

SOME CHEMICAL AND STRUCTURAL PROPERTIES OF PARAMYOSIN*

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Since the first descriptions of the structure of paramyosin by electron microscopy (1, 2) and x-ray diffraction (3, 4) were reported, important new evidence concerning the structure and properties of this fibrous protein has been obtained (5-7). Also the entire concept of the composition and properties of the "myosin" of muscle has been completely altered by the pioneering work of the Szent-Györgyi school (10) and subsequently by many other investigators. Because paramyosin presents certain very distinct structural and biochemical advantages it seems desirable to reinvestigate this protein in the light of current concepts of the contractile mechanism. The present note records certain preliminary observations on the enzymic properties of the protein and on the physical properties of the highly elongated macromolecules as evidenced by their ability to aggregate in highly oriented form from slightly alkaline solutions of medium ionic strength.

Experimental

Paramyosin fibrils were prepared from the white portions of the adductor muscle of the quahog, *Venus mercenaria*, after the method of Hodge; *i.e.*, by fragmentation of the muscle in a blender, followed by repeated differential centrifugation in 0.2 M KCl. The lighter fraction appeared as a homogeneous suspension of rigid, very long, thin needle-shaped structures as viewed in the darkfield microscope. The heavier fraction contained chiefly large aggregates of undissociated fibrils plus small amounts of granular or membranous material. Substantially similar results were obtained with the colored muscle.

The ATPase activity was measured at constant enzyme concentration using the Lowry and Lopez (12) method of phosphate estimation.

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The ultracentrifugation experiments were carried out in a Spinco model E analytical ultracentrifuge.

The electron microscopy was done with the Philips model EM-100 instrument.

RESULTS

Solubility Properties:

Confirming the findings of Hodge (5, 6) and the recent work of Bailey (8), the fibrils are not dissolved by increase of ionic strength, even up to 1.0 in the pH range of 6-7. They are insoluble in buffer solutions (*e. g.*, 0.3 M KCl + 0.15 M phosphate, pH 6.5) commonly used to extract myosin from vertebrate muscles. This has been considered as supporting evidence for the view that paramyosin is distinctly different from myosin although it is possible that myosin solubility may vary considerably over the various phyla of animals.

TABLE I
Solubility Properties of Paramyosin

Preparation	Medium	Residue
		<i>Per cent</i>
a	0.4 per cent acetic acid	4
	0.2 N NaHCO ₃	11
	0.2 N NaHCO ₃	11
b	0.4 per cent acetic acid	4
	0.2 N NaHCO ₃	4
	0.6 M KCl + 0.05 M phosphate pH 7.5	10

Hodge (5, 6) found paramyosin to be readily soluble, except for a small fraction which remained as a residue, in 0.4 per cent acetic acid and also in dilute alkali (5). He used acid solutions to produce the long-spacing form by variation of ionic strength and pH and to carry out physicochemical experiments on the shape and dimensions of the paramyosin macromolecules.

In the present work it was found that the solubility suddenly increases on the alkaline side of neutrality (in 0.6 M KCl + 0.05 M K phosphate, pH 7.5, or in 0.1 M NaHCO₃) although, as shown in Table I, an insoluble residue remains in all cases.

After centrifugation of the KCl solution of paramyosin for 30 min. at 20,000 R.P.M. in the preparative ultracentrifuge, a faintly opalescent solution is obtained in which no fibrils are observed when examined in the darkfield microscope or in the electron microscope (although no effort has been made as yet to apply refined methods, (14, 15), to attempt to visualize the individual macromolecules directly).

Reduction of ionic strength of this solution to a certain point results in the

precipitation of the fibrous protein in a condition which is determined by the manner in which the experiment is performed. Dilution by simple addition of 3 parts of water causes no visible precipitation. Addition of 4 parts of water with rapid stirring produces a silky sheen due to the presence of needle-like crystals which can readily be seen in the darkfield microscope. A sudden dilution by mixing the solution with 5 parts of water produces a flocculent amorphous precipitate which becomes crystalline after standing a few hours. Dialysis of the solution against 0.1 M KCl (in the presence of 0.01 M K phosphate, pH 7.5) also produces acicular crystals but dialysis against 0.03 M KCl (in the presence of 0.003 M K phosphate, pH 7.5) or against distilled water produces a thixotropic gel. In this process the elongated macromolecules aggregate in a characteristic

