LIGHT MEROMYOSIN PARACRYSTAL FORMATION

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ABSTRACT

Studies of paracrystal formation by column purified light meromyosin (LMM) prepared in a variety of ways led to the following conclusions: (*a*) different portions of the myosin rod may be coded for different stagger relationships. This was concluded from observations that paracrystals with different axial repeat periodicities could be obtained either with LMM fragments of different lengths prepared with the same enzyme, or with LMM fragments of identical lengths but prepared with different enzymes. (*b*) Paracrystals with a 14-nm axial repeat periodicity are most likely formed by the aggregation of sheets with a 44-nm axial repeat within the sheets which are staggered by 14 nm. All of the axial repeat patterns expected from one sheet or aggregates of more than one sheet, on this basis, were observed in the same electron micrograph. (*c*) C-protein binding probably occurs preferentially to LMM molecules related in some specific way. This was concluded from the observation that the same axial repeat pattern was obtained in paracrystals formed from different LMM preparations in the presence of C-protein, regardless of differences in the axial repeat obtained in the absence of C-protein. (*d*) Nucleic acid is responsible for the 43-nm axial repeat patterns observed in paracrystals formed by the ethanol-resistant fraction of LMM. In the absence of nucleic acid, paracrystals with a 14-nm axial repeat are obtained. (*e*) The 43-nm axial repeat pattern observed with the ethanol-resistant fraction of LMM is different for LMM preparations obtained by trypsin and papain digestion.

The myosin molecule is essentially a rod with two globular heads at one end (35). The rod portion of the molecule is made up of two regions, the light meromyosin (LMM) portion which is insoluble at low ionic strength (0.1) and the S2 portion which is soluble (17). The LMM portion is presumably responsible for the aggregation of myosin molecules to form myosin filaments. It is therefore likely that the aggregation properties of LMM are related to the aggregation of myosin molecules in the myosin filament.

Although some information has been obtained, from X-ray diffraction studies, about the arrangement of myosin cross bridges on the surface of the filament (9), very little information is available concerning the packing of myosin molecules in the shaft of the filament. Huxley (8) has shown, from studies of the aggregation of myosin molecules from solution, that the myosin molecules in one-half of the filament are all oriented opposite those in the other half of the filament, with the rod (or tail) portions of oppositely oriented molecules overlapping in the middle of the filament and with head-to-tail overlap of similarly oriented molecules occurring along the rest of the filament. No further information concerning how the tails are arranged in the shaft of the filament is available from these studies.

Studies of the aggregation of the rod fragment of myosin have been made under conditions where only tail-to-tail aggregation occurs, forming bipolar "segment" aggregates (3, 7, 11, 13). Similar aggregates were obtained with both LMM and myosin. Tail-to-tail overlaps of 43 nm, 90 nm, and 130 nm were observed in these studies. This suggests that such overlaps may also be present in the middle of the myosin filament where tail-to-tail overlap of myosin molecules occurs.

In most of the studies that have been made of paracrystal formation by LMM, axial repeat patterns with an axial repeat periodicity of about 14 nm or 43 nm have been observed (2, 8, 10, 12, 16, 19, 21, 36, 37). More complicated aggregates in the shape of hollow tubes (14) and nets (8, 10, 12, 16) have also been observed. The 43-nm and 14-nm axial repeats are consistent with X-ray diffraction studies which indicate that myosin cross bridges occur at intervals of 14 nm along the myosin filaments with an axial repeat of 43 nm (9). Both the LMM studies and the X-ray diffraction studies therefore indicate the presence of 14-nm and 43-nm stagger between myosin molecules, but none of them give any information about how these stagger relationships are arranged in the paracrystals or in the shaft of the myosin filament.

The primary considerations of this work are: (*a*) to determine whether LMM obtained by trypsin digestion of myosin behaves like that obtained by papain digestion of myosin on paracrystal formation. We have found that the two enzymes can produce fragments with identical chain weights but different aggregation properties, suggesting that they represent different portions of the myosin rod. (*b*) To determine the influence of length on the aggregation properties of the LMM. We have found that LMM fragments about 91 nm or longer always produce paracrystals with an axial repeat periodicity of about 14 nm, whereas fragments about 86 nm in length produce different axial repeat periodicities with more complex patterns. (*c*) To determine the arrangement of the 14-nm and 43-nm stagger relationships in the LMM paracrystals. We have evidence that the 43-nm stagger relationship occurs entirely in one plane producing thin sheets with a 43-nm axial repeat, and that the 14-nm stagger relationship occurs between these planes. (*d*) To determine the effect of nucleic acid impurities on LMM paracrystal formation. We have found that nucleic acid impurities are responsible for the characteristic 43-nm axial repeat pattern observed in paracrystals formed by the ethanol-resistant fraction of LMM. Column purification of the LMM results in paracrystals with a 14-nm axial repeat. Recombination of the nucleic acid impurities with the column purified LMM restores the characteristic 43-nm axial repeat. Ribonuclease treatment destroys the effect of the nucleic acid impurities. (*e*) To determine the effect of C-protein on paracrystal formation. We have found that the presence of C-protein produces a 43-nm axial repeat pattern instead of the 14-nm axial repeat observed with column purified LMM alone.

MATERIALS AND METHODS

The LMM was prepared by trypsin or papain digestion (15, 16) of chicken pectoralis muscle myosin. The myosin used for digestion was purified by reprecipitation (18) or by ammonium sulfate fractionation using the 40% saturated ammonium sulfate fraction of crude myosin. The ethanol-resistant fraction (37) of the LMM was obtained and was further purified on a *O*-(diethylaminoethyl) (DEAE) Sephadex A-50 column (32). Protein concentrations were determined by biuret.

Papain Digestion

The myosin was dialyzed against 0.5 M KCl, 0.03 M phosphate buffer pH 7.0. Digestion was carried out at room temperature for the required length of time, using 0.3 mg of papain (Worthington, 2 × crystallized [Worthington Biochemical Corp., Freehold, N. J.]; activity of 1.1×10^{-6} mol/min/mg enzyme) per milliliter of myosin solution at a concentration of 5 mg/ml. The reaction was stopped by the addition of 0.1 M iodoacetic acid to give a final concentration of 0.001 M and by adjusting the pH to 6.0 (17). The reaction mixture was then precipitated by dialysis against 10 vol of 0.007 M phosphate buffer pH 7.0, collected by centrifugation, and dissolved in 0.5 M KCl, 0.03 M phosphate buffer pH 7.0. The ethanol-resistant fraction of this solution was isolated (37).

Trypsin Digestion

The myosin (8–10 mg protein/ml) was dialyzed against 0.5 M KCl, 0.03 M phosphate buffer pH 6.2; and for each 150 mg of myosin, 1 milliliter of 0.05% trypsin (Worthington, 2 × crystallized) in the same buffer was added, and digestion was allowed to proceed at room temperature for the required length of time (15). The reaction was stopped with 1 milliliter of 0.1% soybean trypsin inhibitor (Worthington) for each 150 mg of myosin. The activity of the trypsin was 6,500 U/mg enzyme where 1 U is the activity that causes a change in OD of 0.001 per minute, using Benzoyl-L-arginine ethyl ester (BAEE) as a substrate. The LMM together with undigested myosin was precipitated by dialysis, and the

ethanol-resistant fraction was obtained as described above for the papain digests.

Column Purification

The ethanol-resistant protein fractions prepared as described above by either papain or trypsin digestion of myosin were purified by column chromatography, using the procedure described by Richards et al. (32) for purification of myosin. About 200 mg of the protein in 0.15 M phosphate buffer pH 7.5 containing 10 mM EDTA was applied to a 2.5 × 90-cm column packed with DEAE Sephadex A-50. The flow rate was approx. 30 ml/h, and samples were collected at 0.5 h intervals. A linear KCl gradient was used to elute the protein. The gradient was made from 500 ml of 0.5 M KCl (or 1 M KCl), 0.15 M phosphate buffer pH 7.5 containing 10 mM EDTA and 500 ml of 0.15 M phosphate buffer pH 7.5 containing 10 mM EDTA. In all cases, only the peak portion of the eluted protein was used. The eluted column purified protein was dialyzed against 20 vol of water, and then the pH was adjusted to 6.5. The precipitated protein was dissolved in 0.5 M KCl, 0.03 M phosphate buffer pH 7.0, and dialyzed against the same buffer. The protein peak was sometimes followed by a peak containing nucleic acid, identified as described in Results. The nucleic acid was concentrated by pressure dialysis, using an Amicon UM 2 filter (Amicon Corp., Scientific Sys. Div., Lexington, Mass.).

