The Axial Repeats in Paracrystals of Light Meromyosin and Its Complex with C-Protein

Z. A. Podlubnaya, N. A. Freydina and V. V. Lednev

Institute of Biological Physics, USSR Academy of Sciences,
142292 Pushchino, Moscow Region, USSR

Abstract. We examined the axial repeats in electron micrographs of three types of negatively stained paracrystals (two tactoid- and one sheet-like type) of rabbit light meromyosin (LMM) and its complex with C-protein characterized previously by similar axial period of about 43.0 nm. Assuming for the axial repeat in type II tactoids the value of 42.93 $\pm$ 0.05 nm as it was determined by X-ray diffraction technique (Yagi and Offer 1981), we found average axial repeats in type I tactoid and in sheet-like paractrystal of 42.93 $\pm$ 0.75 nm and 43.50 $\pm$ 0.62 nm respectively. Analyzing the micrographs where the two types paracrystals are located side-by-side we determined rather accurately the average ratio of axial repeat in sheet-like paracrystal to that in type I tactoid (1.013 $\pm$ 0.002). Taking 42.93 nm as the axial repeat in type I tactoid, the axial repeat in sheet-like paracrystal was found to be 43.50 $\pm$ 0.08 nm. C-protein binds to LMM with the period of the underlying LMM paracrystals and independently of the value of their axial repeats. Two different axial repeats (42.9 nm and 43.5 nm) revealed for LMM paracrystals in this study precisely coincide with the average repeat periods of myosin crossbridges along the thick filaments found for different physiological states of skeletal muscles (Lednev and Kornev 1987). Molecular basis for the appearance of two structural states in LMM paracrystals and in the shafts of thick filaments are discussed.

Key words: Light meromyosin paracrystal — Axial periodicity — C-protein — Skeletal muscle

Introduction

It is known that Ca^{2+}-dependent structural change of actin-containing filaments from inhibited to activated state is a condition necessary for triggering the actin-myosin interaction (Ebashi et al. 1969). However, some X-ray diffraction data have indicated that the chain of events in Ca^{2+}-mediated regulation of muscle contraction includes also structural change of the myosin-containing, or

thick filaments. According to these data stimulation of contractile activity of muscles or their transition in rigor are accompanied by an increase in the spacing of meridional reflection of 14.3 nm, corresponding to the average axial repeat of the structure of thick filaments, by approximately 1 % (Huxley and Brown 1967; Haselgrove 1975; Lednev et al. 1981). It has been noted, that this effect does not necessarily result from the true increase in the axial period of the levels of myosin crossbridges along the thick filament; alternatively, it can be due to the change in the angle of the myosin bridges in relation to the thick filaments axis (Rome 1972a, b; Squire 1981; Lednev et al. 1981). However, a rigorous analysis of the whole set of meridional reflections, including those at moderate diffraction angles, allowed us to conclude that at least the transition of skeletal muscle from relaxing to rigor state is accompanied by the elongation of the thick filament backbone by approximately 1.3 % (Lednev and Kornev 1987).

Details of the structural changes underlying the reversible transformation of the thick filament backbone remain obscure. In particular, it is not clear whether the elongation results from some unfolding of the helical structure of the whole backbone, or whether it is due to some change in the structure of the myosin coiled-coil rod-parts forming the backbone. The correct interpretation of the nature of the structural transition is of direct relevance to the solution of the problem concerning the packing periods of C-protein and myosin in skeletal muscle thick filaments. Data concerning the period of the C-protein lattice formed on its copolymerization with myosin in thick filaments are contradictory. According to the results of Squire et al. (1982) the period of C-protein lattice in human skeletal muscle exceeds that of myosin packing in thick filaments by approximately 1.2 %. On the other hand, the axial repeats of C-protein and myosin in the backbones of thick filaments of frog and rabbit skeletal muscles have been found to be similar (Craig 1977; Shpagina et al. 1983).

Herein we present the results of a comparative analysis of axial repeats in electron micrographs of paracrystals of pure LMM and its complex with C-protein. We could show that the reversible 1.3 % change of the thick filament backbone structure during transition of skeletal muscle from relaxed state to contraction or rigor may be due to the ability of the LMM-moiety of myosin molecules to form aggregates with two axial repeats, differing also by about 1.3 %.

This work was presented in a preliminary form at the 8th All Union Conference on Biophysics and Biochemistry of Biological Motility (Podlubnaya et al. 1987).

Materials and Methods

Preparation of myosin, LMM and C-protein

Myosin and C-protein were prepared from rabbit skeletal muscles according to Offer et al. (1973). LMM was obtained as described previously (Podlubnaya 1973).

