Paramyosin: Molecular Length and Assembly

Abstract. *Paramyosin paracrystals formed with divalent cations have a 725-angstrom axial period and show simple negative staining patterns in the electron microscope. The structure of the aggregates is interpreted in terms of an array of polar molecules about 1275 angstroms long, with "gaps" and "overlaps" in the molecular assembly. Antiparallel relations between molecules lead to the assembly of paracrystals with opposite polarity at either end. Implications of the in vitro structures for filaments containing paramyosin in muscle are discussed.*

Paramyosin is a fibrous protein found mainly in molluscan muscles specialized for prolonged tension maintenance. Its wide-angle x-ray diagram is that of an α-protein, and paramyosin is completely α-helical as shown by optical rotatory dispersion (*1*). In these aspects, it resembles two other muscle proteins, tropomyosin and light meromyosin fraction I (the helical subunit of myosin) (*2*). The x-ray diagram from muscles containing paramyosin has been interpreted on the basis of a molecular structure consisting of a two-chain α-helical coiled-coil (*3*). This model is in agreement with light-scattering and hydrodynamic measurements on paramyosin which indicate a rod-shaped molecule about 1350 Å in length and 220,000 in molecular weight (*4*). A two-chain structure is also indicated by the observation that the molecular weight of paramyosin is reduced by half in denaturing and reducing solvents (*5*).

Muscle filaments containing paramyosin show a 725-Å axial period, with a prominent 145-Å spacing, observed by small-angle x-ray diffraction (*6*) and, under certain conditions, by electron microscopy (*7*). Aggregates produced by solubilizing paramyosin in neutral salts, or at acid *p*H, and precipitating the protein at low ionic strength, show a variety of large periods in the electron miscroscope, and, in particular, a 145 Å repeat is often found. The molecular length deduced from these periodicities has been estimated as about 1400 Å (*8*), but a more precise evaluation of the length was not obtained. Divalent cations have been shown to produce a highly ordered fibrous form of tropomyosin (*9*). Here we report studies of periodic aggregates of paramyosin produced by divalent cations. Interpretation of the negative staining of these structures permits a deduction of the molecular length from the electron microscope image. Biologically significant features of the in vitro molecular assembly are described.

