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The Adenosine-Triphosphatase Activity of Dissociated Acto-Heavy-Meromyosin

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1. At low ionic strength, when turbidity and viscosity measurements indicated dissociation of acto-heavy-meromyosin, its adenosine triphosphatase was strongly activated by Mg^{2+} and Ca^{2+}. 2. The characteristics of the adenosine triphosphatase of dissociated acto-heavy-meromyosin in the presence of Mg^{2+} were similar to those reported for myofibrils and actomyosin. 3. In the presence of Ca^{2+} the adenosine-triphosphatase activity was much less sensitive to ionic strength than was the case with Mg^{2+}. 4. At low ionic strength Mg^{2+} was more effective in maintaining the dissociation of acto-heavy-meromyosin in the presence of ATP than was Ca^{2+}. This difference was not apparent when ATP was replaced by ITP. 5. Although the recovery of viscosity was complete on reassociation of acto-heavy-meromyosin the turbidity did not return to the original value. 6. The general implications of Mg^{2+} activation of acto-heavy-meromyosin when classical interpretation indicates dissociation of the complex are discussed.

When myosin interacts with actin two effects can be clearly distinguished. One is that aspect of the interaction responsible for the increases in viscosity and light-scattering that accompany complex-formation in the sol state at ionic strength greater than 0·3 and that are reversed by low concentrations of ATP. The other effect occurs only at low ionic strength and takes the form of a change in the enzymic behaviour of the myosin ATPase* in that it is strongly activated by Mg^{2+}, a property not possessed by myosin alone. If myosin is replaced in this system by heavy meromyosin (H-meromyosin), both aspects of the interaction can be demonstrated under conditions of low ionic strength, for acto-H-meromyosin is soluble under these conditions whereas actomyosin is not. Previous investigations on the acto-H-meromyosin system suggested that the two aspects of the interaction are independent (Leadbeater & Perry, 1963; Perry & Cotterill, 1964) in that when the viscometric data were compatible with the breaking of the interaction between the proteins, Mg^{2+} strongly activated the ATPase. The present work is a more detailed study of the interaction between actin and H-meromyosin and the characteristics of the Mg^{2+}-activated enzyme-catalysed hydrolysis of ATP that occurs when the classical interpretation of viscosity and turbidity measurements indicates that acto-H-meromyosin is dissociated. A preliminary note of some of the findings has been published (Perry & Cotterill, 1965).

METHODS

Preparation of muscle proteins. L-myosin was prepared from the back and leg muscles of the rabbit as described by Perry (1955), and F-actin and G-actin preparations were obtained by the method of Straub (1943) as modified by Leadbeater & Perry (1963). F-actin solutions were used within 7 days of preparation from the acetone-dried fibre.

The preparation of H-meromyosin by chymotryptic digestion was based on the original method of Szent-Györgyi (1953) involving tryptic digestion. To 300ml. of myosin solution (E_{280} 6; approx. 9mg./ml.) in 0·5M-KCl, 30ml. of 0·1M-borate buffer, pH 8·6 (0·1M-boric acid–25mM-sodium tetraborate), was added and the whole brought to a temperature of 25°. Then 30ml. of 10mM-borate buffer, pH 8·6, containing crystalline α-chymotrypsin (Sigma Chemical Co., St Louis, Mo., U.S.A.) (0·5mg./ml.) and thrice-crystallized soya-bean trypsin inhibitor (Sigma Chemical Co.) (0·05mg./ml.) was added. After digestion for 10min., at 25°, 30ml. of 10mM-di-isopropyl phosphorofluoridate in 10mM-borate buffer, pH 8·6, was added and the whole cooled to 0°. The digest was dialysed overnight against 4l. of 6·7mM-Sörensen phosphate buffer, pH 7·0, and the precipitate of L-meromyosin centrifuged down. The H-meromyosin, which was precipitated from the supernatant in the 40–55%-saturated $(NH_4)_2SO_4$ fraction, was dialysed against water to remove the bulk of the $(NH_4)_2SO_4$ and finally exhaustively against 25mM-tris–HCl buffer, pH 7·6 (Leadbeater & Perry, 1963). Stock solutions were stored at 15–25mg. of protein/ml. and used as fresh as possible, usually within 14 days. The ATPase activity of these preparations was in the range 220–280μg. of phosphate P liberated in 5min. by 1ml. of H-meromyosin (E_{280} 1·0) when measured in 5mM-ATP–5mM-$CaCl_2$–0·2M-KCl–50mM-tris–HCl buffer, pH 7·6.

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[*] Abbreviation: ATPase, adenosine triphosphatase.

Viscosity measurements. These were carried out in an Ostwald viscometer with a capacity of 3·0ml. and a flow time for water at 25° of approx. 30sec. Readings were taken in triplicate and averaged except when continuous measurements were made after the addition of ATP to acto-H-meromyosin solutions (see below). In the latter case the time of the measurement was taken as the time when the viscosity measurement commenced plus half the flow time. Zero time corresponded to the moment when ATP was added to the system. A little octanol was added to the solution in the viscometer to prevent frothing.

Turbidity measurements. The turbidity changes occurring in the acto-H-meromyosin solutions were followed at $350\,\mathrm{m}\mu$ with the Beckman model DB recording spectrophotometer.

For experiments in which the turbidity, viscosity and the rate of hydrolysis of ATP were followed simultaneously the procedure was as follows. To 1vol. of an acto-H-meromyosin solution (1·55mg. of H-meromyosin/ml. and 0·75mg. of actin/ml.), usually in 25mM-tris–HCl buffer, pH7·6, with further additions as indicated in the text, $\frac{1}{9}$ or 0·1vol. of tris–ATP (0·1–10mM) was added. The solution was mixed, 3ml. immediately pipetted into the viscometer and approx. 10ml. transferred to a large spectrophotometer cuvette with a 1cm. light-path. Continuous viscosity and extinction measurements were started as soon as possible and 0·5ml. samples withdrawn from the cuvette at intervals. These were pipetted into 1ml. of 15% (w/v) trichloroacetic acid and inorganic phosphate estimations were carried out by the method of Fiske & Subbarow (1925). Both viscosity and spectrophotometric measurements were carried out at 25°. Duration of dissociation of the complex was taken as the time from the addition of ATP until the E_{350} reached the upper stationary value. In some cases when the form of recovery was consistent, and the rapid rise in E_{350} occupied only a small fraction of the total time, the dissociation time was taken as the time required for the E_{350} to reach the mid-point between its initial and final values.

Unless otherwise stated tris–ATP, prepared by the method of Schwartz, Bachelard & McIlwain (1962) from the disodium salt supplied by the Sigma Chemical Co., was used throughout. ATP concentrations were determined by estimation of the inorganic phosphate liberated in 10min. at 100° by N-HCl. The ATP content of the ADP preparations (Sigma Chemical Co.) was estimated from the inorganic phosphate liberated on prolonged incubation with H-meromyosin.

Other procedures and materials were those described by Perry & Cotterill (1964).

RESULTS

Systems containing magnesium chloride. The addition of ATP to acto-H-meromyosin solutions brought about an immediate fall in turbidity and at the protein concentrations used in this study the solutions became completely clear. After a time, which depended on the ATP concentration and the ionic conditions, the solution became turbid again, and on the further addition of ATP the cycle could be repeated. Measurement of the viscosity on samples of the acto-H-meromyosin while the E_{350} was being recorded with the spectrophotometer indicated that the changes in these two properties were occurring simultaneously (Fig. 1). This correlation was as close as the precision of the method, which involved viscosity measurements on a separate sample of the original solution maintained at the same temperature, would permit.

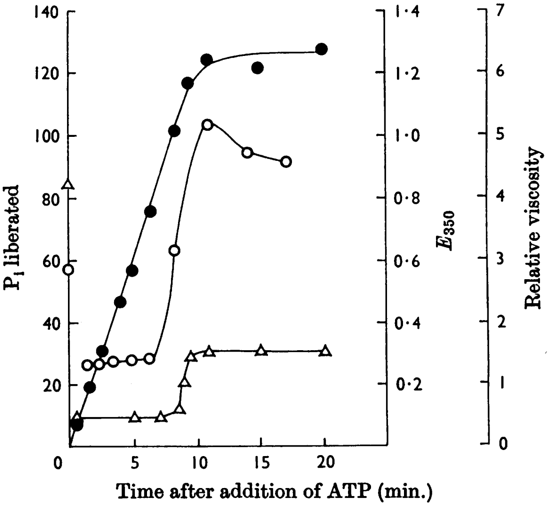

Fig. 1. Correlation of phosphate liberation, turbidity and viscosity changes after the addition of ATP to acto-H-meromyosin in the presence of $MgCl_2$. To 15ml. of acto-H-meromyosin solution containing H-meromyosin (1·5mg./ml.), F-actin (0·75mg./ml.), tris–HCl buffer, pH7·6 (54·2mM), $MgCl_2$ (5mM) and KCl (10mM), 1·5ml. of 50mM-tris–ATP was added at zero time. A 3ml. sample was used immediately for viscosity determinations and 9·0ml. for turbidity and inorganic phosphate estimations. ●, Inorganic phosphate liberated; ○, relative viscosity; △, E_{350}.

Typical results from experiments in which samples for inorganic phosphate determination were withdrawn during the continuous recording of E_{350} are presented in Fig. 1. Immediately after the addition of ATP, throughout the period when viscosity and turbidity remained constant at minimum values, and during the early stages of their increase, hydrolysis continued at a steady rate. By the time the rapid changes in viscosity and turbidity had ceased the liberation of inorganic phosphate had virtually stopped. When higher ATP concentrations (5mM or above) were used the inorganic phosphate liberated at this point corresponded to slightly less (about 90–95%) than would be expected for complete hydrolysis of the terminal phosphate group of the ATP present. At lower ATP concentrations (less than 1mM) this stage appeared to correspond more closely to complete hydrolysis of the triphosphate as estimated from the acid-labile phosphate values. Thence afterwards occurred a very slow, barely significant, increase in inorganic phosphate, which in the case of the lower initial ATP concentrations was usually paralleled by a further, very gradual increase of E_{350}.

The fall in viscosity and light-scattering is normally taken as evidence of dissociation, but nevertheless the ATP was split at a high rate with Mg^{2+} as the only added bivalent cation. At low ionic strength the rate of Mg^{2+}-activated hydrolysis was comparable with that obtained with Ca^{2+} under otherwise identical conditions, but with the former ion it fell off sharply with increasing ionic strength. This effect is illustrated in Fig. 2, which also shows that the initial rate of ATP hydrolysis is in an inverse relationship with the time taken for reassociation.

The time taken for the turbidity to rise to the stationary value after the initial fall produced by the addition of ATP to a final concentration of 2·5mM depended on the magnesium chloride concentration in the system. The shortest times were obtained when the ratio of ATP and magnesium chloride concentrations was slightly greater than unity (Fig. 3), and as might be expected the time the acto-H-meromyosin remained dissociated was in inverse relationship to the initial rate of inorganic phosphate liberation. The relation between ATPase activity and the relative concentrations of magnesium chloride and ATP present was very similar to that demonstrated with actomyosin and myofibrils by Perry & Grey (1956*a*).

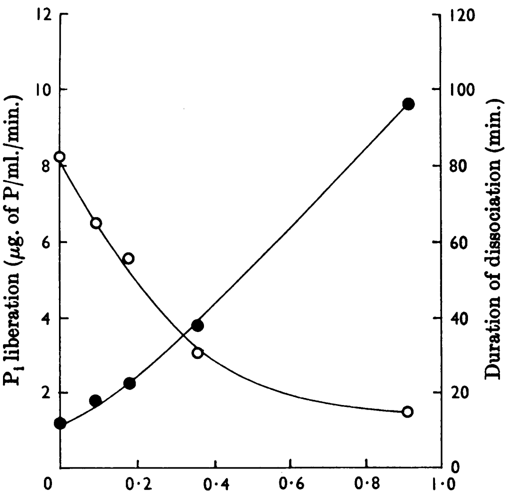

Fig. 2. Effect of ionic strength on the Mg^{2+}-activated ATPase and the duration of dissociation of acto-H-meromyosin after the addition of ATP. To 8·0 ml. of sample containing H-meromyosin (1·5 mg./ml.), F-actin (0·75 mg./ml.), tris–HCl buffer, pH 7·6 (54·2 mM), MgCl$_2$ (5 mM) and KCl as indicated, 0·8 ml. of 50 mM-tris–ATP was added at zero time. Samples were withdrawn for estimation of the rate of ATP hydrolysis over the first 3–5 min., during which period turbidity indicated that the complex was dissociated. ○, ATPase activity; ●, time for 50% of recovery of E_{350} after the addition of ATP.

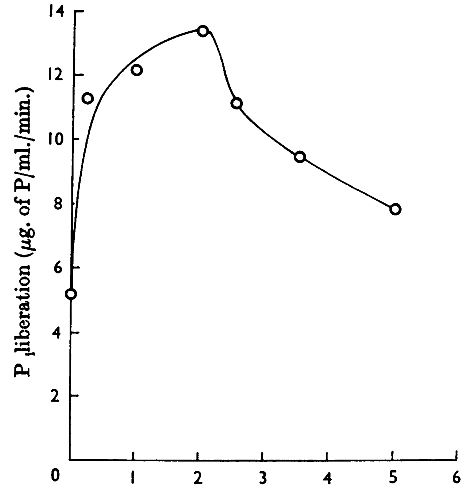

Fig. 3. Effect of Mg^{2+} on the ATPase activity of dissociated acto-H-meromyosin. ATP was added at zero time to the system so that the final concentrations were: H-meromyosin (1·5 mg./ml.), F-actin (0·75 mg./ml.), tris–HCl buffer, pH 7·6 (50 mM), ATP (2·5 mM) and MgCl$_2$ as indicated. The initial rates of ATP hydrolysis were determined as given for Fig. 2.

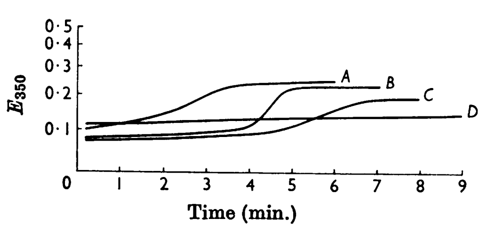

Fig. 4. Effects of Mg^{2+} and Ca^{2+} on the changes in turbidity after the addition of ATP to acto-H-meromyosin. The final concentrations were: H-meromyosin (1·5 mg./ml.), F-actin (0·75 mg./ml.), tris–HCl buffer, pH 7·6 (50 mM), tris–ATP (2·5 mM) and the additions indicated. