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SOME PHYSICO-CHEMICAL PROPERTIES OF MYOSIN B FROM THE HORSESHOE CRAB, *LIMULUS POLYPHEMUS*

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Abstract—1. Natural actomyosin (myosin B) is the usual protein extracted from horseshoe crab muscle with methods used to extract myosin A from rabbit muscle.

2. Myosin B from the crab has been obtained which is almost ultracentrifugally pure having an S_{20w} ($c = 0$) of about 40 S changing to 5·7 S in the presence of ATP, an intrinsic viscosity of 4 to 5 changing to 1·7 with ATP, and an approximately 4-min superprecipitation time with NaATP and no divalent cations.

3. Although apparently inseparable during extraction, both myosin and actin can be recovered when myosin B is dissociated with ATP in the presence of $MgCl_2$

4. The Weber-type treatment results in a myosin with an S_{20w} of about 5·17 S, an actin with an S_{20w} of about 2·36 S and a schlieren pattern for the mixture which becomes altered when ATP is introduced.

INTRODUCTION

FAILURE in our efforts to extract myosin from glycerinated myofibrils of the primitive horseshoe crab using methods comparable to those applied to rabbit glycerinated muscle, and failure in our many different experiments in which we attempted to extract myosin directly, led to the belief that actin and myosin are differently and perhaps more firmly combined in cold-blooded animal muscle than in the muscle of the warm-blooded rabbit or chicken (de Villafranca *et al.*, 1959; de Villafranca & Naumann, 1964). Similar difficulties with other cold-blooded animals have led Maruyama (1959) and others to similar conclusions. Because direct extraction of myosin was apparently impossible, at least with the generally used methods, attention shifted to the study of actomyosin (myosin B) itself and to the question of whether, in fact, it was possible to dissociate myosin B from the horseshoe crab into actin and myosin using A. Weber's technique (1956; Noda & Maruyama, 1960; Holtzer *et al.*, 1960) of ATP dissociation followed by separation through ultracentrifugation. While most extractions of myosin B involve prolonged treatment (24 hr or more) at alkaline pH's which may result in myosin B which is quite heavily contaminated with other proteins (Snellman & Erdos, 1949; Gellert *et al.*, 1959; Tonomura & Sekiya, 1961; Johnson & Rowe, 1964), even a short extraction at slightly alkaline pH of horseshoe crab muscle yields directly a fairly homogeneous myosin B preparation. As a matter of fact this myosin B, perhaps because of a more tight binding of actin to myosin, behaves almost as a single molecular species.

MATERIALS AND METHODS

Extraction and purification

By a method previously described (de Villafranca & Naumann, 1964) myosin B was extracted with 0·6 M KCl at pH 8 for 5 min on ice from muscle removed from all parts of freshly killed horseshoe crabs (*Limulus polyphemus*), shipped from the Marine Biological Laboratory, Woods Hole, Mass. In early studies purification was attempted only by dilution with 10 vol. of cold ion-free water repeated two or three times: each time the precipitate was dissolved in 0·6 M KCl at pH 7·2. This method resulted in preparations which quite frequently exhibited three components in schlieren ultracentrifuge patterns: a major component, myosin B sedimenting at 30–40 S, and two smaller, more slowly sedimenting peaks at 6 S and 3 S. Some results presented here utilized this procedure and are identified, when presented, as "unpurified myosin B". All recent work used myosin B which was subjected to at least one precipitation at a KCl concentration just below 0·30 M (more often two or three such precipitations), which, in general, removed significant amounts of the 6 S and 3 S components: the latter an apparent tropomyosin A contaminant (Rüegg, 1961; de Villafranca & Leitner, 1967).

The final residue of myosin B (after one or two cycles of 0·3 M KCl precipitation) dispersed in 0·6 M KCl–5 mM histidine buffer at pH 7·2 by hand homogenization in a Teflon–glass homogenizer was dialyzed at least 2 hr against about 5 vol. of 0·6 M KCl–5 mM histidine, which was followed by centrifugation at 50,000 rev/min in a Spinco Model L for 15 min to clarify the solution. When checked at 59,780 rev/min in a Spinco Model E centrifuge, a single peak was usually observed (Fig. 1). The concentration of myosin B consistently hovered around 4–5 mg/ml when the residue was taken up in about a single volume of KCl–histidine (1 ml/g fresh wt.) so that this fraction represented approximately 0·4–0·5 per cent of the muscle fresh weight.

Rabbit proteins

Rabbit myosin was prepared essentially according to the procedure of Szent-Györgyi (1950) through two precipitations with 10 vol. of ion-free water and two precipitations by lowering the salt concentration to only 0·25 M KCl with ion-free water to remove actomyosin, followed by further dilution to precipitate myosin at about 0·06 M KCl. Actin was prepared from rabbit by the method of Barany *et al.* (1957) as modified by Carsten & Mommaerts (1963) but with the omission of a second polymerization step.

Procedures

Protein determinations were by semi-micro Kjeldahl distillation into boric acid followed by titration with standard sulfuric acid. Calculations assumed 16 per cent nitrogen content of the proteins. ATPase determinations were as previously described (de Villafranca & Naumann, 1964). All analytical centrifugation was performed on a Spinco Model E centrifuge without electronic speed control, with the AnD rotor, and at temperatures between 3–9°C, held constant for each centrifuge run by the Spinco temperature-controlling system. Calculations of S_{20w} and molecular weights by the Archibald method were carried out as described by Schachman (1959), using the $\bar{V}$ value of 0·724 (Kay, 1960a) for both myosin and myosin B and 0·716 (Kay, 1960b) for actin. Viscosity was determined at 20°C in an Ostwald–Cannon–Fenske viscosimeter with an outflow time of 60 sec for water. Superprecipitation experiments on actomyosin were carried out with a recording Beckman DB Spectrophotometer at a wavelength of 660 mμ essentially according to the procedure of Yasui & Watanabe (1965).

All solutions were reagent grade chemicals in "ion-free water" (distilled water which had been run through a mixed bed resin). ATP was purchased from P-L Laboratories, but no attempt was made to remove calcium which might be present since milliliter quantities of 50 mM ATP did not show any color with Calcein indicator (G. B. Smith Co.). Cleland's reagent (dithiothreitol) was obtained from Calbiochem.

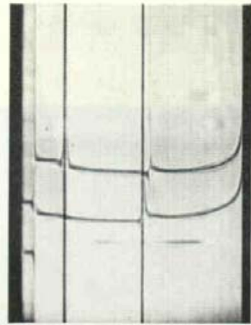

FIG. 1. Schlieren pattern of *Limulus* myosin B with (upper trace) and without 3·8 mM ATP (lower trace) in 0·54 M KCl–4·5 mM histidine, pH 7·0. The picture was taken 36 min after reaching a speed of 59,780 rev/min or 56 min after ATP addition at bar angle of 70 and temperature of 8·0°C. Protein concentration was 3·47 mg/ml in both wedge window and standard cells. S_{20w} of upper trace's major peak = 4·09 S, while S_{20w} of lower trace's major peak = 13·40 S.

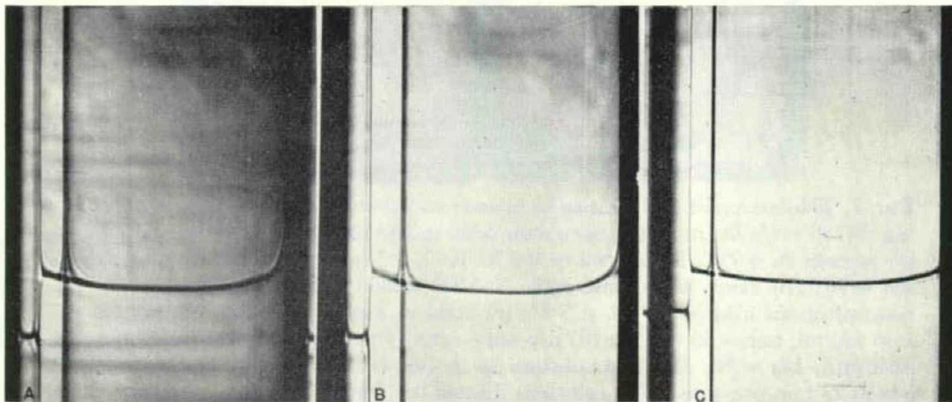

FIG. 2. Schlieren patterns of rabbit myosin A at 59,780 rev/min in 0·6 M KCl–5 mM histidine pH 7 (A and C), or 0·54 M KCl–4·5 mM histidine, pH 7 (B): (A) 24 min at speed, bar = 65, temp. = 8°C, protein = 2·19 mg/ml; (B) 32 min at speed or exactly 63 min after ATP addition to a final concentration of 2·9 mM, bar = 65, temp. = 8°C, protein = 1·98 mg/ml; (C) 32 min at speed, bar = 75, temp. = 5·6°C, protein = 7·25 mg/ml. See text for description of preparations.

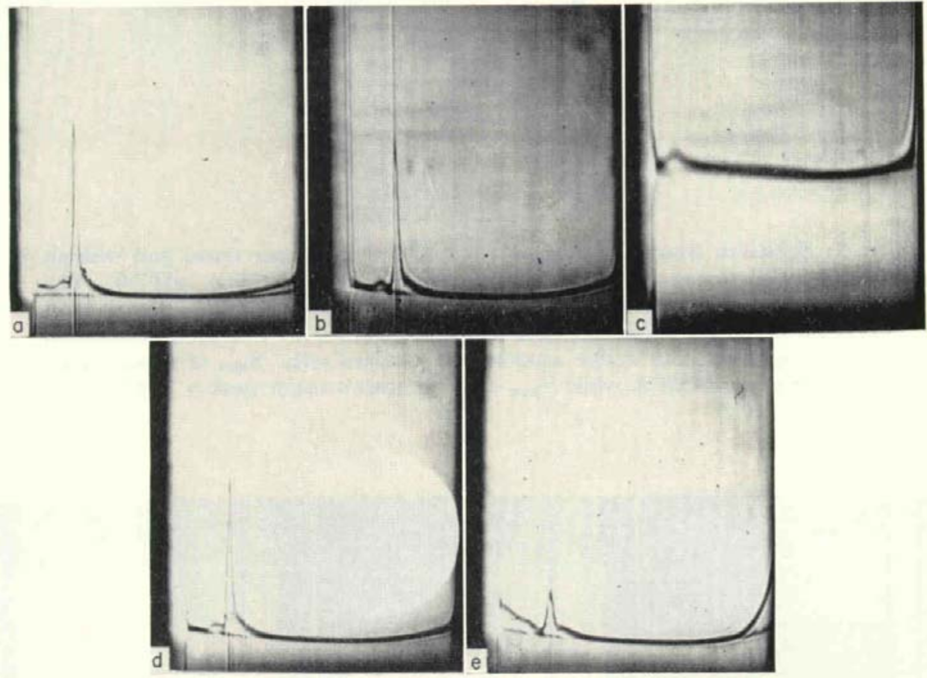

FIG. 7. Schlieren patterns of *Limulus* myosin and actin all taken 40 min after reaching 59,780 rev/min and in double-sector cells, except (c) which was taken at 32 min: (a) myosin A, 4·7°C, 3·8 mg/ml in 0·4 M KCl, 3·3 mM histidine, 0·7 mM Tris, bar = 70; (b) same as (a) but with NaATP added 84 min previously and all concentrations diluted by 0·1 at 7·8°; (c) actin in 2 mM Tris, pH 8·5, protein = 1·46 mg/ml, bar = 50, 8·2°C; (d) myosin + actin (3·8 mg myosin/ml and 0·486 mg actin/ml), bar = 70, 5°C and solution as in (a); (e) same as (d) but with ATP added 73 min previously and solutions diluted 0·1, bar = 70, 6·6°C. All runs were performed in two successive days; (b), (d) and (e) on one day with (a) and (c) on the next.

RESULTS

Rabbit myosin

As previously stated, methods which commonly extract myosin from rabbit muscle fail altogether in extracting myosin from the horseshoe crab muscle (de Villafranca *et al.*, 1959). In all cases the extract is predominantly actomyosin (myosin B). The converse situation also seems to be true: that is, the method employed for extracting actomyosin from the crab (i.e. short extraction with 0·6 M KCl, pH 8) yields *myosin* when used on rabbit muscle which had been rapidly removed from the animal and immediately extracted. The Model E schlieren patterns in Fig. 2 illustrate rabbit muscle preparations of this sort after purification by two water precipitations. Little change in the sedimentation rate occurs when ATP is added (compare Fig. 2B with A) and the rate is about the same as that of rabbit myosin prepared in the more conventional manner of Szent-Györgyi (1950) (Fig. 2C). The ATP sensitivity (H. H. Weber & Portzehl, 1952) in the viscosimeter of the material shown in Fig. 2A and B is negligible (3·9 per cent at a concentration of 3·50 mg/ml), while ATPase activity is Ca^{2+} activated (maximally at 1 mM $CaCl_2$ with a value of 5·5 μmoles P/mg protein per 5 min at 25°C and pH 7) and inhibited by Mg^{2+}. In other words, parallel experiments indicate that actin binding to myosin is probably different and stronger in horseshoe crab than in rabbit muscle. Apparently this is true within the muscle, but, as subsequent experiments show, myosin A can be obtained from the crab by dissociation of isolated actomyosin with ATP in a manner quite comparable to that used with rabbit actomyosin (A. Weber, 1956).