TABLE II
Effect of Ca and Mg on ATPase

Medium	pH	Added ions (0.003 M)	Q _p (20°)
0.075 M glycine	9.0	—	8
0.075 M NaCl		Mg	4
0.0025 M ATP		Ca	167
		Mg + Ca	8
0.03 M veronal	7.0	—	0
0.04 M KCl		Mg	2
0.0025 M ATP		Ca	22
0.02 M veronal	7.0	Mg	0
0.6 M KCl		Ca	21
0.0025 M ATP		—	

pattern as is indicated by the structure of the fibrils in the electron microscope. It should be pointed out that the homogeneity of the protein in this suspension of acicular crystals has not been established, although the ultracentrifuge data do not indicate the presence of impurities.

ATPase Activity:

The suspension of native fibrils obtained after differential centrifugation as described above was found to have weak ATPase activity. The values shown in Table II were obtained by plotting phosphate liberation against time at constant enzyme concentration.

It will be seen that at pH 9.0 the ATPase is calcium-activated and strongly inhibited by magnesium. This is true also at neutral reaction, at high and low ionic strengths, although the activity is much lower. The enzyme is specific for the terminal phosphate of ATP and cannot contain myokinase as is demonstrated by the fact that 96 per cent of the theoretical amount of phosphate for

one terminal group is liberated after 17 hours incubation with calcium ions at pH 9.0. A suspension of paramyosin fibrils retained substantially all of its ATPase activity for 4 days in the cold; a solution in 0.1 M NaHCO₃ having the same initial activity as the suspension of fibrils declined in activity to zero in 2 days.

The activity is a linear function of time at 20° but at 37° the activity falls off rapidly after 10 to 15 minutes. The Q_P at 20° was found to be 167 which would correspond to about 500 at 37°. This is only about 10 per cent of the activity of mammalian myosin ($Q_P = 3000$ to 6000).

Experiments performed subsequently by A. G. Szent-Györgyi¹ may indicate that the weak activity observed in our experiments is to be attributed to a small amount of actomyosin contaminant rather than to the paramyosin. When he removed actomyosin from his whole muscle extracts by treatment with ethanol, the ATPase activity in the remaining paramyosin was zero. These results suggest that the method of preparation of paramyosin as employed in our experiments is rather effectual in separating the actomyosin from the paramyosin since the ATPase activity of the preparation was so low.

Action of Trypsin:

It was of interest to determine whether, as in the case of myosin, tryptic action on paramyosin liberates two characteristically different fractions comparable to light and heavy meromyosin (9).

Under conditions comparable to those used for myosin (9) it was found that at 25° trypsin causes a rapid decline in viscosity in the first few minutes leveling off to about 30 per cent of initial relative viscosity in about 15 minutes. This result encouraged us to study the reaction in the analytical ultracentrifuge in the following manner. To each of three 5 ml. aliquots of paramyosin solution (0.6 M KCl + 0.05 M phosphate, pH 7.5) containing 6.2 mg./ml. of protein was added 0.05 ml. crystalline trypsin (0.05 per cent) at 23°C. The reaction was stopped by the addition of 0.05 ml. of a 1 per cent soybean trypsin inhibitor to each of the three aliquots 2.5, 7, and 20 minutes after addition of the trypsin. These solutions, together with the control solution untreated with trypsin or inhibitor, were spun in pairs in the ultracentrifuge using wedge-window cells. The control material shows a single hypersharp boundary typical of solutions containing interacting, very long macromolecular polymers (see top record in Fig. 1). The material after 2.5 minutes tryptic action behaved similarly. After 7 minutes tryptic action the peak, though at first sharp and traveling with the same velocity as that of the control, spread by diffusion (see lower record in Fig. 1). A similar spreading peak was obtained with material after 20 minutes of tryptic action. These results demonstrate that a profound change in the

¹ We are grateful to Dr. Andrew G. Szent-Györgyi for communicating to us these unpublished results.

protein occurs between 2.5 and 7 minutes of tryptic action. No further analysis of the effect was possible at the time although it seemed clear that the enzyme increased the diffusion rate without appreciable change in the sedimentation velocity.

Although the effect of trypsin on paramyosin bears superficial resemblance to that on myosin, there are certain distinct differences, particularly in the transformation of the hypersharp peak to a spreading peak with the same velocity. This behavior is identical with that found by Macfarlane (13) in the decomposition of tropomyosin by an enzyme from *Clostridium oedematiens* and interpreted by Keckwick (see 13) as a general breakdown of the molecule. This is interesting in view of Bailey's (8, 11) observation that the amino acid composition of the crystalline protein of the adductor muscles of *Ostrea* and *Pinna* is characteristic of a tropomyosin.

