernier mechanism in thick filament length determination (Huxley and Brown, 1967). A third possibility is that C-protein has some role in the thick filament structural changes observed by Haselgrove (1975) when muscle is activated for contraction.

Recent structural studies of synthetic filaments prepared from column-purified myosin have indicated that, with certain limitations, these aggregates can be used as a model system for the study of thick filament protein interactions (Koretz, 1979). In the present work, the effects of C-protein on synthetic filament structure when added before or after filament formation have been investigated at molar ratios of C-protein to myosin that bracket and include the approximate physiological ratio found in the band region of natural filaments (Morimoto and Harrington, 1973; 1974).

MATERIALS AND METHODS

Myosin was prepared as described previously (Koretz, 1979). C-protein was obtained during the myosin column purification step and further purified by ammonium sulfate precipitation, resuspension, and final clarification in the centrifuge as described by Offer et al. (1973). Solutions for dialysis to form synthetic filaments were prepared using the extinction coefficients reported by Offer et al. for column-purified myosin and C-protein. Myosin in 0.5 M KCl was mixed with C-protein, also in 0.5 M KCl, for a final myosin concentration of 1.0 mg/ml. C-protein concentrations were calculated such that the molar ratios of C-protein to myosin in the dialysate were 1:13, 1:6.6, 1:4.3, 1:3.3, and 1:2,4 (2.5, 5.0, 7.5, 10.0, and 12.5% C-protein by weight). Aliquots of myosin alone at a final concentration of 2.0 mg/ml and C-protein alone at 0.2 mg/ml were also placed in dialysis tubing.

The solutions were dialyzed against a covered 0.1-M ammonium acetate solution, pH 7.0, at 4°C for 24 h with gentle stirring and with a ratio of dialysis medium volume to bag volume of at least 80:1. After filament formation, equal volumes of the solution containing myosin alone and C-protein alone were mixed and allowed to stand for 60 s; the ratio of the 2 proteins was equivalent to filaments prepared at a ratio of 1:3.3. Some of the filaments prepared from myosin alone were also kept and investigated as a control. Electron microscopy, measurements of filament diameters, and analysis of optical diffraction patterns were performed as previously described (Koretz, 1979).