Limulus *myosin B* S_{20w}

Myosin B, when purification included precipitation below 0·3 M KCl (Fig. 1), behaves much like a single molecular species giving a sedimentation coefficient of about 40 S at infinite dilution (Fig. 3); a somewhat arbitrary value because the values below concentrations of about 1·5 mg/ml were so high that accuracy in their determination became increasingly difficult and because the line of Fig. 3 is drawn by inspection. At concentrations above about 4 mg/ml the slope evidently changes drastically due, perhaps, to the increased viscosity of the solution itself: no attempt was made to correct for this situation. Plotted as separately identifiable points are results obtained after actomyosin had been stored in approximately 50% glycerol in the deep freezer. From the results at the higher concentrations it would appear that some change has occurred in the structure of the protein to give higher S_{20w}'s than those with material freshly prepared and kept less than 8 days (cf. Levy & Ryan, 1967). Whether this is real and due to glycerination or not cannot be definitely stated at present because it has not been systematically investigated. It is, however, worth noting that actin prepared from stored actomyosin also shows anomalous behaviour in sedimentation studies and the ATPase activity of the actomyosin also seems to be changed qualitatively. In any case because of the uncertainty here no lines were drawn for the S_{20}-concentration curve of Fig. 3 at the

higher protein levels. It may well be that the true curve is a single smooth line for all data rather than the linear relationship shown here.

Included in Fig. 3 are seven points obtained from a single preparation of actomyosin (one horseshoe crab) on a single day with material only 2 days old. These data, if the lowest concentration value is valid, suggest a curve running to infinity,

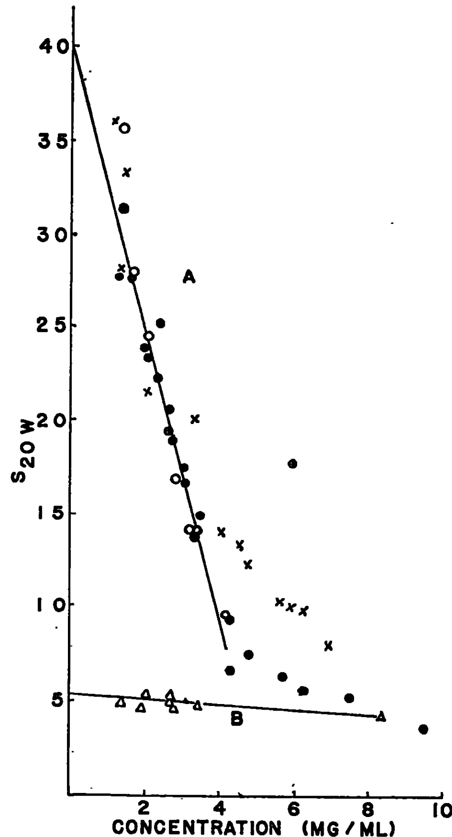

FIG. 3. S_{20w} curves of *Limulus* myosin B with and without ATP. Curve A: × = myosin B stored for a week or more in 50% glycerol–KCl; ● = myosin B used not more than 8 days after sacrificing the animal; ○ = myosin B from an animal sacrificed 2 days previously and all sedimentation data collected in a single day. Curve B obtained on freshly prepared myosin B with measurements obtained about 30 min after the addition of ATP in final concentrations of 2–4 mM. All measurements were made in 0·6 M KCl–5 mM histidine, pH about 7, at 59,780 rev/min, bar angles of 60–75 and temperatures 4–11°C. Lines drawn by inspection.

but ignoring that point the curve can be drawn linearly to $c = 0$ at $S_{20w} = 40$ S. It should be noted that at the concentrations above 5 mg/ml myosin B has a sedimentation constant within the range of myosin A, and within the range of the major peak when ATP is present in an actomyosin solution (Fig. 3, curve B).