Electron Microscopy of Reprecipitated Fibrils:

A detailed study of the structure of paramyosin fibrils is in progress in this laboratory and will be reported in due course. The present brief description will concern only the material precipitated from slightly alkaline solutions by reduction of ionic strength.

From the work of Hodge (5, 6) it is probable that, in acid solutions, paramyosin fibrils break up into their constituent long, thin macromolecules and that, when the ionic strength is raised, the macromolecules aggregate in an antiparallel arrangement to form the symmetrically structured fibrous long-spacing modification. The possibility that a second uncharacterized component may be involved was also mentioned.

Electron microscope examination² of the acicular crystals formed by dilution of a slightly alkaline solution and after staining with phosphotungstic acid (pH 4.5), shows that they are also of long-spacing type (see Fig. 2). The band pattern is symmetrical, suggesting an antiparallel packing of the constituent molecules. Measurement of several hundred reprecipitated fibrils shows relatively small spread of the axial period, varying chiefly between 1700 and 2000 Å. This is significantly higher than the value of 1400 Å reported by Hodge for the long-spacing fibrils precipitated from acid solution. The band pattern and intensities differ also somewhat, suggesting possible differences in the interaction patterns when macromolecular aggregation is induced by elevation of ionic strength (from acid solution) than by reduction of ionic strength (from faintly alkaline solutions).

Several other features of the long-spacing band pattern may also be mentioned. A tendency was noted for the formation of fibrils with an axial period one-fifth that of the long-spacing, *i.e.* about 360 to 400 Å. Both types of struc-

² Thanks are due to Mr. J. W. Jacques for technical assistance in the preparation and examination of material with the electron microscope.

ture are shown in Fig. 2 side by side. It seems probable that these two forms represent different types of packing of the same elongated macromolecules. It may also be suggested that this explains the first observations (1) on paramyosin fibrils which showed a simple band pattern with an axial period of about 360 A. It was supposed at that time that the fibrils under observation in these earliest observations were of the native type; however, under the conditions of the experiment it is possible that some of the fibrils observed were in fact reprecipitated from dissolved paramyosin.

Another phenomenon frequently observed is a variation in density along a given band or interband, *i.e.* perpendicular to the long axis of the fibril. This may be seen in Fig. 2, but it is particularly marked in Fig. 3 where the effect leads to a pronounced diagonal staggering. It would seem that the effect is due to a lateral aggregation of very thin fibrils or elongated tactoids whose fine structure may be out of phase with each other by a discrete fraction of the axial period.

The relationship between the various axial periods reported from electron microscopy and x-ray diffraction studies of intact adductor muscles, of individual native fibrils and of various forms of reprecipitated protein is not clear. Discussion of this matter will therefore be postponed until more evidence is available.

SUMMARY

Paramyosin fibrils from the adductor muscles of *Venus mercenaria* are soluble above neutrality at relatively high ionic strength. From this viscous solution it is possible, by reduction in ionic strength, to reprecipitate acicular crystals of paramyosin. In the electron microscope these fibrils manifest a symmetrical band pattern similar to that previously described by Hodge but differing in some details. The axial periods observed under the conditions of the experiment varied between 1700 and 2000 A and a simple band pattern of one-fifth the main period was frequently observed. ATPase activity of the myosin type but of much lower intensity was demonstrated. Tryptic fission of the protein occurs but the characteristics differ from those of myosin.