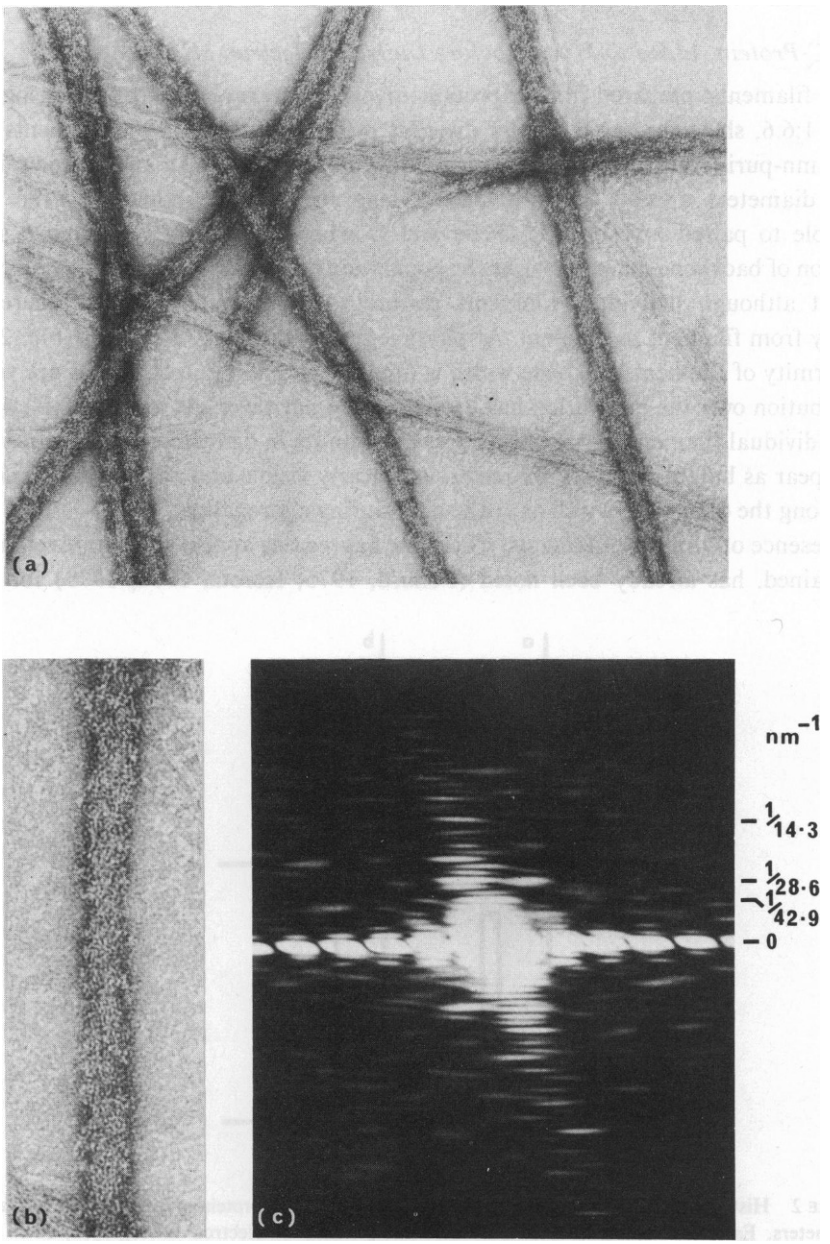

FIGURE 1 Electron microscopy and optical diffraction pattern of synthetic filaments prepared from C-protein and myosin in a molar ratio of 1:6.6. (*a*) A typical electron microscope field (×105,000). Although each filament appears to have fairly uniform backbone diameter, there is great variation from filament to filament. Note the presence of a poorly stained, apparently frayed species; these filaments can be attributed to collapse on the grid (Koretz, 1978). (*b*) Enlargement of part of a well-stained synthetic filament from *a* (×210,000). (*c*) Optical diffraction pattern of the filament in *b*. The apparent disorganization seen in *b* is confirmed by optical diffraction studies; although meridional reflections at 14.3 nm^{-1} and 28.6 nm^{-1} are generally observed, the 43-nm^{-1} off-meridional reflection is usually very weak or not visible.

RESULTS

C-Protein Added to Myosin before Dialysis: Electron Microscopy

Synthetic filaments[1] prepared from C-protein–myosin mixtures below the physiological ratio, 1:13 and 1:6.6, show the same type of disorder previously observed for filaments prepared from column-purified myosin alone (Koretz, 1978; 1979). As Fig. 1*a* and *b* shows, individual filament diameters appear constant over a long length and show the 4-nm striations attributable to paired myosin rods (Pepe and Dowben, 1977). Fig. 2 *a* and *b* shows the distribution of backbone diameters over the population of filaments at these protein ratios; it is clear that although individual filaments maintain a regular appearance, there is great variability from filament to filament. At physiological ratios (Fig. 3 *a* and *b*, Fig. 2 *c* and *d*) the uniformity of filament backbone width is maintained and 4-nm striations are visible, but the distribution over the population has become much narrower. At a ratio of 1:2.4 (Fig. 4 *a* and *b*), individual filaments demonstrate great variability in diameter; areas of greater width, which appear as bulges along the diameter, are clearly visible and may be due to addition of protein along the diameter as well as at the ends during aggregation.

The presence of "frayed" filaments, where the aggregates appear to be unravelling and are poorly stained, has already been noted (Pollard, 1975; Koretz, 1978; 1979) for filaments

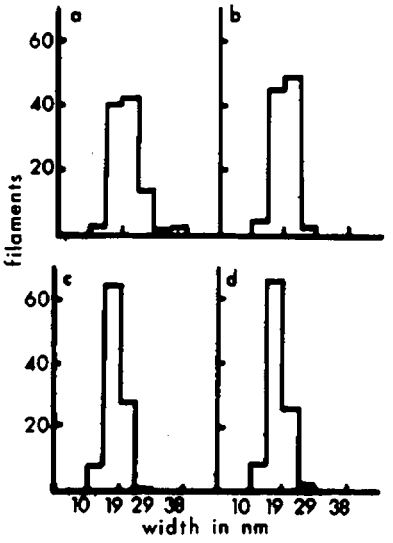

FIGURE 2 Histograms of filament diameter distributions for each C-protein:myosin ratio, plotted as *n* vs. nanometers. Each bin represents 1 mm on 3× enlargements of electron micrographs, such that 1 mm = 4.7 nm; n is > 100 for each histogram, but was normalized to 100 for easy comparison. (*a*) 1:13, (*b*) 1:6.6, (*c*) 1:4.3, (*d*) 1:3.3. Note that at the approximate physiological ratio of C-protein:myosin in the banded region of natural thick filaments the distribution is narrower and the average diameter is smaller than with filaments formed at ratios below the physiological.

[1]For ease of discussion, filaments will be referred to in terms of their protein composition; i.e., 1:13 filaments are those filaments formed from a solution containing C-protein and myosin in a molar ratio of 1:13. Physiological ratio refers to the ratio of C-protein to myosin in the banded region of natural thick filaments, ~1:3–4 (Morimoto and Harrington, 1973; 1974).

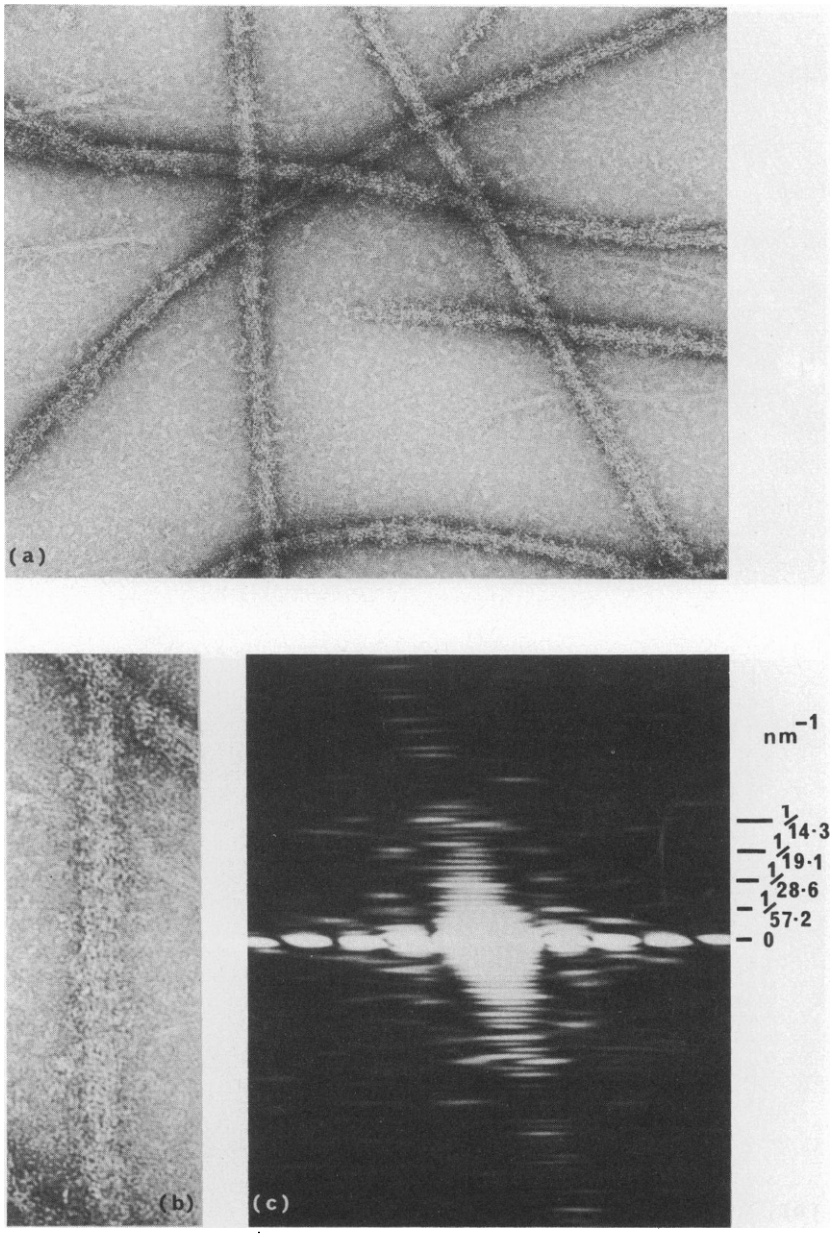

FIGURE 3 Electron microscopy and optical diffraction pattern of synthetic filaments prepared from C-protein and myosin in a molar ratio of 1:3.3. (*a*) A typical electron microscope field (×105,000). The distribution of filament diameter over the field is much narrower than that observed in Fig. 1*a*, and there are far fewer frayed filaments. Note instead the presence of small poorly stained aggregates. (*b*) Enlargement of part of a synthetic filament from *a* (×210,000). (*c*) Optical diffraction pattern of the filament in *b*. The layer line spacings indicate a radical change in synthetic filament organization, from a 43-nm axial repeat to a 114.4 (8 times the subunit repeat) axial period. The 14.3-nm subunit spacing remains unchanged.