Although some attempt was made to determine the molecular weight of myosin B, unfortunately even at very low speeds such as 8225 rev/min the boundary showed no tendency to broaden due to diffusion, and the hypersharp peak remained, thus making it impossible to obtain c_0 curves for Archibald molecular weight determinations. The converse was also true: low protein concentrations where a

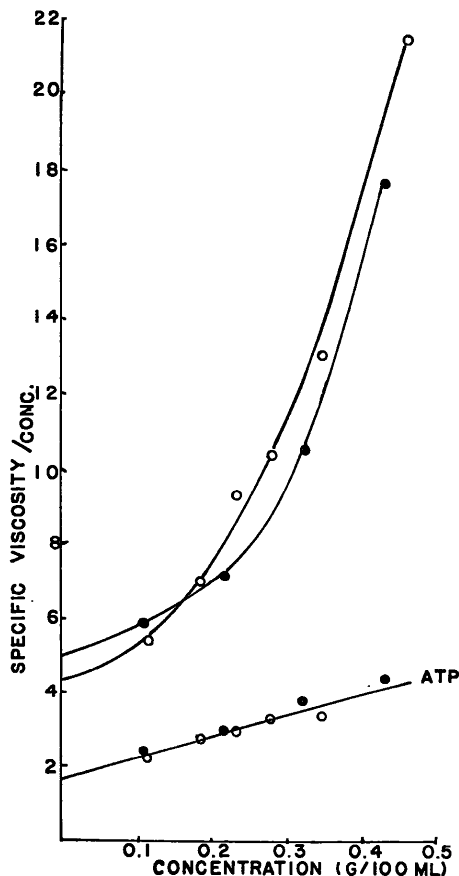

FIG. 4. Sample viscosity curves of *Limulus* myosin B measured at 20°C in 0·6 M KCl–5 mM histidine, about pH 7, giving intrinsic viscosities of 4·4 and 5·1 without ATP (upper curves) and 1·7 with ATP (lower curve).

c_0 run was possible gave boundaries which even at low speeds did not remain at the meniscus but migrated rapidly.

Intrinsic viscosity

The viscosity data (Fig. 4) demonstrate similar difficulties in attempting precision with a protein as complex and large as actomyosin. Myosin B's well-known

anomalous viscosity behaviour is quite apparent with horseshoe crab myosin B, i.e. the relation of viscosity to concentration is not linear. Through extrapolation from a variety of determinations, only two of which are shown, one obtains an intrinsic viscosity of 4–5. Myosin B in the presence of ATP (Fig. 4), however, has a more precisely determinable intrinsic viscosity, $[1{\cdot}7]_\eta$, the same figure we obtain for purified rabbit myosin A.

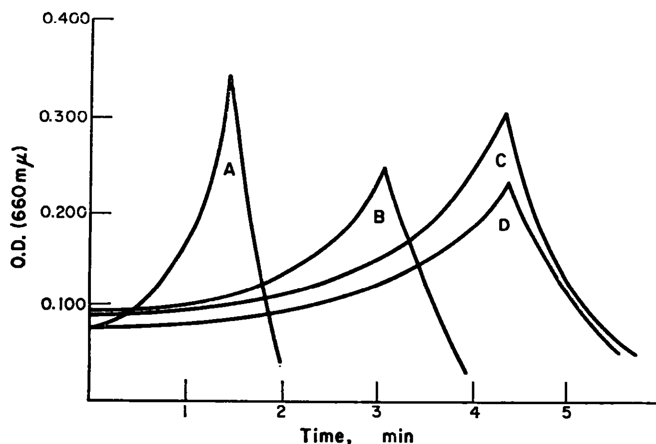

FIG. 5. Sample superprecipitation experiment with *Limulus* myosin B. Curves originally obtained during the experiments in a Beckman DB spectrophotometer with recorder are redrawn here with compressed time scale (abscissa). Superprecipitation was induced by ATP to a final concentration of 1 mM in a medium of 0·06 M KCl–5 mM histidine, pH 6·8; protein = 0·32 mg/ml and temp. = 25°C; plus 2 μM $MgCl_2$ (A), 5 μM EGTA (B), no cation or chelator (C) and 2 μM EDTA (D).

Superprecipitation

Limulus myosin B's behaviour in the presence of ATP, like other actomyosins, depends upon the KCl concentration, i.e. whether or not myosin B is in solution. Below about 0·3 M KCl the horseshoe crab myosin B precipitates while at 0·06 M KCl the precipitate changes to a "superprecipitate" when one adds ATP. In preliminary studies (Fig. 5) data clearly indicate that superprecipitation of this material occurs fairly rapidly even in the absence of added divalent cations, i.e. in 0·06 M KCl–5 mM histidine buffer, pH 6·8, at 25°C with 1 mM disodium ATP added. The optical density or turbidity rises to a maximum as the "plug" forms in the light path and then decreases as the "plug" falls to the bottom of the cuvette. EDTA in the experiment shown has little effect but at other times did hasten the clumping. Both Mg^{2+} and EGTA increased the rate of superprecipitation. Although the myosin B used here had been reprecipitated three times, it may well have adsorbed or bound divalent cations whose inhibitory effects might be overcome either by EGTA or Mg^{2+} in a manner comparable to that reported by Levy

& Ryan (1967). In similar experiments but with different preparations both Ca^{2+} and Mg^{2+} increased the rate when added separately.