Redigestion of Column Purified Preparations

In some cases, the column purified product of digestion was redigested with either trypsin or papain, using the same procedure as described above for digestion of myosin. Two column purified papain preparations were redigested with trypsin (Fig. 4*a* and *b*). One column purified trypsin preparation was redigested with papain (Fig. 4*c*). After redigestion with papain, the protein was again column purified as described above, and two peaks were eluted. One of these peaks contained a component with a chain weight of 30,000 which did not aggregate under any of the conditions used in this study. Only the second peak containing the components shown in Fig. 4*c* was used in this work.

Preparation of C-Protein

C-protein was prepared as described by Offer et al. (22), except that the ammonium sulfate fractionation of the crude chicken pectoralis muscle myosin extract was done in the presence of 10 mM magnesium ATP. This modification was not used in the preparation of rabbit muscle C-protein (leg and back muscles of the rabbit were used). The purified C-protein was dialyzed against 0.01 M potassium phosphate and lyophilized for storage.

Paracrystal Formation

Paracrystals were formed at a protein concentration of 0.8 mg/ml by dialysis against the appropriate buffer solution. Protein concentration was determined by the biuret technique. Paracrystals were always formed in buffers with a total ionic strength of 0.1 and pH 7.35. The buffer ion was either phosphate or imidazole, and the buffer ion contribution to the total ionic strength was varied, keeping the total ionic strength constant by adding KCl.

For paracrystals formed in the presence of C-protein, the lyophilized C-protein was dissolved in 0.5 M KCl, 0.03 M potassium phosphate buffer pH 7.0. Protein concentration was determined by the biuret technique. C-protein was added to the column purified LMM (in the same solvent) in a 1:5 mol ratio before dialysis against the buffer used to produce the paracrystals.

For recombining column purified LMM with the nucleic acid containing impurities, 18.4 μg of the nucleic acid impurities in 0.5 M KCl, 0.03 M potassium phosphate buffer pH 7.0 was added per milligram of LMM before dialysis against the buffer used to produce paracrystals. The concentration of the nucleic acid containing impurities was estimated by assuming an extinction coefficient of 20 (1 mg/ml, 1 cm path) at 260 nm.

Electron Microscopy

The suspension of paracrystals was diluted to approx. 0.16 mg of protein per milliliter with the buffer used to produce the paracrystals. The diluted suspension was placed on copper grids which were previously coated with a carbon film. The excess suspension was washed off with the buffer used to produce the paracrystals. The wash was followed immediately by 1% uranyl acetate solution in water for negative staining, and the excess uranyl acetate solution was drained off with filter paper. Electron micrographs were obtained at a magnification of 40,000 on a Siemens Elmiskop I electron microscope.

SDS-Polyacrylamide Gel Electrophoresis

The procedure used for SDS-gel electrophoresis is essentially that described by Weber and Osborn (38), except that dithioerythritol (DTE) was used instead of β-mercaptoethanol. The protein solution was dialyzed against 10 mM sodium phosphate pH 7.0. After dialysis, the protein solution was made 1% in DTE, 1% in SDS, 15% in sucrose, with a final protein concentration of 1 mg/ml. This solution was then incubated at 100°C (boiling water bath) for 3 min and applied to 6% polyacrylamide gels. In all cases, a set of gels was purposely overloaded (about 30 μg of protein) to detect minor components (Figs 2–4). The running time was about 1 h and 10 min. In some cases, two preparations were run on the same gel. In these cases, a glass divider was present only at the top of the gel tubes, and the two samples were placed one on each side of the divider. The running time

for these split gels was longer in order to accentuate small differences in chain weights.

RESULTS

Column Purification of LMM

An example of the elution profile obtained when the ethanol-resistant fraction of light meromyosin (LMM) is purified on a DEAE Sephadex A-50 column is shown in Fig. 1. This preparation of LMM was obtained by trypsin digestion of myosin for 17 min as already described. The largest peak obtained contains the column purified LMM. The trailing peak of impurities contains nucleic acid.

The presence of nucleic acid in the trailing peak of impurities was identified as follows: (*a*) using a quantitative orcinol determination for RNA (34), the peak was found to contain about 40% RNA. (*b*) The 280 nm to 260 nm ratio of OD was 0.4–0.5. (*c*) Using an extinction coefficient of 20 (1 mg/ml, 1 cm path) at 260 nm, 5 μg of yeast RNA and 5 μg of the nucleic acid trailing peak were compared in polyacrylamide gel electrophoresis, and similar bands were observed on staining the gels with methylene blue. (*d*) No DNA was detectable by fluorimetric ultramicroanalysis (33).

SDS-Polyacrylamide Gel Electrophoresis

The results obtained on gel electrophoresis of the column purified products of digestion used in this work are shown in Figs. 2–4. These gels are heavily loaded to make minor components visible.

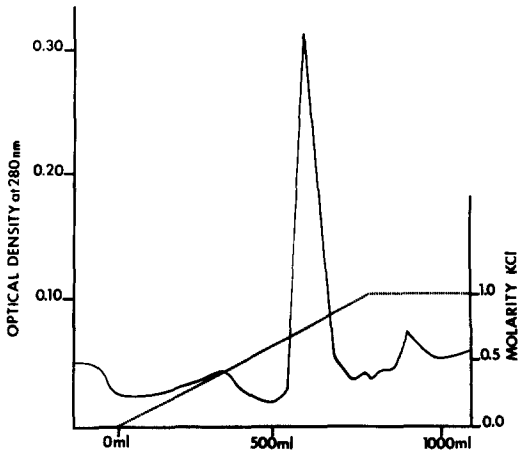

FIGURE 1 Column purification of the ethanol-resistant LMM produced by proteolytic digestion of myosin. This representative preparation was obtained by 17-min trypsin digestion of myosin. Approx. 200 mg was applied to a 2.5 × 90-cm column of DEAE Sephadex A-50 and was eluted with a KCl gradient (see text).

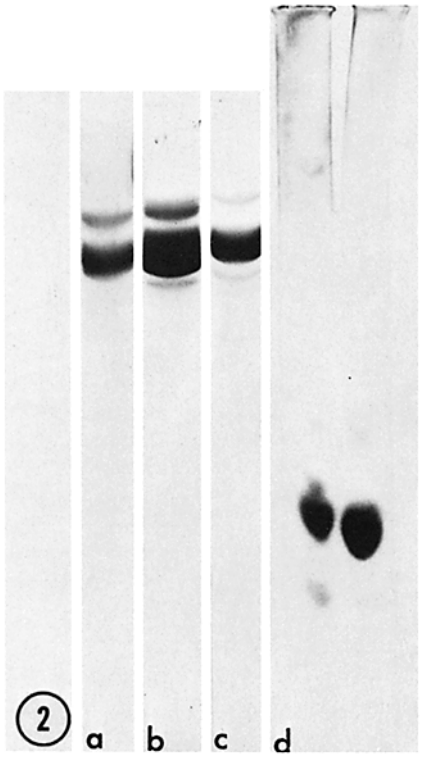

FIGURE 2 SDS-polyacrylamide gel electrophoresis of column purified LMM prepared by trypsin digestion of myosin. These gels are purposely heavily loaded to aid in detection of minor components. (*a*) LMM prepared by 5-min digestion of myosin. The 80,000 chain weight major component represents a rod 96 nm long.[1] (*b*) LMM prepared by 17-min digestion of myosin. The major component has a chain weight of 76,000 (91 nm long). (*c*) LMM prepared by 16-min digestion of myosin. The major component has a chain weight of 72,000 (86 nm long). (*d*) A split gel run with the preparation in (*b*) on the left and (*c*) on the right.

COLUMN PURIFIED TRYPSIN AND PAPAIN FRAGMENTS OF MYOSIN: On digestion with either trypsin or papain, one major LMM component with a chain weight in the range of 72,000–80,000 is present (Figs. 2 and 3), unless the time of digestion is prolonged (Fig. 3*c*). When papain is used, rod is present in addition to

[1] A chain weight of 80,000 represents an LMM with a mol wt of 160,000. The length of 96 nm was calculated using 0.6 nm as the length of a coiled-coil with a mol wt of 1,000 (5).

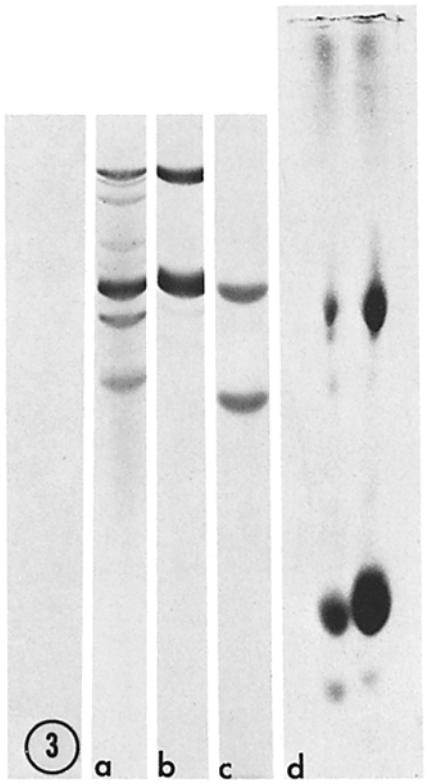

FIGURE 3 SDS-polyacrylamide gel electrophoresis of column purified preparations obtained by papain digestion of myosin. These gels are purposely heavily loaded to aid in detection of minor components. (*a*) Preparation obtained by 15-min digestion of myosin. A major LMM component with a chain weight of 76,000 (91 nm long) and rod with a chain weight of 111,000 (133 nm long) are present. (*b*) Preparation obtained by 16-min digestion of myosin. A major LMM omponent with a chain weight of 76,000 (91 nm long) and rod with a chain weight of 111,000 (133 nm long) are present. (*c*) Preparation obtained by 40-min digestion of myosin. Two major LMM components with chain weights of 73,000 (88 nm long) and 52,000 (62 nm long) are present. (*d*) A split gel run with the preparation in (*a*) on the left and (*b*) on the right.

the major LMM (Fig. 3*a* and *b*); and the rod is absent after prolonged digestion (Fig. 3*c*).

It is important to note that when the same batch of enzyme was used, the LMM produced under similar conditions was completely reproducible. However, different batches of enzyme of the same quality obtained from the same supplier sometimes produced slightly different LMM fragments. The LMM preparations in Fig. 2*b* and *c* were obtained with different batches of trypsin under similar conditions. After 16-min digestion, the major LMM component obtained with one batch of enzyme had a chain weight of 72,000 (Fig. 2*c*), while after 17-min digestion with a different batch of enzyme a 76,000 dalton component was obtained (Fig. 2*b*). That this difference in chain weight is significant is verified by running the two preparations side by side in a split gel (Fig. 2*d*). With papain, although the chain weight of the major LMM component obtained with different batches of enzyme under similar conditions (Fig. 3*a* and *b*) was not significantly different (Fig. 3*d*), one batch of enzyme produced some additional minor components (Fig. 3*a*).

REDIGESTION OF COLUMN PURIFIED LMM: When LMM was prepared with both trypsin and papain sequentially, the second digestion was always done after column purification of the ethanol-resistant products of the first digestion. The LMM in Fig. 4*a* was obtained by trypsin digestion of the preparation in Fig. 3*a*, and that in Fig. 4*b* by trypsin digestion of the preparation in Fig. 3*b*. The papain and trypsin used to obtain the LMM in Fig. 4*a* were different from the batches of enzyme used to obtain the LMM in Fig. 4*b*. Each of the two LMM preparations has a 73,000–74,000 chain weight components. One (Fig. 4*a*) has an appreciable amount of a 52,000 chain weight component, and from the split gel in Fig. 4*d* there is a small difference in the higher chain weight component in the two preparations. Both of these preparations give aggregates with a 60-nm axial repeat periodicity (Table I). Therefore, the presence of the additional LMM component in the preparation in Fig. 4*a* has little effect on paracrystal formation.

The two major components of LMM in Fig. 4*a*, produced by papain digestion followed by trypsin digestion, are identical in chain weight to the two components obtained after prolonged papain digestion (Fig. 3*c*), as can be seen from the split gel in Fig. 4*f*. However, these LMM preparations (Fig. 3*c* and 4*a*) form paracrystals with quite different axial repeat patterns under the same conditions (Table I). This suggests that the LMM components in the two preparations represent different portions of the myosin rod.

The LMM in Fig. 4*c* was prepared by first digesting the myosin with trypsin under conditions identical to those used to produce the LMM in Fig. 2*b* and then by following this by papain digestion. Three components are produced with chain weights of 75,000, 67,000, and 52,000. In

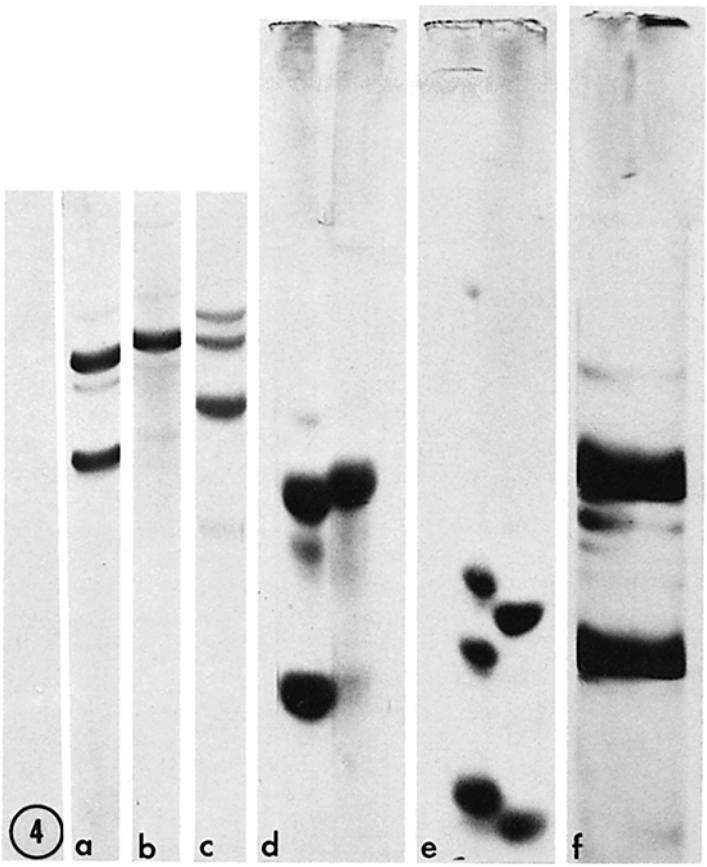

FIGURE 4 SDS-polyacrylamide gel electrophoresis of column purified LMM prepared by digestion with both trypsin and papain. These gels are purposely heavily loaded to aid in detection of minor components. (*a*) Prepared by redigestion of the column purified preparation in Fig. 3*a* (15-min papain digestion) for 25 min with trypsin. Two major LMM components with chain weights of 73,000 (88 nm long) and 52,000 (62 nm long) are present. (*b*) Prepared by redigestion of the column purified preparation in Fig. 3*b* (16-min papain digestion) for 25 min with trypsin. One major LMM component with a chain weight of 74,000 (89 nm long) is present. Both the trypsin and papain used for the preparation of this LMM were different batches but were of the same quality as those used in (*a*). See text. (*c*) Prepared by redigestion of a column purified LMM preparation obtained as in Fig. 2*b* (17-min trypsin digestion) for 25 min with papain. The three major LMM components have chain weights of 75,000 (90 nm long), 67,000 (80 nm long), and 52,000 (62 nm long). (*d*) A split gel run with the preparation in (*a*) on the left and (*b*) on the right. (*e*) A split gel run with the preparation in (*c*) on the left and in Fig. 3*c* on the right. (*f*) A split gel run with preparation in (*a*) on the left and in Fig. 3*c* on the right.

Fig. 4*e*, this preparation is compared with that obtained by prolonged papain digestion (Fig. 3*c*). These preparations (Figs. 3*c* and 4*c*) form paracrystals with identical axial repeat patterns under the same conditions (Table I). This suggests that the LMM fragments in these two preparations represent similar portions of the myosin rod, and that small differences in the chain weight of these portions of the rod have little effect on paracrystal formation.

Paracrystal Formation by Column Purified Preparations

Paracrystals were formed in either phosphate or imidazole buffers with an ionic strength of 0.1 and pH 7.3. The buffer ion contribution to the ionic strength was varied, using KCl to maintain the total ionic strength at 0.1.

The paracrystals formed in the phosphate buffers, in general, were more loosely aggregated

TABLE I

Summary of the Axial Repeat Periodicities Observed with the Column Purified Fragments of the Rod Portion of Myosin

Preparation (figures)	Enzyme used*	Length of fragments‡	Axial repeat periodicity
		nm	*nm*
1. 2*a*	Trypsin (5)	96	15§
2. 2*b*, 5*a*	Trypsin (17)	91	15§
3. 2*c*, 5*b–d*	Trypsin (16)	86	30‖
			60¶
			7**
4. 3*a*	Papain (15)	133	15§
		91	
5. 3*b*	Papain (16)	133	15§
		91	
6. 3*c*, 6*a*	Papain (40)	88	44‡‡
		62	
7. 4*a*, 6*b*	Papain (15)	88	60§§
	Trypsin (25)	62	
8. 4*b*	Papain (16)	89	60§§
	Trypsin (25)		
9. 4*c*	Trypsin (17)	90	44‡‡
	Papain (25)	80	
		62	

* Time of digestion is in parentheses. Where both enzymes were used, the order represents the order in which they were used.