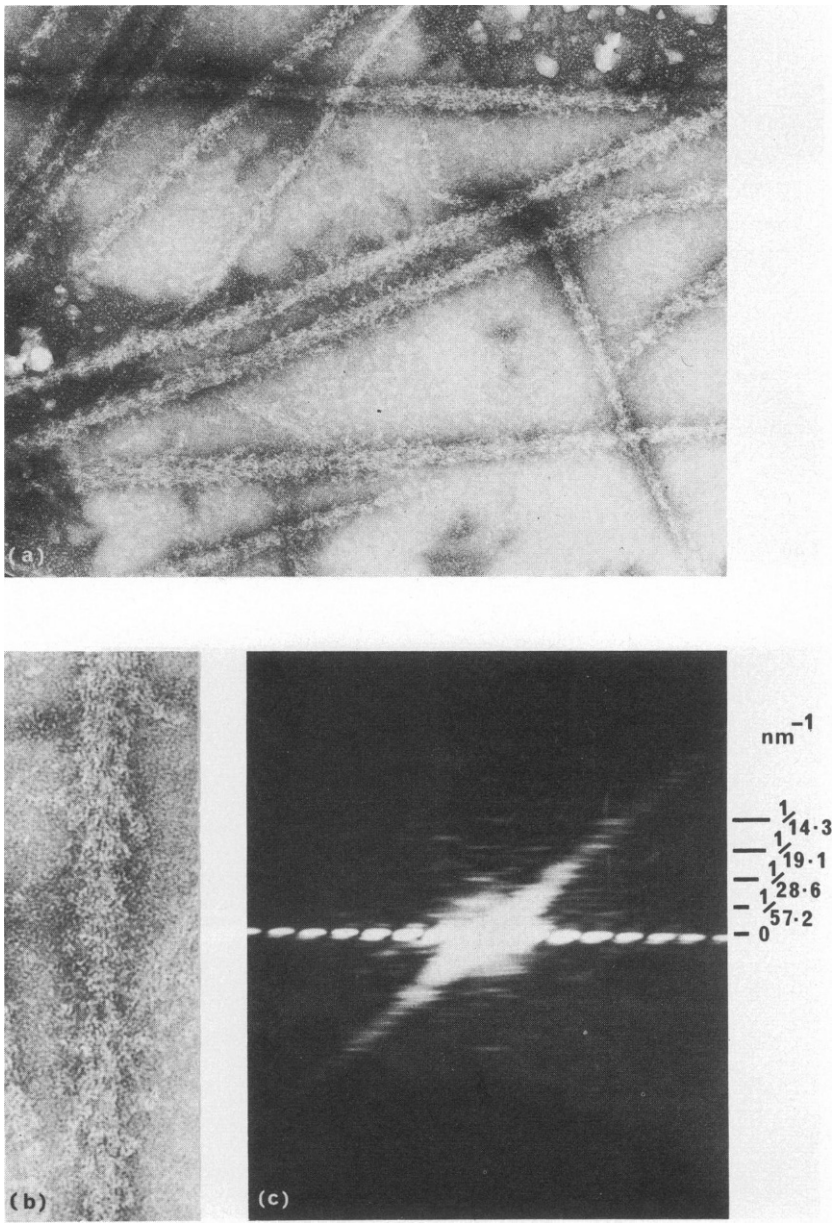

FIGURE 4 Electron microscopy and optical diffraction pattern of synthetic filaments prepared from C-protein and myosin in a molar ratio of 1:2.4. (*a*) A typical electron microscope field (×105,000). Each filament shows great variability in backbone diameter along its length, possibly due to lateral myosin addition during aggregation. Note the absence of both frayed filaments and poorly stained protofilaments. (*b*) Enlargement of part of a synthetic filament from *a* (×210,000). These filaments present a poorly organized appearance at high magnification. (*c*) Optical diffraction pattern of the filament in *b*. Despite the apparent lack of organization noted in *b*, the pattern demonstrates that these filaments nevertheless retain most of the elements of structure observed for the 1:3.3 and 1:4.3 filaments.

prepared from myosin alone. The addition of C-protein at ratios lower than the physiological seems to have no effect on their presence or the relative percentage in the population (22% for 1:13 and 26% for 1:6.6 filaments). At physiological ratios, however, there is a dramatic change in the areas observed by electron microscope (Fig. 3 *a*). The percentage of fraying filaments decreases to 6% for the 1:4.3 filaments and 9% for the 1:3.3 filaments. Tiny aggregates of approximately the same appearance as the frayed filaments are visible, however, and may arise from incomplete filament formation due to the solution reaching its critical concentration. Above the physiological ratio, the 1:2.4 filament micrographs show neither fraying (<1%) nor the tiny protofilaments seen at physiological ratios (Fig. 4 *a*).

C-Protein Added before Dialysis: Optical Diffraction

Filaments prepared from column-purified myosin alone demonstrate a 14.3-nm subunit repeat (Koretz, 1979) and a 43-nm axial period similar to natural filaments. Addition of C-protein to myosin for a final ratio of 1:13 results in filaments that have a greatly weakened 43-nm^{-1} reflection. In some diffraction patterns, a meridional 28.6-nm^{-1} reflection was visible, and could be attributed to the occasional absence of a subunit level. At a ratio of 1:6.6 (Fig. 1 *b* and *c*), the 43-nm^{-1} reflection was almost never visible, although the 14.3-nm subunit repeat was maintained. It should be noted here that, if equal proportions of C-protein and myosin are preserved throughout the precipitation-resuspension method of myosin purification, the molar ratio of C-protein to myosin in the final solution is approximately 1:6–8. The present results indicate that C-protein disrupts the higher organization observed with filaments prepared from column-purified myosin alone at ratios below physiological and thus explain the poorly organized filaments observed previously.

At ratios of 1:3.3 and 1:4.3, the subunit periodicity was again apparent. However, there was

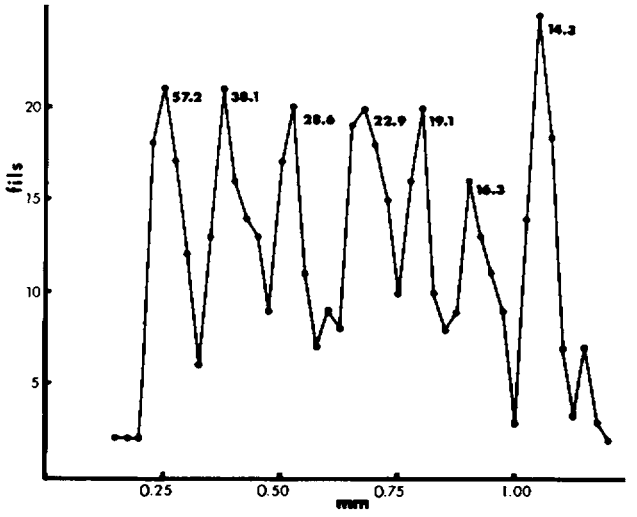

FIGURE 5 Modified histogram representation of distribution of reflections from optical diffraction patterns of synthetic filaments (fils) prepared from a mixture of C-protein and myosin in a 1:3.3 ratio, plotted as a function of distance from the equator. Assuming that the reflection located at ~1 mm is the subunit spacing of 14.3 nm; the spacings of other peaks can be assigned. Layer line separation is consistent with an axial period of 114.4 nm, or 8 times the subunit repeat; because of the 19-nm filament diameter, these results are interpreted as arising from 6 myosin helices, each with 16 subunits per turn.