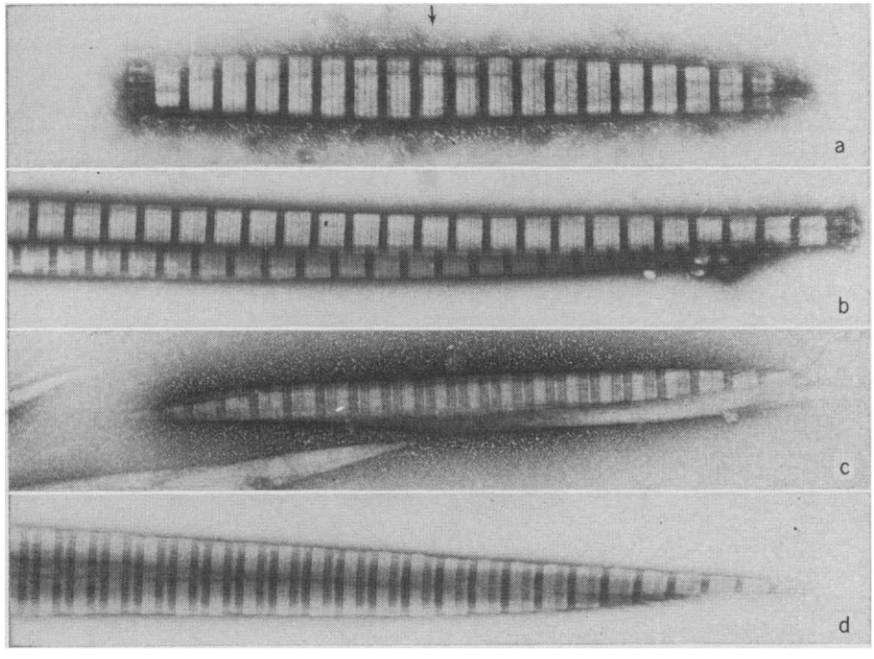

Fig. 1. (a) Paramyosin from *Mercenaria mercenaria* white muscle dissolved in 0.05*M* tris (*p*H 8), 1*M* urea, and precipitated with 0.05*M* $BaCl_2$. Marker points to nonpolar (dihedral) period. (b) Smooth musc'e paramyosin *Pecten irradians*, dissolved in 0.05*M* tris (*p*H 8), 0.1*M* KSCN, and precipitated with 0.05*M* $BaCl_2$. (c) Striated paramyosin from *P. irradians* digested briefly with insoluble papain, dissolved in 0.05*M* tris (*p*H 8), and precipitated with 0.05*M* $MgCl_2$. (d) Smooth muscle paramyosin from *P. irradians* dissolved in 0.05*M* tris (*p*H 8), 1*M* urea, and precipitated with 0.05*M* $MgCl_2$. All preparations negatively stained with 1 percent uranyl acetate (× 50,000).

Pecten irradians and *Mercenaria mercenaria* were obtained live and dissected to isolate the "individual" muscle types: in *P. irradians* the striated adductor was separated *in situ* from the translucent and opaque portions of the smooth adductor; in *M. mercenaria*, the white and red-tinted portions of the smooth adductors were separated from each other. Paramyosin was usually prepared from the freshly dissected muscles, but in a few cases, glycerinated muscle was used. The smallest yield of paramyosin was obtained from *P. irradians* striated muscle; this amounted to less than 2 percent of the myofibrillar fibrous proteins (*10*). The paramyosin was prepared as described previously (*11*) and stored by lyophilization.

To produce fibrous aggregates by divalent cations, the following procedure was used: paramyosin precipitated three times was dissolved (0.1 to 0.2 percent) in 0.6*M* KCl, 0.01*M* phosphate buffer (*p*H 7), and then dialyzed at 4°C against 0.05*M* tris (hydroxymethyl)amino methane-hydrochloride (tris-HCl) *p*H 8 (23°C). The protein was precipitated by dialysis against 0.05*M* $MgCl_2$ or $BaCl_2$ in 0.05*M* tris-HCl, *p*H 8. The tactoids formed by divalent cations were frequently aperiodic or polymorphic when negatively stained with 1 percent uranyl acetate. Correspondingly, ultracentrifugation showed the presence of aggregates in the alkaline tris-HCl solvent, prior to the addition of divalent cations. There were marked differences in the aggregation of paramyosins from different sources.

In order to produce a high proportion of periodic structures with a simple staining pattern, further dispersion of the paramyosin prior to precipitation by divalent cations was necessary. The following methods were employed to disperse the aggregates: (i) digestion for a short time with insoluble papain (*12*); (ii) treatment with urea (0.4 to 1*M*); (iii) treatment with adenosine triphosphate (ATP) (2 to 5 m*M*); and (iv) treatment with thiocyanate (0.02 to 0.1*M*). These treatments reduce molecular interactions in the low ionic

strength medium, and essentially monomeric protein preparations may be obtained. Optical rotatory dispersion studies indicated that the helix content of paramyosin so "solubilized" was not affected by these agents. Ultracentrifugation revealed a symmetric 3*S* peak and no other components. It should be noted that addition of monovalent salts, such as KCl, in concentrations necessary to disaggregate paramyosin also prevents precipitation of the protein by divalent cations. Barium was a more effective precipitant than magnesium, but could not be used with ATP. Both these cations formed paramyosin paracrystals of similar structure.

The most commonly occurring structure has a period of 725 ± 35 Å, divided into a lightly stained region and a smaller darkly stained band. The lightly stained regions varied slightly in width depending on the source of paramyosin. Paracrystals from *P. irradians* had a light region about 550 Å in width; in *M. mercenaria* this region appeared to be somewhat shorter. There were also slight differences in the appearance of the lightly stained regions of the paramyosin tactoids from *M. mercenaria* and *P. irradians*, but the general distribution of stain was similar (Fig. 1, a and b). The details of the staining pattern within the light region indicate a polarity of the band pattern over most of the paracrystal. We call this polar form type PI.

In these same fibers, additional structural features may be noted when most of the fiber can be observed. In these cases, a few periods at the center have nonpolar banding. Moreover, the band patterns at either end of these fibers show opposite polarity. The lightly staining region of the nonpolar central periods appear slightly smaller in width than the corresponding region in adjacent type PI periods. We call this nonpolar (dihedral) form type DI (Fig. 1a).

Another band pattern frequently observed also has a period of 725 Å, but a different stain distribution. In paramyosin from *P. irradians* the light zone is about 275 Å or less in width, and the dark zone is often intersected by a very thin, light line (Fig 1, c and d). This band pattern is nonpolar. We call this dihedral form type DII. Fibers are often seen with the type DII band pattern at the center, followed by a transition zone where the staining progressively changes, until the type PI band pattern is seen at the ends of the fiber (Fig. 1, c and d). The polarity is thus reversed at either end of the fiber.

These patterns can be accounted for by interpreting the negative staining pattern in terms of "overlaps" (where stain is largely excluded) and "gaps" (where stain is bound) in the molecular assembly. This kind of paracrystal formation has been demonstrated for collagen (*13*) and may also occur in light meromyosin Fr I (*14*). Based on this interpretation of the pattern, the molecular length equals an integral multiple of the period plus the length of the overlap region. A two-dimensional representation of the polar molecular array,

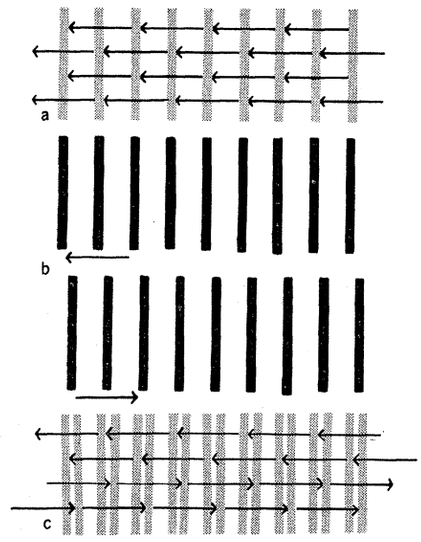

Fig. 2. (a) Two-dimensional representation of fundamental polar molecular array. Arrows represent paramyosin molecules. Shading shows "gap" regions where uranyl acetate penetrates paracrystal. This array corresponds to the type PI pattern, illustrated by the ends of the paracrystal in Fig. 1, a and b. Note that the translation of 725 Å between neighboring rows is half the repeat period of 1450 Å along the individual rows, that is, the molecular length plus the gap. This arrangement suggests that the molecules in neighboring rows are related, at least locally, by parallel twofold screw axes. (b) As (a), showing oppositely directed arrays related by perpendicular diads. The position of the diads defines the relative displacement of the two arrays. This diagram relates the type PI pattern with types DI and DII. (c) Superposition of the two PI arrays as shown in (b), which are related by perpendicular diads about 50 Å from one edge of the light zone. By symmetry, there is another class of diads about 137 Å from the other end of the light zone. This leads to the appearance of a 275 Å "shift" of the two PI arrays. The diagram corresponds to the type DII patterns. This representation is not intended to illustrate the pairwise side-to-side relation of neighboring molecules.

type PI, deduced from the electron micrographs is shown in Fig. 2a. The type PI pattern thus shows that the smallest length for the molecule is 725 Å + 550 Å = 1275 Å for paramyosins from *P. irradians* (Fig. 2a). The molecular length may be about 30 Å less in *M. mercenaria* paramyosin. This figure gives a molecular weight of 200,000 daltons for paramyosin, if we assume a two-chain α-helical model, and agrees well with hydrodynamic and light-scattering data. The next possible length for the molecule would be 2000 Å—a figure excluded by the light-scattering measurements.

The dihedral type DI periods would be composed of sets of molecules oriented in opposite directions related by perpendicular diads so that the arrays appear "shifted" relative to one another by 80 Å. The type DII form is similarly interpreted, but in this case the "shift" of oppositely oriented arrays is frequently about 275 Å (or half the length of the overlap region) in the paramyosin from *P. irradians* (Fig. 2c). Note that the superposition of oppositely directed polar arrays of identical period does not alter the period, but the band pattern produced depends on the position of the twofold axes (Fig. 2b). This assembly scheme is readily seen by inspection of Fig. 1, c and d. The nonstaining zone becomes progressively more dominant from the center to the end of the fiber. This appearance may be interpreted to mean that, at the center of the fiber, two oppositely directed arrays of the type PI are present, as in Fig. 2c. Moving from the center to the end of the fiber, one polar molecular array predominates, as successively fewer molecules of the other array are present.