Preparation of paracrystals

Paracrystals were formed by overnight dialysis at 4 °C of LMM solution (0.1 – 0.05 mg/ml) against a solution containing 0.1 mol/l KCl, 0.01 mol/l phosphate buffer, pH 7.0. Paracrystals of LMM with C-protein were formed either by dialysis of the two proteins against the above solution or by treating preformed LMM paracrystals with C-protein directly on electron microscope grids. There was no difference in C-protein decoration of LMM paracrystals between these two methods.

Electron microscopy and analysis of electron micrographs

A drop of the suspension of paracrystals was applied to a carbon-coated collodion grid, washed with a few drops of freshly prepared 0.1 mol/l ammonium acetate solution and then stained with 1% uranyl acetate solution. The preparations were examined in a JEM-100B electron microscope operating at an accelerating voltage of 80 kV, and 35 μm objective aperture at nominal magnifications up to 50,000 ×.

The axial repeats in LMM paracrystals were measured on original micrograph negatives using an optical magnifier (Carl Zeiss JENA). The average axial periods for each of the three paracrystal types as well as the standard deviation (SD) and standard error of the mean (SEM) were calculated. The method of inner calibration of magnification was employed. The axial period in one paracrystal type (type II of LMM tactoid) has recently been determined with a high accuracy by X-ray diffraction (42.93 ± 0.05 nm) (Yagi and Offer 1981). This period was used as the reference value for the periodicity estimation in the two other paracrystal types.

For more accurate measurement of axial periodicity in paracrystals decorated with C-protein, eleven micrographs were selected in which LMM paracrystals with and without C-protein were located in close contact with each other. The analysis of such micrographs provided a higher accuracy of the results because in this way experimental artifacts due to differences in fixation and photography were largely eliminated.

Results

Axial periods in LMM paracrystals

It has been shown earlier that under certain conditions LMM can form paracrystals with different axial periods, and among them three types of paracrystals with axial repeats of approximately 43.0 nm (Podlubnaya et al. 1968; 1969; Nakamura et al. 1971; Podlubnaya 1973; Katsura and Noda 1973; Podlubnaya 1974; Chowrashi and Pepe 1977; Bennett 1981; Strzelecka-Golaszewska et al. 1985). The three paracrystal types show different fine striation patterns within the main repeat. Two of them have the appearance of typical tactoids (Figs. 1*A*, *B*, 2*A*, and Fig. 3, *bottom*) and one is sheet-like (Figs. 1*C*, 2*B*, Fig. 3, *top*). As already mentioned these three types of paracrystals can coexist in the same preparation. Using the value of 42.93 nm for the period in type II tactoid, we

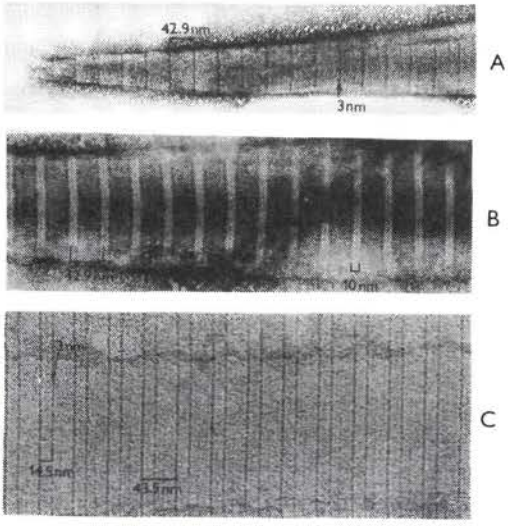

Fig. 1. Electron micrographs of different types of LMM paracrystals. *A* — type I LMM tactoid with axial periodicity of 42.93 nm, formed by narrow dark dividing lines about 3 nm wide. Under the conditions used, C-protein does not bind to this type of LMM tactoid. *B* — Type II LMM tactoid with axial periodicity of 42.93 nm, formed by light dividing bands about 10 nm wide. The period in this tactoid type was used as reference value. *C* — Sheet-like LMM paracrystal with axial periodicity of 43.5 nm, formed by double dividing bands consisting of two dark 3 nm wide lines, about 14.5 nm apart. Magnification 147,500 × (*A*); 194,100 × (*B*); 216,000 × (*C*).

established that the average axial period in type I LMM tactoid was 42.93 ± ± 0.75 nm (Table 1). The axial repeat in sheet-like LMM paracrystals was 43.5 ± 0.62 nm, i.e. it exceeded the axial period in LMM tactoids by 1.33 %.

Axial periods of C-protein lattice in paracrystals of LMM-C-protein complex

At least under the conditions of our experiments C-protein bound to sheet-like LMM paracrystals and to one of the two LMM tactoids (type II). In electron micrographs of LMM paracrystals compactly decorated with C-protein, the period of C-protein decoration is usually distinctly seen whereas that of LMM packing in paracrystal is masked by C-protein (Fig. 3, *top*). We succeeded in obtaining electron micrographs with rare C-protein decoration in which the periodic patterns, reflecting both the LMM packing in paracrystal and the arrangement of C-protein along the paracrystal, were well discernible (Figs. 2*A*, *B*). The analysis showed that the periods of C-protein lattice on the surface of LMM paracrystals precisely coincide with the axial periods of LMM packing

Table 1. Axial periods in different paracrystal types of pure LMM and LMM with C-protein. Statistical parameters

Paracrystal type	Average period (nm)	Standard deviation (S. D.)	Number of paracrystals analyzed (N)	Standard error of the mean (S. E. M.)
LMM paracrystal with dark narrow (3 nm) dividing lines (type I tactoid)	42.93	0.75	142	0.07
LMM paracrystal with 10 nm light dividing bands (type II tactoid)	42.93*			
LMM paracrystal with two 3 nm-dividing lines by about 14.5 nm apart (sheet-like)	43.50	0.62	59	0.09
Sheet-like LMM paracrystal decorated with C-protein	43.54	0.48	33	0.08

* The axial period in this type of paracrystals was used as reference value.

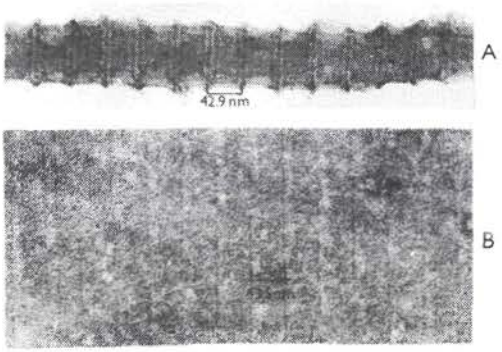

Fig. 2. Electron micrographs of different types of LMM paracrystals decorated with C-protein. *A* Type II LMM tactoid. C-protein binds to 10 nm-dividing bands, 42.93 nm apart. *B* Sheet-like LMM paracrystal with rare C-protein decoration. C-protein is located in the space between two dark 30 nm-lines, forming the period of 43.5 nm.
Magnification 211,800× (*A*); 212,000× (*B*).

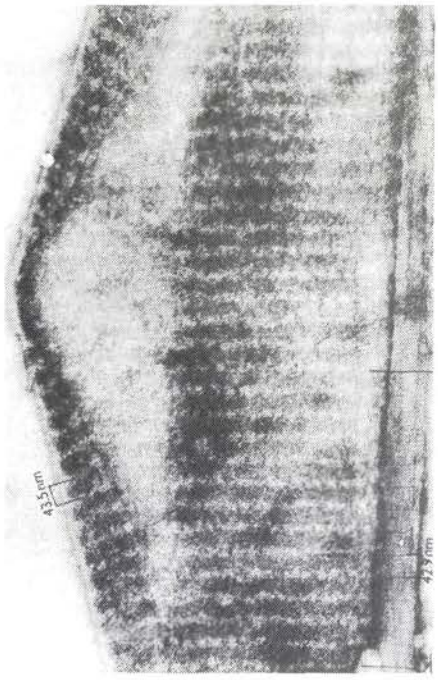

Fig. 3. Electron micrographs of two types of LMM paracrystals. *Bottom* Type I LMM tactoid not decorated with C-protein. *Top* Sheet-like LMM paracrystal compactly decorated with C-protein. The lower paracrystal with axial period of 42.93 nm was used to measure C-protein decoration repeats in the upper paracrystal (43.5 nm). Magnification 141,100 ×.

in paracrystals, being 43.54 ± 0.48 nm for sheet-like paracrystal and 42.93 nm (S. D. was not determined) for type II tactoid.