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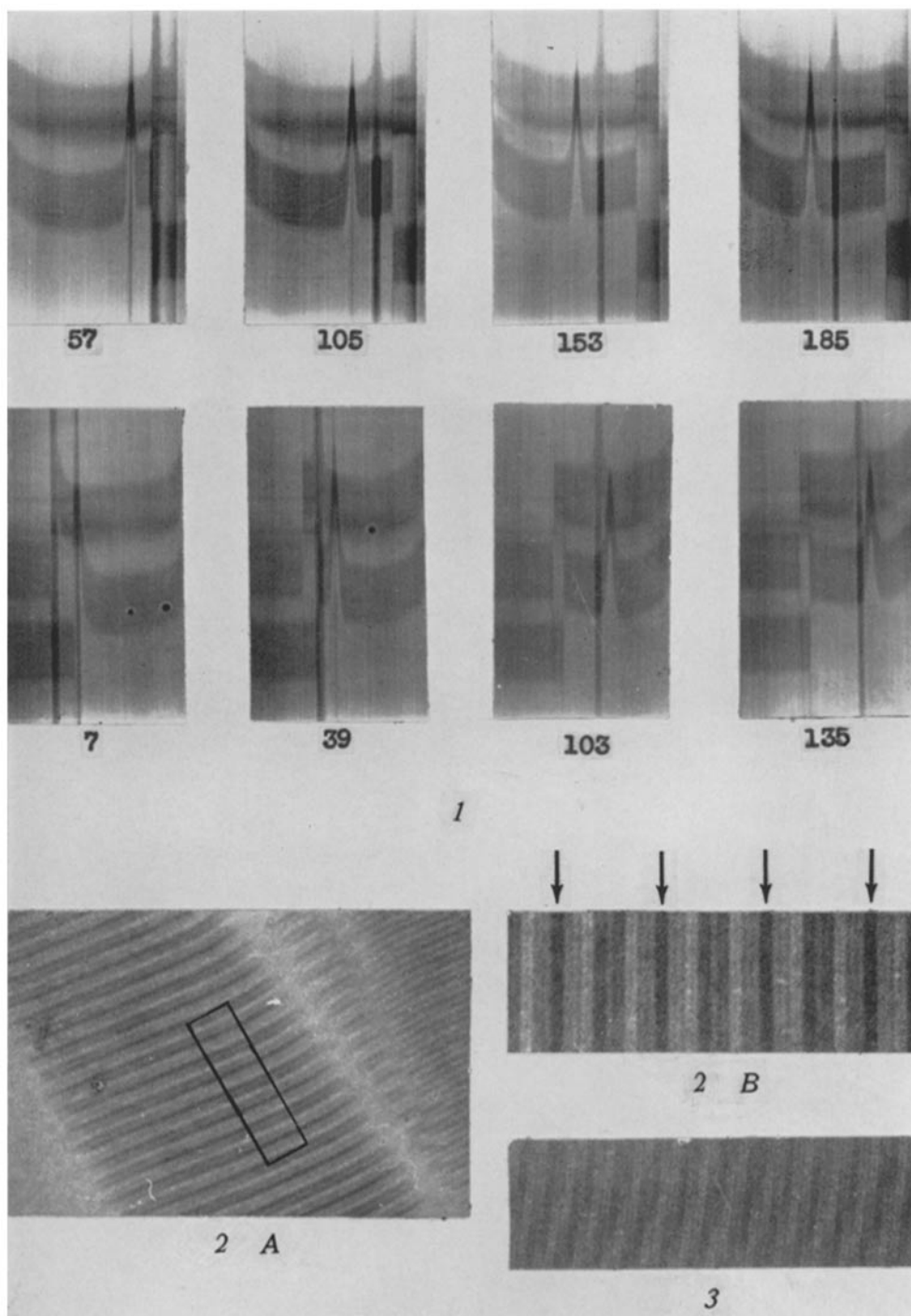
EXPLANATION OF PLATE 289

FIG. 1. Ultracentrifuge record of paramyosin preparation treated with crystalline trypsin. The series at top (with meniscus at right) shows the untrypsinized paramyosin control (upper Schlieren pattern) and the 7 minute sample (lower pattern), the frames being taken at 57, 105, 153, and 185 minutes after reaching top speed (60,000 R.P.M.). The series at the bottom (with meniscus at left) are of the 2½ minute and 20 minute trypsinized samples (upper and lower patterns respectively), taken at 7, 39, 103, and 135 minutes after reaching top speed.

FIG. 2 A. Paramyosin long-spacing fibrils precipitated from slightly alkaline solution by reduction in ionic strength to about 0.15. Stained with phosphotungstic acid. Magnification, 31,000.

FIG. 2 B. Enlargement of region enclosed in rectangle in Fig. 2 A. Note 12-banded, axially symmetrical band pattern and adjacent fibril with simple band pattern with axial period one-fifth that of the large fibril. Repeating periods marked above. Magnification, 81,000.

FIG. 3. Same as Fig. 2. Note stagger of band densities leading to a diagonal, pseudo-helical structure, due presumably to a slight shift in axial position of the macromolecules. Magnification, 58,000.



(Locker and Schmitt: Properties of paramyosin)