no evidence of a 43-nm axial repeat; instead a number of strong reflections indicates a radical change in overall filament organization. As Fig. 3 *b* and *c* shows, the reflections visible at these protein ratios indicate an axial periodicity of 114.4 nm, or eight times the subunit repeat. A histogram averaging observations of at least 25 diffraction patterns obtained from filaments demonstrates this more clearly (Fig. 5). An interpretation consistent with this pattern suggests that the 3rd and 5th layer line reflections arise from myosin packing into 6 helices, each with 16 subunits per turn, and thus an apparent repeat of 8 times the subunit repeat. The other reflections, some of which enhance weaker myosin reflections, arise from C-protein forming three helices with a subunit repeat of 28.6 nm and an axial repeat of 114.4 nm, or eight C-protein molecules per turn (Fig. 6 *a*). As the 4-nm backbone striations attributable to paired myosin rods are clearly visible at all C-protein:myosin ratios, it is probable that C-protein is affecting myosin interactions primarily through interpolation into the backbone.

Above physiological ratios, the synthetic filaments again appear disordered in the electron microscope, showing nonuniform filament diameters and lateral bulges. However, the 1:2.4 filaments still clearly show the 14.3 and 114.4-nm periods (Fig. 4 *b* and *c*). This suggests that the bulges may arise from radial addition of myosin molecules during aggregation due to extra available sites on the C-protein at this ratio. This point and the nature of the structural transition observed will be discussed more fully later.

Addition of C-Protein to Filaments Prepared from Column-Purified Myosin: Electron Microscopy and Optical Diffraction

C-protein was added to synthetic filaments prepared from column-purified myosin alone in a ratio of approximately 1:3.3 myosin molecules. The appearance of these filaments in the electron microscope was very different from that seen when the same amount of C-protein was added before dialysis. Filaments demonstrated a fuzzy outline, with 4-nm backbone striations

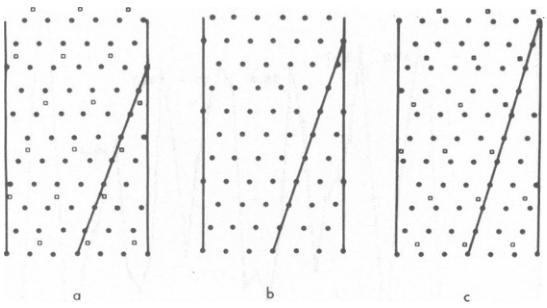

FIGURE 6 Possible helix nets for the three filament types. All are based on the assumption of a six-start myosin helix aggregate and can be interconverted by a small distortion either clockwise or counterclockwise. In all cases, the apparent axial period is less than the assumed true helical repeat. (*a*) Model for the 1:4.3 and 1:3.3 filaments. Each myosin helix has 16 subunits per turn, with an apparent axial repeat for the aggregate of 8 times the subunit repeat (114.4 nm). The C-protein periodicity is suggested to be three helices, each with a subunit spacing of 28.6 nm and an axial repeat of 8 times the subunit repeat. (*b*) Helix net for filaments prepared from column-purified myosin alone (Koretz, 1978). The apparent axial period is 43 nm, analogous to natural thick filament periodicity, whereas the true repeat is 18 times the subunit repeat. (*c*) Model for filaments prepared by adding C-protein after filament formation in a molar ratio of C-protein to myosin of ~1:3.3. Each myosin helix has 20 subunits per turn, with an apparent axial repeat of 10 times the subunit repeat. The C-protein, which is assumed to lie primarily on the surface of the filament in the striations formed by myosin rod packing, is probably arranged in three helices, each with a subunit repeat of 28.6 nm and an axial repeat of 10 times the subunit repeat.

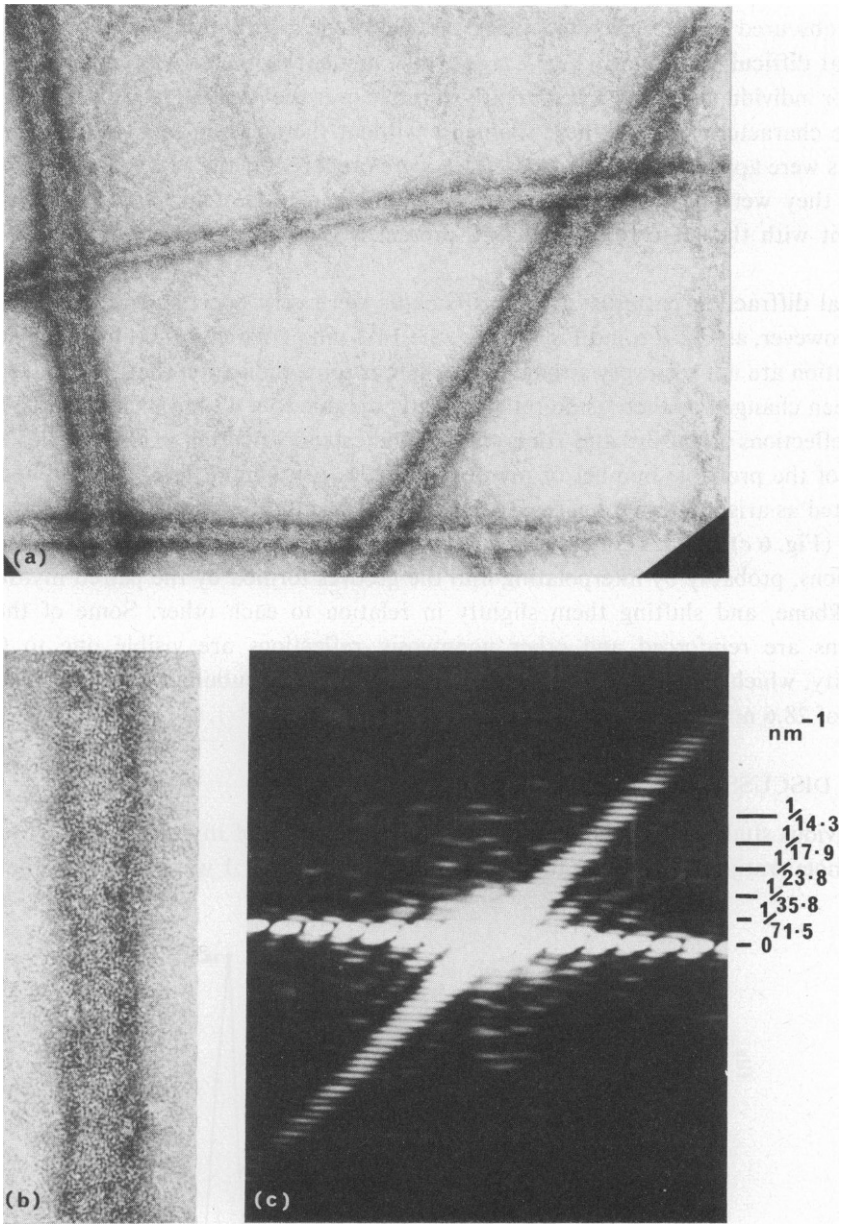

FIGURE 7 Electron microscopy and optical diffraction pattern of synthetic filaments prepared from column-purified myosin alone, then mixed with C-protein for a C-protein:myosin ratio of 1:3.3. (*a*) A typical electron microscope field (×105,000). The filaments demonstrate a fuzzy appearance, and both subunit levels and backbone striations are obscured. This is interpreted as due to C-protein binding and covering the filament surface. There are no fraying filaments or protofilaments present, either because C-protein stablizes filament structure or because it causes deaggregation of poorly organized filaments. (*b*) Enlargement of a synthetic filament from *a* (×210,000). Myosin subunit levels are faintly visible. (*c*) Optical diffraction pattern of synthetic filament from *b*. Although the patterns obtained from these filaments are not very good, they nevertheless demonstrate a periodicity different from both the 1:3.3 filaments and filaments prepared from column-purified myosin alone. The layer line spacings are consistent with an axial repeat of 10 times the 14.3-nm subunit repeat.

usually obscured and projections from the backbone attributable to the myosin heads somewhat difficult to discern (Fig. 7 *a* and *b*). Filament diameter was constant over a long length for individual filaments, but greatly variable over the population, as would be expected from the characterization of these filaments without the addition of C-protein. No fraying filaments were apparent, either because they were obscured by the fuzzy C-protein overlay or because they were completely disrupted by addition of C-protein. These observations are consistent with the interpretation that C-protein is binding almost wholly to the filament surface.

Optical diffraction patterns of these filaments were very poor, and reflections generally weak. However, as Fig. 7 *c* and Fig. 8 show, the 14.3-nm periodicity is visible. Higher levels of organization are not seen very strongly, but there is some indication that the axial repeat has again been changed as there is no reflection attributable to a 43-nm axial repeat. Relatively strong reflections on the 3rd and 7th layer lines indicate an apparent axial period of 1,430 nm; in light of the probable number of myosin molecules per subunit level, these reflections are interpreted as arising from 6 helices, each with 20 subunits per turn and a subunit spacing of 14.3 nm (Fig. 6 *c*). These results suggest that C-protein is externally affecting myosin–myosin interactions, probably by interpolating into the grooves formed by the paired myosin rods of the backbone, and shifting them slightly in relation to each other. Some of the myosin reflections are reinforced and other nonmyosin reflections are visible due to C-protein periodicity, which is suggested to be 3 helices, each with 10 subunits per turn and a subunit spacing of 28.6 nm (Fig. 6 *c*).

DISCUSSION

In a previous study of C-protein effects on the aggregation of myosin in vitro (Moos et al., 1975), molar ratios of C-protein to myosin of approximately 1:1 were used, and the resultant

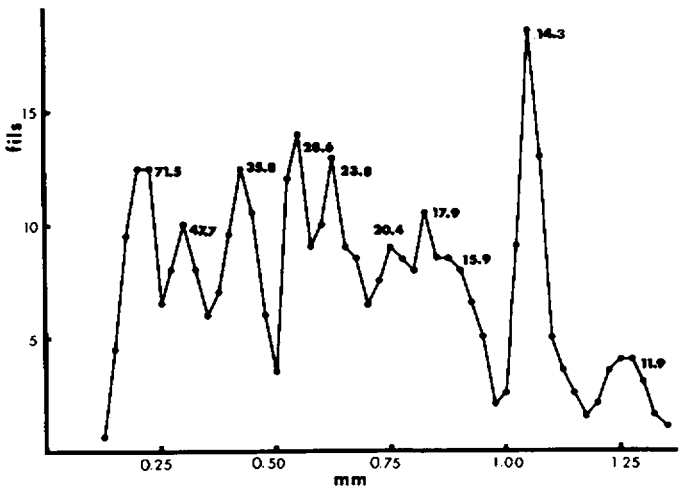

FIGURE 8 Modified histogram representation of distribution of reflections from optical diffraction patterns of filaments (fils) prepared from myosin alone, then mixed with C-protein for a C-protein:myosin ratio of ~1:3.3. Although the patterns were, on the whole, quite poor, the spacings of the peaks indicate a periodicity of 10 times the subunit repeat.