For more accurate measurements of the axial repeats in sheet-like LMM paracrystals decorated with C-protein eleven electron micrographs were selected, in which type I LMM tactoids, not binding C-protein (Fig. 3, *bottom*) were arranged in tight contact with the sheet-like paracrystals decorated with C-protein (Fig. 3, *top*). In such micrographs the difference of axial repeats in paracrystals is seen especially clearly. Analyzing these electron micrographs we established with a high accuracy the ratio of period values for each pair of paracrystals. The average ratio was 1.013 ± 0.022 (Table 2), i.e. the axial periodicity in sheet-like paracrystal decorated with C-protein exceeded the axial repeat in type I of LMM tactoids not decorated with C-protein by 1.33 ± 0.2 %. Using the obtained ratios of axial periods in the compared paracrystals and

Table 2. Axial periods in two types of paracrystals located side-by-side in electron micrographs (sheet-like type with C-protein and type I tactoid, not decorated with C-protein). Statistical parameters

N/N	Ratio of axial periods in the two paracrystal types	Axial period in sheet-like LMM paracrystals, decorated with C-protein* (nm)
1	1.011(9)	43.44
2	1.011(6)	43.42
3	1.015(5)	43.59
4	1.015(5)	43.59
5	1.012(3)	43.46
6	1.012(3)	43.46
7	1.015(5)	43.59
8	1.012(0)	43.45
9	1.012(0)	43.45
10	1.016(0)	43.62
11	1.011(9)	43.44
Average	1.013(3)	43.50
S. D.	0.022	0.08
S. E. M.	0.0006	0.02

* The axial period in type I LMM tactoid was taken as 42.93 nm.

taking the value of axial repeat in LMM tactoids not decorated with C-protein as 42.93 nm, the value of average axial periodicity in sheet-like LMM paracrystals decorated with C-protein could be estimated as 43.5 ± 0.08 nm (Table 2). As seen from Table 1, the axial periodicity measured in the same paracrystal type varies significantly from paracrystal to paracrystal (and even along the same paracrystal) as evidenced by the values of standard deviations (S. D. = = 0.75; 0.62; 0.48 nm). However, as it might be expected, the ratio of the periods in the two side-by-side types of paracrystals, compared remains effectively constant. Thus an improved accuracy of the determination of the axial periodicity (about 10 times) is obtained. It is worth mentioning that Safer and Pepe (1980) also compared axial periods in two types of LMM paracrystals (sheet-like paracrystals and type II tactoids). According to their data the difference in the axial periodicity between the two LMM paracrystal types was about 1 nm, (44 nm and 43 nm) with a standard deviation of 1 nm. Our comparative method of estimation provides an almost tenfold better accuracy.

Discussion

It follows from our data that two of the three LMM paracrystal types analyzed have identical axial repeats of 42.93 nm (type I and II tactoids), whereas the period in sheet-like paracrystals is 43.5 nm i.e. the axial repeats in the LMM paracrystals can differ by 1.33 %. We concluded also that C-protein binds to LMM paracrystals with a periodicity directly related to the LMM organization.

As mentioned in Introduction, longitudinal cryo-sections of human skeletal muscle fibers fixed in relaxed state revealed two axial periods in C-zones of thick filaments: 42.9 nm and 43.4 nm. (Squire et al. 1982). These authors suggested that the axial repeats of C-protein lattice and LMM packing in thick filaments differ from each other, and that the larger of the two periods corresponds to that of C-protein lattice. On the contrary, the results of the present investigation show that for the same type of paracrystals the axial repeats in decorated and not decorated LMM paracrystals coincide. Our results are in agreement with the data obtained by Safer and Pepe (1980). Moreover, based on own earlier electron microscopic studies of the thick filament structure in isolated A-disks and on longitudinal sections of rabbit and frog skeletal muscles, we could also conclude that the axial repeats of myosin and C-protein in C-zones of thick filaments coincide with each other (Shpagina et al. 1983; see also Craig 1977).

Thus, the rod segments of the myosin molecule can form in vitro filamentous paracrystalline aggregates with and without C-protein showing two axial repeats (42.9 nm and 43.5 nm), precisely coinciding with the average repeat periods of myosin crossbridges along the thick filaments found for different physiological states of vertebrate (frog, rabbit) skeletal muscles (Lednev and Kornev 1987). It is reasonable to suppose that the two discrete structural states of the thick filaments in vertebrate skeletal muscles are due to the properties of myosin molecules themselves and, in particular, their LMM-parts. We believe that the existence of the two slightly different periods in the sequence of the acidic and basic amino acid residues in LMM- and S2-fragments of rabbit myosin, which include either 27.1 or 27.7 residues per period (Parry 1981), may provide the molecular basis for the appearance of two structural states in LMM paracrystals and in the shafts of thick filaments.

References

Bennett P. M. (1981): The structure of spindle-shaped paracrystals of light meromyosin. J. Mol. Biol. **146,** 201–221

Chowrashi P. K., Pepe F. A. (1977): Light meromyosin paracrystal formation. J. Cell Biol. **74,** 136–152

Craig R. (1977): Structure of A-discs from frog and rabbit skeletal muscle. J. Mol. Biol. **109,** 69—81

Ebashi S., Endo M., Ohtsuki I. (1969): Control of muscle contraction. Quart. Rev. Biophys. **2,** 351—384

Haselgrove J. C. (1975): X-ray evidence for conformational changes in the myosin filaments of vertebrate striated muscle. J. Mol. Biol. **92,** 113—143

Huxley H. E., Brown W. (1967): The low angle X-ray diagram of vertebrate striated muscle and its behaviour during contraction and rigor. J. Mol. Biol. **3,** 383—431

Katsura J., Noda H. (1973): Structure and polymorphism of light meromyosin aggregates. J. Biochem. **73,** 257—268

Lednev V. V., Kornev A. N. (1987): The change in axial repeat of the structure of skeletal muscle thick filaments at muscle transitions "relax-rigor" and "relax-contraction". Proc. 8th All-Union Symp. "Biophysics and Biochemistry of Biological Motility". p. 86. Nauka, Tbilisi (in Russian)

Lednev V. V., Srebnitskaya L. K., Kornev A. N., Khromov A. S., Malinchik S. B. (1981): Localization of minor proteins and structural changes in myosin filaments of vertebrate skeletal muscle. Biofizika **26,** 739—748 (in Russian)

Nakamura A., Sreter F., Gergely J. (1971): Comparative studies of light meromyosin paracrystals derived from red, white and cardiac muscle myosins. J. Cell Biol. **49,** 883—898

Offer G., Moos C., Starr R. (1973): A new protein of the thick filaments of vertebrate skeletal myofibrils. Extraction, purification and characterization. J. Mol. Biol. **74,** 653—676

Parry A. D. (1981): Structure of rabbit skeletal myosin. Analysis of the amino acid sequences of two fragments from the rod region. J. Mol. Biol. **153,** 459—464

Podlubnaya Z. A. (1973): Effect of ionic strength on light meromyosin paracrystal structure. Biofizika **18,** 593—599 (in Russian).

Podlubnaya Z. A. (1974): Electron microscope investigation of the light meromyosin strucutre as function of ionic strength. In: Structure and Function of Normal and Diseased Muscle and Peripheral Nerve (Eds. I. Hausmanova—Petrusewicz, H. Jedrzejowska) p. 47—59, Polish Medical Publishers

Podlubnaya Z. A., Kalamkarova M. B., Nankina V. P. (1969): Polymorphism of the light meromyosin crystallization. J. Mol. Biol. **46,** 591—592

Podlubnaya Z. A., Kalamkarova M. B., Nankina V. P. (1969): Polymorphism of the light meromyosin crystallization. J. Mol. Biol. **46,** 591—592

Podlubnaya Z. A., Kalamkarova M. B., Azarashvili T. S., Nankina V. P. (1968): Electron microscopic study of light meromyosin. Biofizika **13,** 835—838 (in Russian)

Podlubnaya Z. A., Freydina N. A., Lednev V. V. (1987): Axial periods in paracrystals of pure light meromyosin and copolymerized with C-protein. Proc. 8th All-Union Symp. "Biophysics and Biochemistry of Biological Motility", p. 31, Nauka, Tbilisi (in Russian)

Rome E. (1972a): Structural studies by X-ray diffraction of striated muscle permeated with certain ions and proteins. Cold Spring Harb. Symp. Quant. Biol. **37,** 331—339

Rome E. (1972b): Relaxation of glycerinated muscle: low angle X-ray diffraction studies. J. Mol. Biol. **65,** 331—345

Safer D., Pepe F. A. (1980): Axial packing in light meromyosin paracrystals. J. Mol. Biol. **136,** 343—358

Shpagina M. D., Orlova A. A., Latsabidze I. L., Podlubnaya Z. A., Lednev V. V. (1983): Study of thick filament structure in isolated A-discs and longitudinal sections of vertebrate skeletal muscle by optical diffraction. Biofizika **28,** 306—310 (in Russian)

Squire J. M. (1981): Structural Basis of Muscular Contraction. Plenum Press, New York and London

Squire J. M., Harford J. J., Edman A. Ch., Sjöström M. (1982): Fine structure of the A-band in cryo-sections. III. Cross-bridge distribution and the axial structure of the human C-zone. J. Mol. Biol. **155,** 467–494

Strzelecka-Golaszewska H., Nyitray L., Bálint M. (1985): Paracrystalline assemblies of light meromyosin with various chain weights. J. Muscle Res. Cell Motil. **6,** 641–658

Yagi N., Offer G. W. (1981): X-ray diffraction and electron microscopy of a light meromyosin tactoid. J. Mol. Biol. **151,** 467–490

Final version accepted November 3, 1989