Other aspects of the electron micrographs related to this interpretation are informative. "Corrugation" at the edges of the fibers is clearly visible owing to the increased width in the light staining regions corresponding to the "overlap" zones. This feature of the structure is also found in negatively stained collagen fibers (*13*). Another significant observation is the presence of "fringes" often seen at the ends of paracrystals (Fig. 1, a and b). The edge of the fringe in the pool of negative stain marks the approximate end of the molecules, and the molecular length deduced from this measurement is consistent with that based on the interpretation of the band patterns.

The two ends of the paramyosin

molecule must be different, since there is no way for nonpolar molecules to produce a polar band pattern. Molecular polarity has already been demonstrated for myosin (*14*) and tropomyosin (*15*). One cannot decide at present, however, whether the two chains in the molecule run in the same or opposite directions. If the two chains are identical in sequence, then the polarity of the molecule requires that they run in the same direction. Although the three-dimensional packing of the molecules cannot be deduced from these observations, it is possible to build a filament where the molecules are related by parallel twofold screw axes to produce the band pattern shown in the two-dimensional plan of Fig. 2. Evidence regarding the three-dimensional packing arrangement of paramyosin in native filaments under certain conditions has been obtained (*16*). It is important to note that although the axial periodicity is very regular in fibrous protein paracrystals, there is no long-range order in the lateral packing (*15*).

The assembly of paramyosin filaments in vitro gives some insight into the structure of the filaments containing paramyosin in muscle. The antiparallel relations between molecules lead to the assembly of a filament with opposite polarity at either end. This kind of packing had been demonstrated for myosin from vertebrate striated muscle (*14*) and is an important aspect of the design requirements of the sliding filament mechanism of contraction. It is possible that, at the center of the native paramyosin filament, the molecules have antiparallel dimer relations similar to those occurring in either the type DI or type DII paracrystals. Growth would proceed at either end of the filament by polar assembly. Another fundamental aspect of the aggregates in vitro is the presence of "gaps" and "overlaps" in the structure. These occur necessarily in forming a filament with an axial repeat of 725 Å from molecules 1275 Å long, and there must therefore be gaps in the molecular packing of the native filament. A pattern of "gaps" which resembles the lattice seen in the native paramyosin filament can, in fact, be produced by a specific staggering of the type PI arrays. Myosin may be located at the surface of the native paramyosin filament (*17*). Myosin and paramyosin have α-helical coiled-coil rod regions very similar in length and aggregation properties (*18*). The interaction of these proteins may be crucial in accounting for the structure of the filament. A paramyosin aggregate could provide a bonding area with the appropriate geometry and polarity for positioning a surface array of myosin molecules.

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Chick Interferon: Heterogeneity of Electric Charge

Abstract. *Chick interferon from allantoic fluid, virus-induced and partly purified, consists of several active components of different charge. The components are separable by elution at different* pH *values from a carboxymethyl-Sephadex column; they also occupy different* pH *zones in electrofocusing gradients. Most of the interferon is, however, isoelectric near neutrality.*

The active moiety in purified preparations of chick interferon is apparently not homogeneous (*1–3*). The purpose of our work was to prove that heterogeneity was not a result of partial adsorption to a solid matrix and to learn something about the relative abundance of factors in the interferon concentrates.

Interferon from allantoic fluid was prepared, partly purified, and concentrated by methods described (*3*). The concentrate in our experiments contained 25,600 interferon units and 780 μg of protein per milliliter; this represented 70-fold purification and an overall recovery of activity of 40 percent. The concentrate (100 ml in 0.1*M* phosphate buffer *p*H 5.9) was applied to a carboxymethyl-Sephadex (C50, medium grade) column (2 g; 190 × 15 mm), that was previously equilibrated with the same buffer. The column was washed with 0.1*M* phosphate buffer (*p*H 6), and the fractions were eluted by means of a steep, rising *p*H-gradient. The gradient was formed by running 0.1*M* trisodium phosphate into 300 ml of 0.1*M* phosphate buffer (*p*H 6.0). The effluent was collected in 10 ml portions in polypropylene tubes. The fractions were assayed for interferon content (*3*), and their *p*H values were determined (Table 1).

In order to obtain fairly concentrated eluate fractions, a steep *p*H-gradient had been chosen. Therefore, elution did not proceed at equilibrium, and the *p*H of a fraction was not directly related to the isoelectric point of its interferon