filaments were obviously disordered. In the present study, protein ratios were selected such that the physiological ratio of 1:3–4 was bracketed above and below. Results suggest that these three categories, below, at, and above physiological ratios, describe three structurally distinct populations. Filament populations formed at C-protein:myosin ratios below the physiological, 1:13 and 1:6.6, demonstrate a broad distribution of filament diameters, although a given filament has a uniform diameter, and contain ~25% fraying filaments. The 43-nm axial period observed by optical diffraction in the absence of C-protein (Koretz, 1979) is greatly weakened or absent, whereas the 28.6-nm^{-1} meridional reflection, an indication of occasional subunit level absences, is usually present. Filaments formed at physiological ratios, 1:4.3 and 1:3.3, are highly organized: they are well-stained and compact (with frays present in a ratio of <1 in 10); they demonstrate a uniform diameter along their length; the distribution of widths over the population is relatively narrow. Their optical diffraction patterns indicate a radical change in axial periodicity to 114.4 nm, whereas the 14.3-nm subunit spacing is preserved. Above the physiological ratio, filaments exhibit diffraction patterns similar to those found at the physiological ratio, but differ in other respects from the other two populations. Although fraying filaments constitute <1% of the population, filaments exhibit bulges, as if small myosin aggregates bound radially to the surface in areas distant from the ends where filament lengthening is presumed to occur.

In terms of the criteria used here to measure orderliness, the most ordered filaments are those formed from a C-protein:myosin ratio of 1:3–4. It has already been noted (Moos et al., 1975; Chowrashi and Pepe, 1977; Starr and Offer, 1978) that C-protein binds strongly to myosin and its fragments. The present results suggest that C-protein may also have a specific number of myosin binding sites, either three or four; this interpretation is consistent with the types of disorder noted for filaments prepared from molar ratios of the two proteins above and below the physiological ratio, as will be developed below.

If there are three or four myosin binding sites on each C-protein molecule, then these sites will be saturated by myosin molecules at ratios below the physiological. Thus, for the 1:13 and 1:6.6 filaments, ~75% and 50% of the myosin molecules, respectively, are not bound to C-protein. This means that myosin will behave as if C-protein were not present during the aggregation process, except where C-protein is concentrated in small patches. It is not possible from the results to determine whether 1:13 and 1:6.6 filaments have areas where C-protein is present and others, say at the ends, where it is absent, or whether C-protein is evenly distributed throughout the filament. Even distribution seems more likely. If all C-protein molecules were localized in one region of the filament, then each filament would exhibit an area of highly ordered appearance—that of the 1:4.3 and 1:3.3 filaments—and a less ordered region with a 43-nm apparent axial period. Such a distinction should be particularly apparent with the 1:6.6 filament, where 50% of the filament length (probably the center) would contain C-protein in the physiological ratio and 50% would have no C-protein at all, and optical diffraction patterns would show either one type of periodicity or the other. In fact, no such distinction is seen either by study of electron micrographs or by optical diffraction, which itself shows disorder that can be interpreted in terms of a structural mixture. In addition, the percentage of frays is approximately the same for the 1:13 and 1:6.6 filaments as for filaments prepared from myosin alone. Thus, filaments with protein ratios below physiological are presumably "mosaics" of the two structures all along their length, the degree of mosaic character being dependent upon the C-protein ratio to myosin.

Above the physiological ratio, myosin binding sites on the C-protein are presumably not saturated. As a result, although a filament will exhibit the altered axial organization observed with 1:4.3 and 1:3.3 filaments, it will also exhibit bulges, or uneven backbone width, along its length. These bulges probably arise from radial addition of small myosin or myosin–C-protein aggregates along the filament backbone rather than from addition at the ends of the filament, due to free exposed binding sites for myosin on the C-protein molecules. Since the backbone surface striations attributable to myosin rod packing are visible at all molar ratios studied, only a portion of the C-protein molecule would be exposed to the surface and available for participation in radial aggregation.

C-protein specificity for myosin is also consistent with the observation that, at a 1:2–4 ratio, almost no fraying filaments are seen. If, as has been proposed (Koretz, 1979), frays are due to weak lateral myosin–myosin interactions, then C-protein at or above concentrations where its myosin sites are saturated will strengthen the lateral binding pattern. Pollard (1975) has suggested that frays are due to filaments being formed from a mixture of two populations of myosin, one of which is activated for interaction with thin filaments; the fraying areas then are composed of myosin whose subfragment-2 region has moved out from the backbone. If this is the case, then a decreased percentage of fraying filaments with saturated C-protein concentrations is due more specifically to C-protein interaction with the subfragment-2 region of myosin. The implication of the latter explanation is that C-protein may be involved in control of cross-bridge movement when muscle has been activated for contraction, as has been suggested by Starr and Offer (1978) on the basis of their analytic ultracentrifugation experiments.