‡ Length was calculated from the polypeptide chain weight (Crick, 1953).

§ Narrow dark bands 4 nm wide at intervals of 15 nm.

‖ Light bands 4 nm wide at intervals of 30 nm.

¶ Group of three light bands at intervals of 60 nm. The outer two of the three light bands are 5 nm wide and separated by 33 nm; the middle band is diffuse.

** Alternating dark and light bands. The width of the bands was difficult to measure.

‡‡ Two dark bands each 3 nm wide, separated by 15 nm, occurring at intervals of 44 nm.

§§ Alternating light and dark bands each 30 nm wide.

than those formed in the imidazole buffers. Also, with increasing phosphate ion contribution to the ionic strength, the LMM in general was more soluble. With increasing imidazole ion contribution to the ionic strength, the LMM in general, was less soluble and the paracrystals appeared more tightly packed.

The axial repeat periodicities observed in paracrystals formed by the preparations used in this study are summarized in Table I.

LENGTH OF THE LMM FRAGMENTS: Three LMM preparations obtained by trypsin digestion of myosin and having major components with chain weights of 80,000 (Fig. 2*a*), 76,000 (Fig. 2*b*), or 72,000 (Fig. 2*c*) were compared. Using the theoretical coordinates of Crick (5) for a two-stranded, coiled-coil of pure alpha helix, the length of these three LMM fragments are 96 nm, 91 nm, and 86 nm, respectively.

The LMM preparations with lengths of 96 nm (Fig. 2*a*) and 91 nm (Fig. 2*b*) always gave paracrystals with a 15-nm axial repeat consisting of a narrow (4 nm) dark band every 15 nm, regardless of the buffer used (Fig. 5*a*).[2] The LMM preparation having a major component with a length of 86 nm (Fig. 2*c*) gave three different axial repeat patterns on paracrystal formation (Fig. 5*b* and *d*). Two of the patterns (Fig. 5*b* and *c*) were observed in the phosphate buffers, with some indication of transitions between them. The pattern in Fig. 5*b* consists of a narrow (4 nm wide) light band at intervals of 30 nm, and that in Fig. 5*c* consists of two narrow (4 nm wide) light bands spaced 33 nm apart with a third diffuse light band midway between them, all three repeating at intervals of 60 nm. These two patterns were also observed in imidazole buffer with an ionic strength of 0.025 in imidazole. On increasing the ionic strength of imidazole to 0.05, some of the paracrystals had a pattern consisting of alternating light and dark bands with an axial repeat of 7 nm. Paracrystals with this axial repeat pattern predominated in buffers with an ionic strength of 0.075 or 0.1 in imidazole (Fig. 5*d*).

We may conclude that although fragments longer than 91 nm always produce paracrystals with the same axial repeat patterns (Fig. 5*a*), a decrease of about 5 nm in length to 86 nm results in different axial repeat patterns which vary depending on the buffer used for formation of the paracrystals (Fig. 5*b–d*).

PREPARATIONS WITH MORE THAN ONE MAJOR LMM COMPONENT: Prolonged (40 min) papain digestion produced an LMM preparation containing two major components with lengths of 88 nm and 62 nm (Fig. 3*c*). In imidazole buffers, this preparation produced paracrystals with an axial repeat pattern consisting of two dark bands each 3 nm wide, separated by 15 nm, and repeating every 44 nm (Fig. 6*a*). In phosphate buffers, no turbidity developed in the LMM solution, and no paracrystals were observed.

The LMM preparation in Fig. 4*a* was obtained by 15-min papain digestion (Fig. 3*a*), then followed by 25-min trypsin digestion of the column purified products of papain digestion. In phosphate buffer, this LMM preparation produced paracrystals with a 60-nm axial repeat consisting

[2] Paracrystals formed from preparations containing rod in addition to LMM (Fig. 3*a* and *b*) produced similar paracrystals with a 15-nm axial repeat, except that the repeat was not so clearly visible as that obtained in the absence of rod (31).

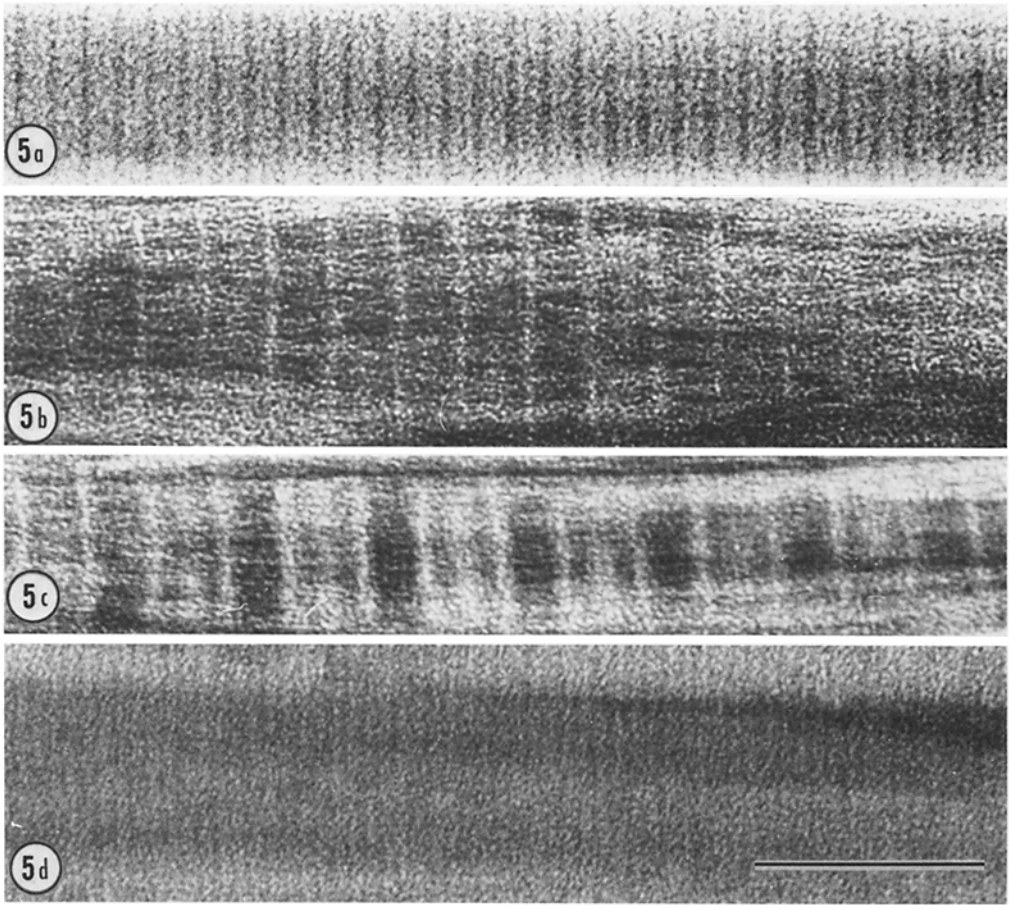

FIGURE 5 Paracrystals formed by column purified LMM obtained by trypsin digestion of myosin (LMM preparations in Fig. 2). Bar, 0.1 μm. × 300,000. (*a*) Representative example of the 15-nm axial repeat often observed. This example is the preparation in Fig. 2*b*, having a major LMM component 91 nm long. The axial repeat consists of dark bands 4 nm wide occurring at intervals of 15 nm. The buffer used in this example has an ionic strength of 0.025 in phosphate and 0.075 in KCl, pH 7.35. (*b–d*) LMM preparation in Fig. 2*c* having a major LMM component 86 nm long. The buffer used in (*b*) had an ionic strength of 0.025 in phosphate and 0.075 in KCl, pH 7.35, in (*c*) 0.025 in imidazole and 0.075 in KCl, pH 7.35, and in (*d*) 0.1 in imidazole pH 7.35. The axial repeats consist of (*b*) a light band 4 nm wide spaced at intervals of 30 nm, (*c*) two light bands 5 nm wide spaced 33 nm apart with a light diffuse band midway between them, the set of three bands repeating at intervals of 60 nm, and (*d*) alternating light and dark bands with a repeat of 7 nm.

of alternating light and dark bands 30 nm wide (Fig. 6*b*). In imidazole buffers, this preparation produced paracrystals with an axial repeat consisting of dark bands 4 nm wide at intervals of 15 nm as in Fig. 5*a*. (The preparation in Fig. 4*b* was prepared in essentially the same way as that in Fig. 4*a* but with different batches of enzymes. Even though the lower chain weight component is missing in the preparation in Fig. 4*b*, paracrystal formation is closely similar to that of the preparation in Fig. 4*a*.)

The LMM preparations in Figs. 3*c* and 4*a* have components of identical chain weights (Fig. 4*f*) but give paracrystals with different axial repeat periodicities (Fig. 6). This suggests that the identical chain weight LMM fragments in the different preparations represent different portions of the myosin rod.

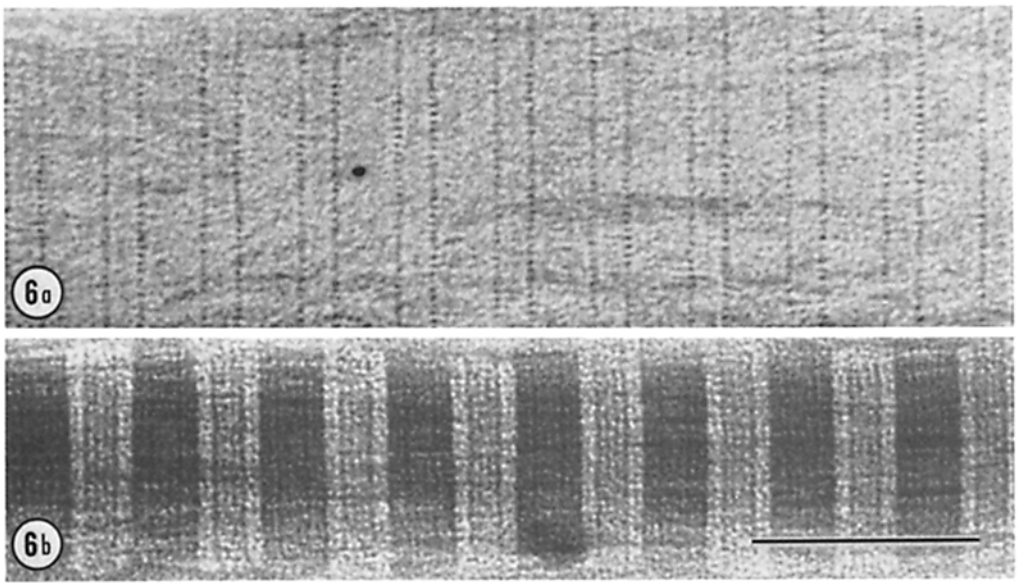

FIGURE 6 Paracrystals formed by the column purified LMM preparations in Figs. 3*c* and 4*a*. Bar, 0.1 μm. × 300,000. (*a*) LMM prepared by 40-min papain digestion of myosin (Fig. 3*c*) having two components with lengths of 88 nm and 62 nm. The buffer has an ionic strength of 0.025 in imidazole and 0.075 in KCl, pH 7.35. The axial repeat consists of two dark bands, 3 nm wide and separated by 15 nm, repeating every 44 nm. This LMM was soluble in all of the phosphate buffers used in this work. (*b*) LMM prepared by 25-min trypsin digestion of a column purified preparation obtained by 15-min papain digestion of myosin (Fig. 4*a*). The buffer has an ionic strength of 0.075 in phosphate and 0.025 in KCl, pH 7.35. The axial repeat consists of alternating dark and light bands 30 nm wide giving a repeat of 60 nm.

The LMM preparation in Fig. 4*c* was prepared by 17-min trypsin digestion of myosin (as in Fig. 2*b*), followed by 25-min papain digestion of the column purified products obtained with trypsin. The products of papain digestion were again column purified. As is evident from the split gel in Fig. 4*e*, this preparation has components similar in chain weight to those in Fig. 3*c*, except that in place of the component with a length of about 88 nm in Fig. 3*c* there are two components in Fig. 4*c* with lengths of about 80 nm and 90 nm. Both of these LMM preparations (Figs. 3*c* and 4*c*) produced paracrystals with the same axial repeat patterns under the same conditions (Fig. 6*a*). This suggests that these represent similar portions of the myosin rod and that variations in the length of these papain fragments in the range of 80–90 nm do not affect paracrystal formation.

From these observations, we can conclude that the method of preparation is the major factor in determining the axial repeat pattern obtained on paracrystal formation.

ORIGIN OF THE 14-NM AXIAL REPEAT PERIODICITY: With some preparations which produced paracrystals with a 15-nm axial repeat of dark bands 4 nm wide (Fig. 5*a*), it was possible to observe stages in the formation of this axial repeat. In Fig. 7, the different patterns observed are all present in the same area of the grid. These consist of a strong dark band at intervals of about 44 nm (with some faint bands detectable within this interval), two dark bands spaced 15 nm apart and occurring at intervals of about 44 nm (upper left corner of Fig. 7), and dark bands at intervals of 15 nm (upper right corner of Fig. 7). These observations strongly suggest that the 15-nm axial repeat periodicity is formed by the overlapping of sheets in which the axial repeat is 44 nm and the stagger between sheets is 15 nm.

PARACRYSTAL FORMATION IN THE PRESENCE OF C-PROTEIN: In general, the presence of C-protein interferes with LMM aggregation so that the paracrystals that do form are less well organized than in the absence of C-protein. This is reasonable if the LMM-C-protein interaction sterically interferes with LMM-LMM interactions.

The LMM preparation in Fig. 2*a*, which gives

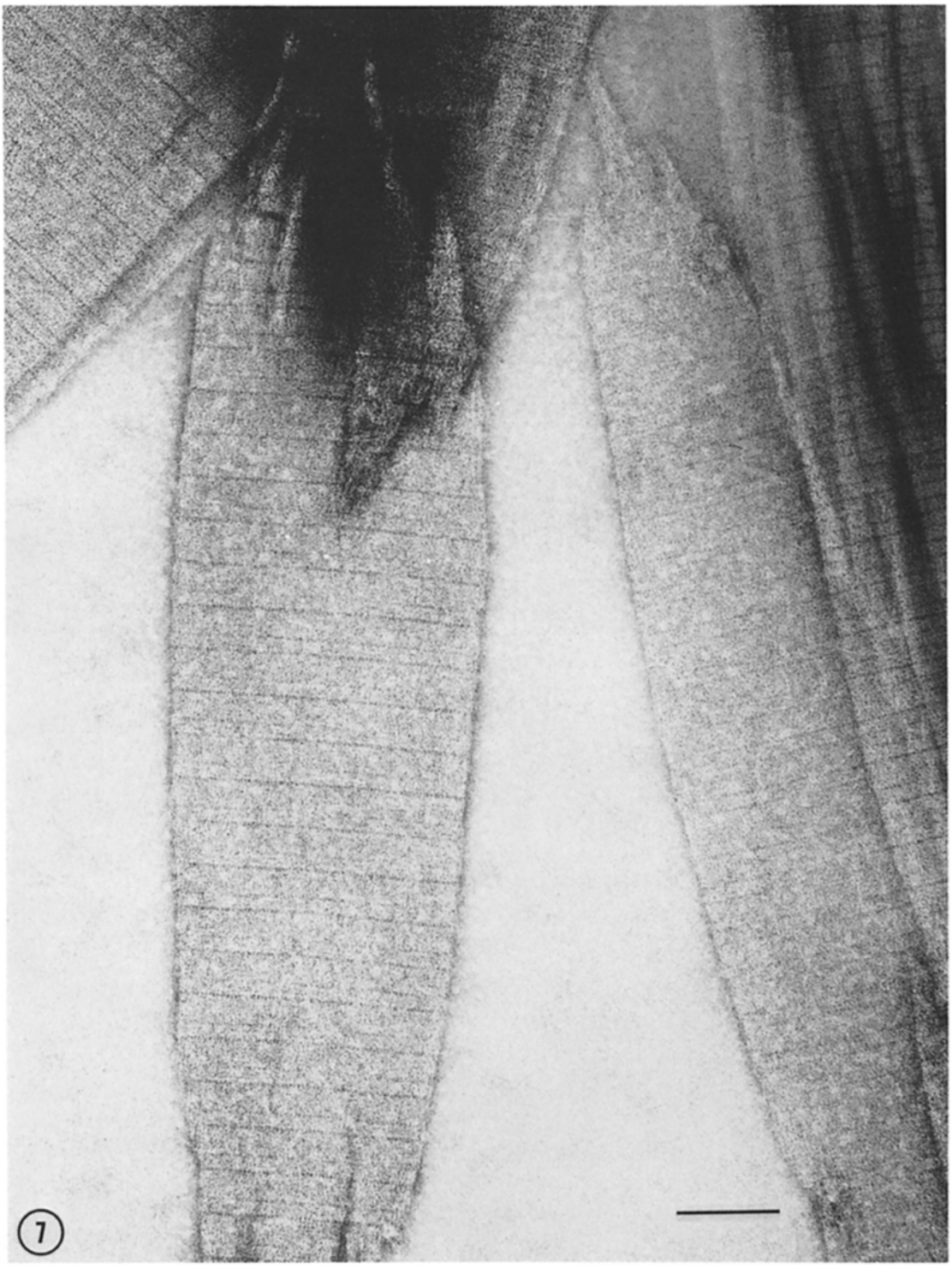

FIGURE 7 Origin of the 15-nm axial repeat in paracrystals of LMM. In this area, three patterns are observed. In the upper left corner, there are two dark bands each 4 nm wide spaced 15 nm apart and occurring at intervals of 44 nm. In the upper right corner are dark bands 4 nm wide occurring at intervals of 15 nm. In the center of the micrograph, strong dark bands 4 nm wide occur at intervals of 44 nm (with some faint bands detectable within this interval). The buffer used has an ionic strength of 0.025 in imidazole and 0.075 in KCl, pH 7.35. Bar, 0.1 μm. × 160,000.

paracrystals with a 15-nm axial repeat periodicity (Fig. 5*a*), in the presence of C-protein gives paracrystals with a 44-nm axial repeat (Fig. 8*a*). The light band (14 nm wide) is presumably due to the presence of C-protein. The LMM preparation in Fig. 3*c*, which gives paracrystals with dark bands spaced 15 nm apart occurring at intervals of 44 nm (Fig. 6*a*), in the presence of C-protein gives the same axial repeat pattern with C-protein superimposed in the space between the dark bands spaced 15 nm apart (Fig. 8*b*). In this case, both the periodicity of the LMM paracrystal and the attached C-protein are clearly observable. This unequivocally determines that the C-protein is binding to the LMM with the axial repeat of the underlying LMM.

PARACRYSTAL FORMATION IN THE PRESENCE OF NUCLEIC ACID CONTAMINANTS: The involvement of RNA in producing the 44-nm axial repeat patterns observed with the ethanol-resistant fraction of the LMM (Fig. 9) was shown in the following ways: (*a*) on recombining the RNA containing impurities in the trailing peak in Fig. 1 with column purified LMM, the 44-nm axial repeat characteristic of the ethanol-resistant fraction of LMM before column purification (Fig. 9) was observed instead of the 15-nm axial repeat observed with the column purified LMM alone under the same conditions (Fig. 5*a*). (*b*) Treating the RNA containing impurities with RNase destroyed their ability to produce the 44-nm axial repeat on recombination with column purified LMM. (*c*) The ethanol-resistant fraction of LMM which gives the 44-nm axial repeat periodicity gives the 15-nm axial repeat periodicity after treatment with RNase. These findings verify that it is the RNA in the impurities which is responsible for the 44-nm axial repeat.

Substitution of the RNA containing impurities with yeast RNA did not result in formation of paracrystals with the 44-nm axial repeat. This suggests that the RNA isolated from the LMM preparation by column chromatography interacts specifically with the LMM.

Two different 44-nm axial repeat patterns were observed in the presence of the RNA containing impurities, one characteristic for trypsin LMM fragments, and the other characteristic for papain fragments. The pattern characteristic for trypsin fragments consists of alternating light (13 nm

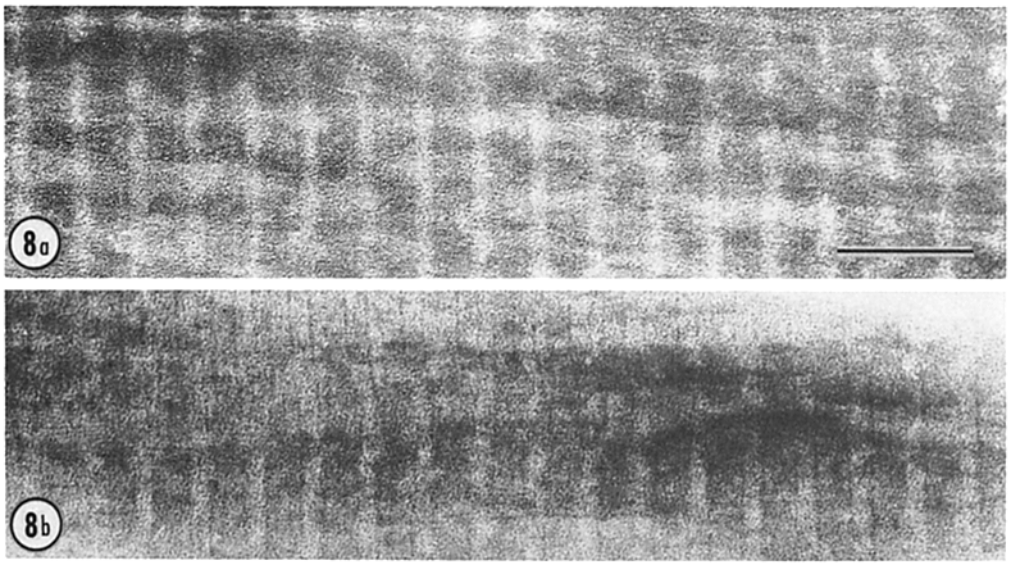

FIGURE 8 Paracrystals formed in the presence of C-protein. Bar, 0.1 μm. × 180,000. (*a*) In the absence of C-protein, the LMM preparation used here (Fig. 2*a*) gave paracrystals identical to those shown in Fig. 5*a*. In the presence of C-protein, the axial repeat consists of a light band 14 nm wide occurring at intervals of 44 nm. The light band represents the presence of C-protein. The buffer is the same as that used in Fig. 5*a*. (*b*) The LMM preparation used here is the same as that shown in Fig. 6*a*, except that in this case the paracrystals were formed in the presence of C-protein. The buffer used is also the same as that used in Fig. 6*a*. The axial repeat pattern of the LMM is not changed. In this case, the C-protein has been introduced in the 15-nm space between the two dark bands occurring at 44-nm intervals observed in Fig. 6*a*.

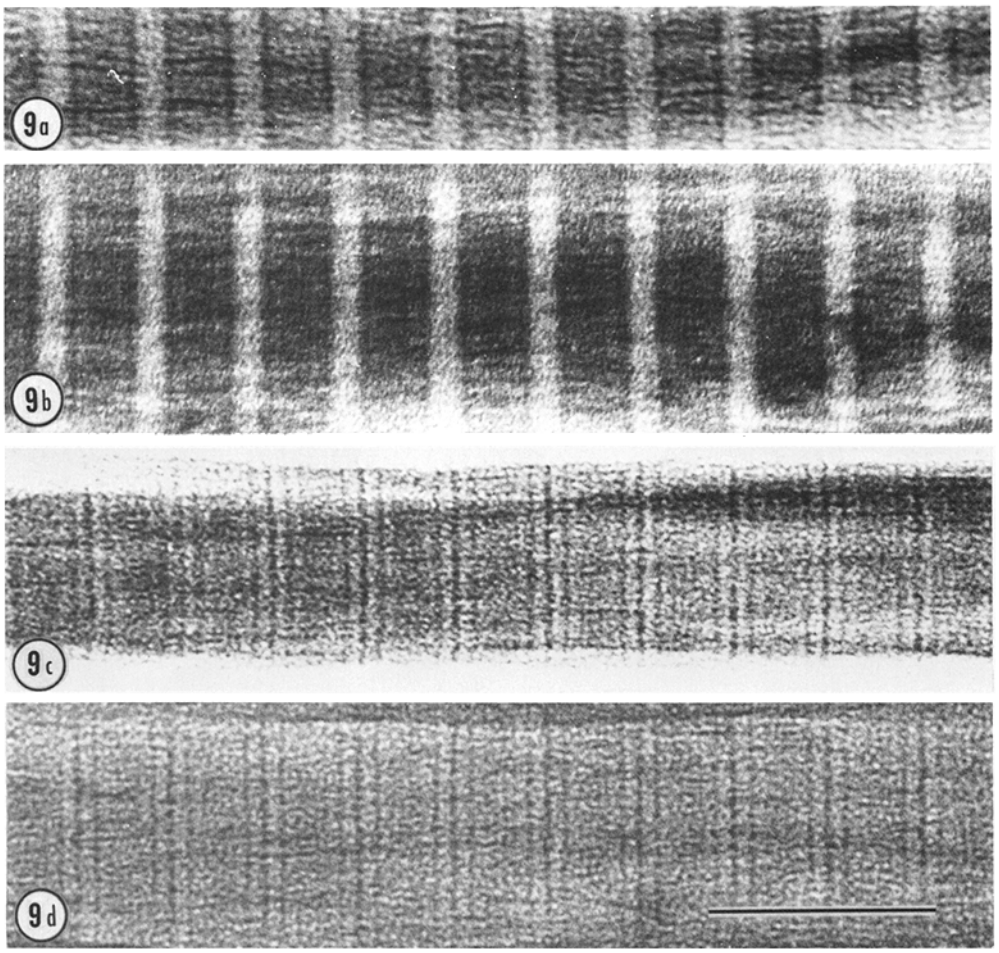

FIGURE 9 Effect of nucleic acid on paracrystal formation. Bar, 0.1 μm. × 300,000. (*a*) Ethanol-resistant fraction of a 5-min trypsin digestion of myosin. The buffer had an ionic strength of 0.025 in phosphate and 0.075 in KCl, pH 7.35 (same as that in Fig. 5*a*). The axial repeat pattern consists of alternating light (13 nm wide) and dark (31 nm wide) bands. Within the dark bands, at the edges neighboring the light bands, are darker bands about 4 nm wide. (*b*) After column purification of the ethanol-resistant fraction of LMM used in (*a*), paracrystals identical to those in Fig. 5*a* were observed under the same buffer conditions. Recombining the column purified LMM with the nucleic acid fraction isolated from the column produced the axial repeat pattern shown here which is identical to that observed before column purification, i.e., in (*a*) above, under the same buffer conditions. (*c*) Ethanol-resistant fraction of an 18-min papain digestion of myosin. The buffer had an ionic strength of 0.025 in phosphate and 0.075 in KCl, pH 7.35. The axial repeat pattern consists of a group of three dark bands occurring at 44-nm intervals. The outer two dark bands are 3 nm wide, and the middle one is 4 nm wide. The center-to-center distance between the outer bands is 16 nm. (*d*) The preparation used in (*c*) was column purified and then redigested for 15 min with trypsin. This preparation gave paracrystals identical to those in Fig. 5*a*, under the same conditions. On recombining this with the nucleic acid fraction isolated from the column, the axial repeat pattern shown here was obtained. This pattern is identical to the axial repeat pattern obtained before column purification, i.e., in (*c*) above, under the same buffer conditions.

wide) and dark (31 nm wide) bands (Fig. 9*a* and *b*). Within the dark bands, at the edges neighboring the light bands, are darker bands about 4 nm wide. The pattern characteristic for papain fragments consists of a group of three dark bands occurring at 44-nm intervals (Fig. 9*c* and *d*). The

outer two dark bands are 3 nm wide, and the middle one is 4 nm wide. The center-to-center distance between the outer bands is 16 nm.

Nucleic acid containing impurities isolated from either a papain or trypsin LMM preparation were capable of producing either of the two patterns (Fig. 9) when combined with column purified LMM. The pattern produced was dependent only on whether the column purified LMM was produced by trypsin or papain digestion.

The presence of substantial amounts of rod in the column purified preparations inhibited production of the 44-nm axial repeat pattern on combination with the nucleic acid containing impurities. Papain preparations containing rod were redigested with trypsin for 15–25 min, after which the 44-nm axial repeat characteristic for the papain fragments was produced on combination with the nucleic acid containing impurities.

DISCUSSION

Studies of the aggregation of the LMM fragment of myosin into paracrystals with a well-defined axial repeat periodicity can be useful for obtaining information about the packing of myosin molecules in the shaft of the myosin filament. Presumably, the LMM fragment of myosin, which carries the solubility characteristics of the myosin molecule, will behave like the intact myosin molecule in its aggregation properties. Axial repeats of about 14 and 43 nm have been reported in LMM paracrystals (2, 8, 10, 12, 16, 19, 21, 36, 37) and in myosin filaments (9). This indicates that stagger relationships of about 14 and 43 nm are involved in LMM and myosin aggregates, but little information is available about the arrangement of these stagger relationships.

Three major pieces of information have come out of this work: (*a*) it is likely that the 15-nm axial repeat in paracrystals results from the stagger of sheets in which the axial repeat in the sheets is 44 nm, and the stagger between sheets is 15 nm. (*b*) It is likely that different portions of the myosin rod are coded for different stagger relationships. (*c*) Nucleic acid impurities and C-protein can affect LMM paracrystal formation.

Stagger Relationships in Paracrystals with a 15-nm Axial Repeat Periodicity

In LMM preparations which give paracrystals with a 15-nm axial repeat periodicity (Fig. 5*a*), in some cases three different axial repeats can be observed in the same electron micrograph (Fig. 7). These consist of a strong dark band at intervals of 44 nm (with some indication of lighter bands between them), dark bands at intervals of 15 nm, and a pattern of two dark bands spaced 15 nm apart occurring at intervals of 44 nm. A possible explanation for the paracrystals observed in Fig. 7 is that they are made up of structural units staggered by 44 nm in one plane to form thin sheets with a 44-nm axial repeat and that there is a stagger of 15 nm between overlapping sheets. Two of these overlapping sheets would give the pattern of two dark bands spaced 15 nm apart and occurring at intervals of 44 nm. Three or more overlapping sheets would produce the 15-nm axial repeat periodicity. Consistent with this is the fact that where the 15-nm axial repeat is observed in Fig. 7, the aggregate appears to be made up of multiple overlapping aggregates.

It is noteworthy that in the model for the myosin filament proposed by Pepe (23, 24), the shaft of the filament is made up of three groups of four structural units. Within each group of four there are two pairs of structural units in which the stagger within a pair is 43 nm and the stagger between pairs is 14 nm. Therefore, the stagger of 43 nm within a pair corresponds to the 44-nm stagger within sheets of the paracrystals, and the stagger of 14 nm between pairs corresponds to the 15-nm stagger between sheets in the paracrystal. Whether or not this correspondence is truly significant will depend upon further studies of the similarities in the fine structure of LMM paracrystals and myosin filaments.

Although the 15-nm axial repeat observed in paracrystals formed from LMM preparations is well defined (Fig. 5*a*), the presence of rod can make this periodicity more difficult to observe clearly (31). In the model for the myosin filament proposed by Pepe (23, 24, 26, 27), about 86 nm of the rod (corresponding to the LMM portion) is packed in the shaft of the filament, and the rest of the rod portion of the molecule (S2 portion) is excluded to the surface of the filament. A similar packing arrangement in the paracrystals would result in exclusion of the S2 portion of the rod from the combined LMM and rod aggregate and the bulky S2 portion would be expected to interfere with the precise packing of LMM in the paracrystal, producing a less clearly defined 15-nm axial repeat, consistent with these findings.

Paracrystal Formation by LMM Fragments Representing Different Portions of the Myosin Rod

No systematic attempts have previously been made to study the effect of length of the LMM on its aggregation properties. We have compared the aggregation properties of three LMM preparations all produced by trypsin digestion and all containing one major LMM component.

The longer LMM fragments (91 nm and 96 nm in length, see Table I) produced paracrystals with a 15-nm axial repeat (Fig. 5*a*) under all of the buffer conditions used in this work. In contrast to this, the shorter LMM fragment (86 nm in length) produced paracrystals with different axial repeat periodicities depending on the buffer used for formation of the paracrystals (Fig. 5*b–d*); and none of these paracrystals showed a 15-nm axial repeat in any of the buffers used in this study. This suggests (*a*) that the portion of the rod corresponding to the difference in length of the 91-nm and 86-nm fragments, i.e., 5 nm, is required to obtain the 15-nm axial repeat under all of the buffer conditions used in this work, and (*b*) that the portion represented by the 86-nm fragment is coded for a different stagger relationship than the entire rod.

The LMM fragments routinely obtained by trypsin or papain digestion of myosin are generally assumed to represent the entire tail end of the myosin molecule. Loss of material on proteolytic digestion is considered to occur at the junction between the LMM and S2 portions of the rod (17, 39). In this study, two preparations containing LMM fragments of identical chain weights (Fig. 4*f*) were compared with respect to aggregation properties. One preparation was obtained by prolonged (40 min) papain digestion (Fig. 3*c*), and the other by short (15 min) papain digestion followed by longer (25 min) trypsin digestion (Fig. 4*a*). These two preparations with components of identical chain weights produced paracrystals with entirely different axial repeat patterns (Fig. 6). In addition, the preparation obtained exclusively by papain digestion was essentially soluble in the phosphate buffers, whereas that involving trypsin digestion was insoluble in both the phosphate and imidazole buffers. This difference in solubility and paracrystal formation strongly suggests that the fragments in the two preparations of identical chain weight components represent different portions of the myosin rod, and that these different portions of the rod are coded for different stagger relationships.

From studies of myosin dimer formation (1, 6) and the formation of antiparallel aggregates of rod "segments" (3, 7, 11), it is clear that considerable overlap of the myosin rod is involved in aggregation. This makes it highly unlikely that differences in terminal peptides resulting from enzyme cleavage could account for the differences in aggregation properties observed in these two preparations (Fig. 6). Therefore, since the two preparations have components of identical chain weight, it is reasonable to assume that the more soluble papain preparation (Fig. 3*c*) corresponds to a portion of the rod closer to the more soluble S2 end of the rod than the trypsin preparation (Fig. 4*a*).

A preparation similar to that in Fig. 4*a* was obtained containing only the higher chain weight component (Fig. 4*b*). These similar preparations had similar aggregation properties (Table I; Fig. 6*b*), indicating that the presence of the lower chain weight component in the preparation in Fig. 4*a* does not have a significant effect on paracrystal formation.

We have been comparing a preparation obtained exclusively by papain digestion (Fig. 3*c*) with preparations obtained by short (15 or 16 min) papain digestion followed by longer (25 min) trypsin digestion (Fig. 4*a* and *b*). It was of interest to compare these double-digested preparations with one obtained conversely by short (17 min) trypsin digestion followed by longer (25 min) papain digestion (Fig. 4*c*). The solubility characteristics and paracrystal formation of this latter preparation were similar to those of the preparation obtained exclusively by papain digestion (Figs. 4*c* and 6*a*), suggesting that they represent similar portions of the myosin rod. This finding strengthens our correlation of papain with the production of an LMM fragment which represents a different portion of the myosin rod than that produced by trypsin.

Without (*a*) a complete determination of the amino acid sequence of the myosin rod, (*b*) a determination of what portions of the rod are represented by each fragment, and (*c*) a determination of how the amino acid sequence in each fragment is related to the aggregation properties, the various factors involved in aggregation cannot be determined unequivocally. However, we have made a start by showing that (*a*) fragments of different lengths produced by trypsin digestion can give paracrystals with different axial repeat pe-

riodicities (Table I: lines 2 and 3), and that (*b*) fragments of identical chain weights but most likely representing different portions of the myosin rod can also form paracrystals with different axial repeat periodicities (Table I: lines 7 and 8). These findings strongly suggest that different regions of the myosin rod are coded for different stagger relationships.

In general terms, these findings are consistent with the scheme proposed by Pepe (24) for the precise determination of the length of myosin filaments. In this scheme, interacting sites with different spacings would interact between neighboring myosin molecules in a vernier-type mechanism involving only myosin molecules. This could be achieved if the molecules are staggered and if the spacing of the interacting sites in one portion of the molecule is different from the spacing of those in another portion. Such differences in spacing of interacting sites in different portions of the rod could account for the different stagger relationships observed with the different LMM fragments studied in this work.

Influence of C-Protein and Nucleic Acid on LMM Paracrystal Formation

C-PROTEIN: By comparing the localization of antibody specific for C-protein observed in *both* fluorescence and electron microscopy, it has been found that C-protein localization is *restricted* to a portion of the myosin filament (30). Localization determined by electron microscopy alone (4) is not sufficient to determine restricted localization because of the special requirements for visibility of untagged antibody in electron microscopy (25, 28, 29). Neither the factors responsible for this restricted binding of C-protein to a portion of the myosin filament nor the functions of C-protein are yet known. The purpose of our studies of the interaction of C-protein and LMM is to try to understand what determines C-protein binding to the myosin filament.

C-protein binds to LMM paracrystals with the same repeat as the LMM to which it is bound. This is demonstrated unequivocally by the simultaneous visualization of the axial repeat of the LMM and the added C-protein (Fig. 8*b*). In previous studies of C-protein binding to LMM paracrystals (19, 20), enhancement of the 43-nm axial repeat of LMM paracrystals was observed on addition of C-protein, but the LMM repeat and the added C-protein were not observed simultaneously.

In all cases when C-protein binding to paracrystals was observed, the C-protein was restricted to a region less than 15 nm wide (Fig. 8). This width is consistent with the width of the stripes observed after labeling of myofibrils with antibody specific for C-protein (4, 30).

An important finding in this work is that LMM preparations which give paracrystals with a 15-nm axial repeat in the absence of C-protein (Fig. 5*a*) give paracrystals with a 44-nm axial repeat when formed in the presence of C-protein (Fig. 8*a*). In the electron micrograph in Fig. 8*a*, there is no evidence of an underlying 15-nm axial repeat, suggesting that the presence of C-protein has altered the formation of the paracrystal in this case. In contrast to this, LMM paracrystals having a pattern of two dark bands spaced 15 nm apart and occurring at intervals of 44 nm in the absence of C-protein (Fig. 6*a*) were not altered when formed in the presence of C-protein, the C-protein adding in the space between the two dark bands spaced 15 nm apart (Fig. 8*b*). The resultant axial repeat patterns observed in the presence of C-protein, in both cases, are strikingly similar, which suggests that C-protein may be binding preferentially to LMM molecules related in some specific way.

The change in axial repeat pattern obtained in the presence of C-protein (Fig. 8*a*) suggests further that C-protein binding can interfere sterically with some LMM-LMM interactions that would have occurred in the absence of C-protein. That C-protein can interfere with LMM-LMM interactions is further suggested by the finding that paracrystals formed in the presence of C-protein were always more poorly organized than those formed in the absence of C-protein.

These findings suggest that the restricted localization of C-protein to a portion of the myosin filament (30) may be related to a specific packing arrangement of the myosin rods in that portion of the shaft of the filament. The possible perturbations of packing resulting from interaction between different portions of the myosin rod coded for different stagger relationships, already discussed above, could be responsible for determining the restricted location of the specific packing region required for C-protein binding. These possibilities are all generally consistent with the possible scheme proposed by Pepe (24) for determination of the length of myosin filaments. The detailed mechanism by which C-protein binds to a restricted portion of the myosin filament must await elucidation of the details of both C-protein-myosin interaction and myosin filament packing.

NUCLEIC ACID: Whenever a 43 to 44-nm axial repeat periodicity has been observed with column purified LMM, the pattern consists of either a single dark band at 44-nm intervals (Fig. 7) or two dark bands spaced 15 nm apart and occurring at 44-nm intervals. The more generally observed 43-nm axial repeat patterns (2, 8, 16, 21, 36) are more complex.

We have found that, if the column purified LMM has not been prepared by extensive proteolytic digestion, paracrystals with a 15-nm axial repeat periodicity will be obtained (Fig. 5*a*) regardless of whether trypsin or papain was used for digestion. However, if the LMM preparation is not column purified, a complex 44-nm axial repeat pattern is observed (Fig. 9). We have shown that contaminating nucleic acid is responsible for this 44-nm axial repeat pattern by the following criteria: (*a*) recombining the nucleic acid containing impurities with the column purified LMM produces paracrystals with the same 44-nm axial repeat pattern that was observed before separation. (*b*) Destroying the nucleic acid by RNase treatment destroyed its ability to produce paracrystals with the 44-nm axial repeat pattern on recombination with column purified LMM. (*c*) Nucleic acid contaminated LMM which produced paracrystals with a 44-nm axial repeat pattern after treatment with RNase produced paracrystals with a 15-nm axial repeat pattern. There is no evidence for the presence of nucleic acid in myosin filaments. Therefore, it is likely that these effects of nucleic acid contamination have no direct relationship to the packing of myosin molecules in the myosin filament.

Two distinct 44-nm axial repeat patterns were observed, one characteristic for trypsin fragments (Fig. 9*a* and *b*), and the other for papain fragments (Fig. 9*c* and *d*). Since the same preparation of nucleic acid containing impurities could produce either pattern depending solely on whether trypsin or papain was used to prepare the LMM, the difference must be related to the LMM rod.

From Fig. 9, it is clear that the ethanol-resistant fraction of the digest before column purification gives the same 44-nm axial repeat pattern as the recombined column purified LMM and nucleic acid containing impurities. This is so, in spite of the fact that the column purified papain digest was redigested briefly (15 min) with trypsin to convert the rod present in the preparation to LMM. The reason why the pattern characteristic for the papain digest was preserved in this case is not clear. The trypsin digestion was required because substantial amounts of rod in the preparation inhibited the effect of the nucleic acid containing impurities. The reason for this inhibition is also not clear.

We have shown that the ethanol-resistant fraction of LMM prepared from myosin purified by reprecipitation will contain contaminating nucleic acid (Fig. 9*a* and *c*). Similarly prepared LMM obtained from the myosin of different muscle types give different 43-nm axial repeat patterns characteristic of the muscle type (21, 36). This has been interpreted as indicating that there is a difference in the primary structure of the myosin rods in the different myosin types. From our findings that: (*a*) nucleic acid contamination is responsible for the 43-nm axial repeat and (*b*) the nucleic acid effect is specific for nucleic acid isolated from LMM preparations, i.e., yeast RNA is ineffective, the possibility that nucleic acid differences could be responsible for the differences in 43-nm axial repeat observed with the LMM from different myosin types must be considered. This possibility is not excluded by the fact that the same nucleic acid containing preparation can give axial repeat patterns characteristic for trypsin or papain LMM fragments (Fig. 9).

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