Dissociation of myosin B

In solution at 0·6 M KCl myosin B preparations exhibit the characteristic dissociation phenomenon in the presence of ATP. ATP above 2 mM changes the rapidly sedimenting peak of myosin B to the more slowly sedimenting peak of myosin as noted previously (Figs. 1 and 3). $MgCl_2$ is not necessary to inhibit the ATP hydrolysis, since dissociation of the actomyosin persists over a considerable time interval (Fig. 6) and after the ATPase activity has hit its maximum. In Fig. 6 it can be seen that actomyosin dissociation, as revealed by ultracentrifugation, lasts several hours although the viscosity recovers (ATP sensitivity decreases) within 1 hr after ATP addition. The amount of ATP hydrolyzed reaches its maximum level (about 10 per cent of the total ATP added is hydrolyzed) by 2 hr from the start. In spite of this, $MgCl_2$ addition to a level of 10 mM aids considerably the ultracentrifugal recovery of myosin and actin after actomyosin dissociation. Perhaps Mg^{2+} is required to polymerize actin (Weber & Portzehl, 1952), making it sediment more readily to the cell bottom rather than being required to inhibit myosin ATPase. It was further noted that Mg^{2+} inclusion makes it possible to use the entire supernatant for myosin preparations rather than the upper third or half of the supernatant from ATP dissociation-centrifugation (Weber, 1956).

Dissociation of *Limulus* actomyosin was accomplished quite successfully by diluting myosin B in 0·6 M KCl–5 mM histidine, pH 7, to about 2–3 mg protein/ml and then adding ATP and $MgCl_2$ to final concentrations of 4 and 10 mM respectively. The entire supernatant (about 14–20 per cent of the original myosin B) after centrifugation at 50,000 rev/min for 3 hr in a Spinco Model L was poured off and dialyzed overnight against water to precipitate myosin and to remove ATP. The precipitate was dissolved in 0·6 M KCl–5 mM histidine, dialyzed against the same solvent and then clarified by centrifugation at 50,000 rev/min for 15 min. Recovery was about 5 per cent of the original myosin B. Figure 7 illustrates the sedimentation pattern of such a myosin preparation both with and without ATP present (Fig. 7b and a respectively) and shows that no change in the pattern or in the sedimentation velocity occurs with ATP thus confirming the absence of myosin B. Purer preparations than that illustrated have been obtained; they do not have the distinct trailing peak of tropomyosin.

Figure 8 further supports the identity of myosin A resulting from ATP-induced dissociation of *Limulus* myosin B. Here the sedimentation coefficient extrapolates by least squares to 5·17 S at infinite dilution. This value is lower than that reported in the literature for various myosins (Holtzer & Lowey, 1959; Kielley & Harrington, 1960; Lowey & Cohen, 1962; Connell, 1963; Woods *et al.*, 1963; Mueller, 1964), but is only slightly higher than the 4·7 S which we obtain from purified rabbit myosin. ATPase activity with $MgCl_2$ is no higher at either pH 7 or 9 than it is in the absence of divalent cations, but is activated by $CaCl_2$ to give a value of 1·20 μmoles P/mg protein per min at pH 9·25, 25°C, in the presence of 10 mM $CaCl_2$

and 0·06 M KCl. From a single Archibald determination, the myosin molecular weight appears to be about 890,000 as compared with the 530,000 we obtain for purified rabbit myosin at a protein concentration of about 1·80 mg/ml.

The pellet from the Weber treatment, when dissolved in ATP and Tris, shows the centrifuge pattern of Fig. 7c. When mixed with its own myosin the patterns d and c of Fig. 7 are obtained suggesting that some interaction may occur. Experiments with rabbit actin in combination with *Limulus* myosin have not, as yet, yielded any conclusive results, nor have we yet tried combining *Limulus* actin with rabbit myosin. Figure 9 indicates a sedimentation coefficient (by least squares) of

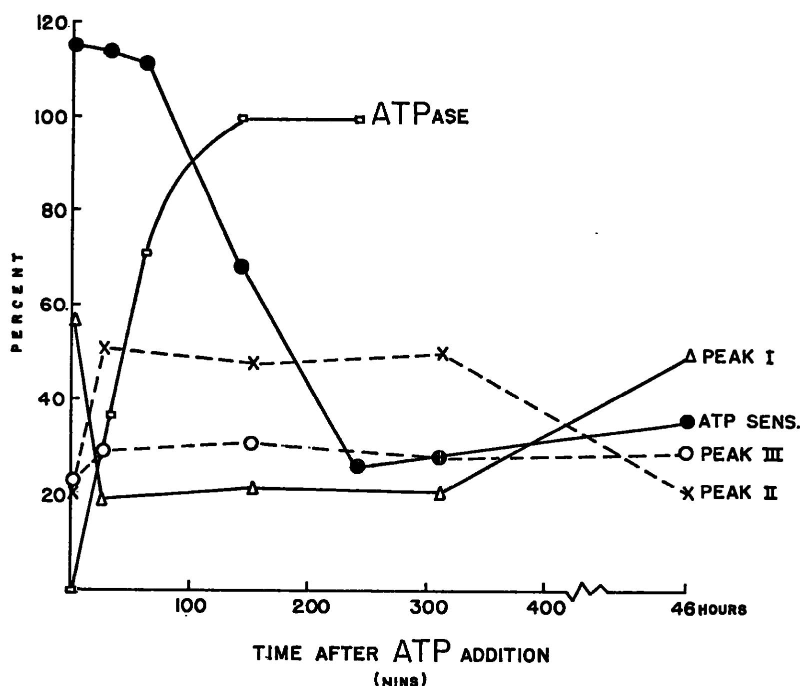

FIG. 6. The ATPase activity, ATP sensitivity and sedimentation characteristics of unpurified *Limulus* myosin B as a function of time after ATP addition. The ATP sensitivity was calculated from the relative viscosity of the myosin B determined at the time intervals after the single original ATP addition and relative to the viscosity before ATP addition. ATPase is the percentage of the maximum amount of inorganic phosphate found after 4 hr. The percentage of protein in each sedimenting peak was calculated from planimeter measurements of photographic enlargements of plates all taken at the time interval indicated after the addition of ATP. No correction was made for radial dilution. Each time interval represents a separate ultracentrifuge run of material to which ATP had been added at zero time. Peak I (40 S component) is presumed to be myosin B; Peak II (6 S component) is presumed to be myosin A; Peak III (3 S component) is presumed to be tropomyosin. All measurements were in 0·6 M KCl–5 mM histidine at pH 7·05 at 59,780 rev/min and approximately 5°C in the ultracentrifuge, 20°C in the viscosimeter, and 25°C for the ATPase with no cation activators.

2·36 S, quite a bit lower than Kay's (1960b) value of 3·02 S. Constants of 3·4, 3·8 and 3·2 for protein concentrations of 2·13, 1·70 and 1·42 mg/ml respectively were obtained but are not included in the line of Fig. 9 because that actin was prepared from actomyosin which had been stored in glycerol in the deep freezer. As noted previously (Fig. 3), glycerol-stored myosin B exhibited anomalous sedimentation behaviour. The actin pellet's identification was supported by experiments in which the viscosity was shown to increase with time after addition of KCl and $MgCl_2$ to final concentrations of 0·1 M and 2 mM respectively.

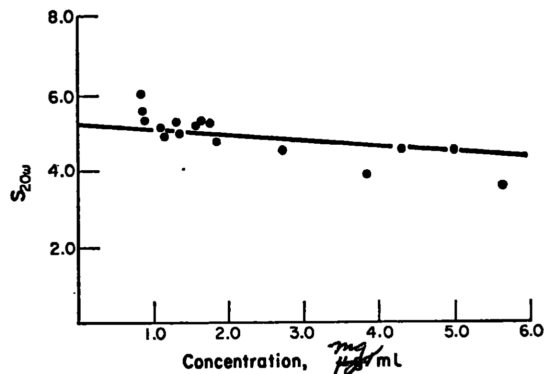

FIG. 8. Sedimentation curve of *Limulus* myosin prepared from fresh myosin B. All measurements were made at 59,780 rev/min between 4 and 9°C in 0·6 M KCl–5 mM histidine, pH 7. Line from least-squares analysis extrapolates to S_{20w}, $c = 0$, at 5·18 S.

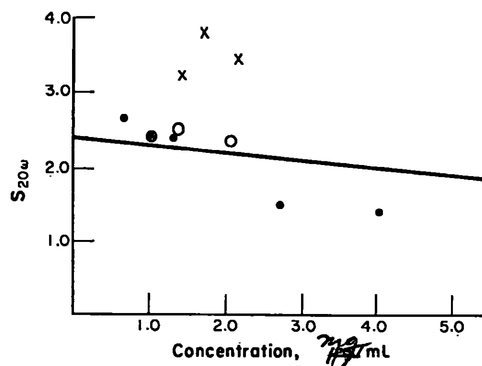

FIG. 9. Sedimentation curve of *Limulus* actin prepared from myosin B. Open and filled circles represent two preparations from fresh myosin B and are used in the least-squares calculation of the line extrapolating to 2·36 S. The points indicated by × are not included in the least-squares line because they represent actin prepared from myosin B which had been stored in glycerol–KCl and were centrifuged in the presence of 0·2 mM ATP only. Conditions for the other points were: 59,780 rev/min, 4–6°C, 0·2 mM ATP–0·2 mM Cleland's reagent (dithiothreitol), pH 7·8.

DISCUSSION

In spite of the difficulties which are apparently inherent in the direct extraction of myosin from marine organisms and contrary to our earlier report (de Villafranca, 1964), horseshoe crab myosin B does dissociate into actin and myosin. The earlier data can be explained, perhaps, by our failure to include Mg^{2+} in the dissociation process, leaving in the supernatant actin which could then recombine with myosin giving our apparent "reversible dissociation" or molecular shape change. This ability to dissociate quite cleanly in the presence of Mg^{2+} now demonstrated in the horseshoe crab myosin B raises the problem of how actomyosin might exist in the intact muscle. According to our earlier findings (de Villafranca *et al.*, 1959), it would appear that actomyosin exists as such in the A bands of the muscle rather than in separate filaments of actin and myosin; a concept more compatible than the present findings with the difficulty in direct extraction of myosin. The almost "molecular behaviour" of myosin B in the ultracentrifuge, however, suggests stoichiometric binding of actin to myosin; or, put another way, actomyosin behaves almost as though it were the parent molecule of actin and myosin subunits rather than a complex of actin and myosin molecules as in the rabbit muscle. This contrasts quite sharply with the behaviour of rabbit myosin B, which seems to have several classes of components apparently interacting with ATP in different ways (Gellert *et al.*, 1959; Tonomura & Sekiya, 1961; Johnson & Rowe, 1964). Moreover, although our S_{20} curve has the same shape as Johnson & Rowe's (1964) the sedimentation coefficient at $c = 0$ is less than half that which they report. We ourselves have noted in rabbit myosin B preparations that a large, amorphous cloud sediments in the Model E, but upon ATP addition this material disappears as a cloud and apparently forms up in the myosin boundary; no such behaviour has been noted with *Limulus* myosin B.

But, to argue both sides, it is rather disquieting to note that experiments with the ATP dissociation of myosin B yield very little myosin A (about 20 per cent of the myosin B assuming the entire supernatant to be myosin A). In most experiments the original myosin B was undoubtedly contaminated with tropomyosin A (de Villafranca & Leitner, 1967) which should also remain in the supernatant; thus the true yield might be less although still more than the 5 per cent protein which precipitates by dialysis against water. We are left, therefore, with the paradoxical situation that even though almost all of the myosin B ultracentrifuge peak can be accounted for in a 6 S (myosin) peak in the presence of ATP (Figs. 1 and 6), the same experiment when performed preparatively does not give the amount of myosin which would be expected from dissociation. It may be, therefore, that two classes of myosin B exist; one dissociable and the other changing shape so that it does sediment differently from "native" myosin B but the same as myosin A (Snellman & Erdos, 1949; Gellert *et al.*, 1959; Johnson & Rowe, 1964).

Even though results on *Limulus* myosin are to some extent preliminary, some comment on its S_{20w} is necessary. Although a value of 5·17 S is the same order of magnitude as reported by Woods *et al.* (1963) for lobster myosin (5·82 S) but low for the values reported by many workers for rabbit or cod myosin (Holtzer & Lowey,

1959; Kielley & Harrington, 1960; Lowey & Cohen, 1962; Connell, 1963; Mueller, 1964), it is close to our very low rabbit myosin figure of 4·7 S. Why the latter is so low cannot be explained unless the histidine buffer has some effect. It does raise the question of whether the 5·17 S for *Limulus* may in fact be about 28 per cent too low. If protein determinations were at fault the $[1{\cdot}7]_\eta$ we obtained for the same rabbit myosin would be too high. The fact that *Limulus* myosin B in the presence of ATP gives a value of about 5·7 S gives some confidence to the myosin figure but begs the question for the rabbit myosin figure. Undue significance should not be attached to the single, high molecular weight determination, since it could easily be a dimer; non-rabbit myosins seem to be even more easily polymerized or dimerized than rabbit myosin (Mackie, 1966).

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