Filaments prepared at about the physiological ratio demonstrate neither the bulges characteristic of the 1:2.4 filaments nor the variability in filament diameter over the population characteristic of the 1:13 and 1:6.6 filaments. The percentage of fraying filaments is $<10\%$. Most important is the observation that filament periodicity has been altered from the 43-nm axial period of aggregates prepared from myosin alone to a new axial repeat of 114.4 nm. This symmetry is not seen at ratios below the physiological, indicating that there is a minimum ratio of C-protein to myosin necessary for the structural shift to occur.

On the basis of the differences between the three filament populations, C-protein is suggested to possess the following characteristics: (*a*) It has binding sites for three or four myosin molecules. This interpretation is consistent with the types of filament disorder observed at ratios above and below the physiological, and with the observation that this is approximately the ratio at which the structural transition occurs. (*b*) It is likely that each binding site is specific for a different portion of the myosin rod, which is consistent with analytic ultracentrifugation studies and will explain the structural transition observed. (*c*) The C-protein molecule is interpolated, at least in part, into the filament backbone, since backbone striations are visible at all C-protein:myosin ratios when C-protein is added before dialysis. It is probable that at least a portion of the molecule is accessible to the surface, because of nonmyosin reflections in the optical diffraction patterns and because it is consistent with the explanation of bulges found at high C-protein:myosin ratios.

When C-protein is added in the physiological ratio after the formation of filaments composed of column-purified myosin alone, it appears to bind almost completely to the backbone surface. It is likely, considering the structural effect it exerts and the resultant

optical diffraction pattern, that C-protein is binding in the grooves of the surface formed by myosin rod packing. The simplest explanation for its effect on structure is that, through its fixed myosin binding sites, C-protein causes slippage between adjacent paired myosin rods, which results in the structure observed.

A comparison of the 1:3.3 synthetic filaments and natural thick filaments in the banded region where C-protein is located reveals few apparent similarities. C-protein binding in synthetic filaments seems to occur at 28.6-nm intervals, whereas in the banded region of natural filaments the spacing is 43–44 nm. Interpolation of C-protein into synthetic filament backbones changes the axial period of the aggregates from 43 to 114.4 nm, whereas C-protein binding in vivo presumably does not affect the observed 43-nm period. The diameter of 1:3.3 filaments is consistent with a myosin content of approximately six myosin molecules per subunit level, whereas natural filaments have only three or four.

On the other hand, it is clear from the synthetic filament studies that myosin will aggregate in the presence of C-protein with a periodicity related to that observed with other in vitro aggregates. The 14.3 nm subunit repeat, which is found in synthetic and natural myosin filaments, in some light meromyosin paracrystals, and possibly in rod aggregates, is preserved when C-protein is present. C-protein itself binds to synthetic filaments with a spacing of 28.6 nm, twice the subunit repeat, and appears to be at least partially interpolated into the backbone. The difference in C-protein spacing between that found with synthetic filaments and the 43-nm spacing found in light meromyosin paracystals is probably due to C-protein recognition of additional sites along the myosin rod in the subfragment-2 region. However, the partial interpolation of the protein into the synthetic filament backbone argues against recognition of surface binding sites alone. It may well be that, in the natural thick filament, the observed bands arising from C-protein periodicity are due to a small portion of the C-protein molecule being exposed to the surface, with the rest of the molecule buried in the filament backbone.

The question of what myosin configuration is necessary for C-protein binding in either natural or synthetic myosin filaments has not been answered. Recent structural studies by Bennett (1976) on the packing organization of light meromyosin paracrystals that had been embedded and sectioned indicate that the molecules pack with spacings of $\frac{2}{8}$, $\frac{3}{8}$, and $\frac{3}{8}$ of the 43-nm axial period (10.75, 16.1, and 16.1 nm). She suggests that this may be the packing arrangement of myosin in the banded region of the natural filament, with the $\frac{2}{8}$ spacing a cue for C-protein binding. It is interesting, and possibly significant, to note that the 28.6-nm spacing of C-protein in vitro is only ~6% greater than $\frac{5}{8}$ of 43 nm (26.8 nm).

Fig. 6 shows helix nets of possible structures for the 1:3.3 filaments, filaments prepared from myosin without C-protein, and the latter filaments with C-protein added after formation. It is apparent that only a small angular shift either clockwise or counterclockwise of the structure prepared from myosin alone will result in the structures observed in the presence of C-protein. The similarity between the first two of these helix nets, and the nets proposed by Haselgrove (1975) to explain the differences in meridional reflections from x-ray diffraction of rigor and resting muscle, is striking. It is possible that C-protein in the natural thick filament forces the shift from a 43-nm axial period to a 114.4-nm period when muscle is activated for contraction.

C-protein changes the structure of synthetic aggregates of myosin whether added before or

after filament formation, exhibiting a maximum organizing effect when added before filament formation at approximately physiological ratio. C-protein may have a more substantial role in natural filament organization and function than had previously been supposed. The results do not support the idea that C-protein binds externally to the natural thick filament in bands (Craig and Offer, 1976; Starr and Offer, 1978), nor that C-protein binds with a periodicity that will act as a vernier mechanism in determination of natural filament length (Huxley and Brown, 1967).

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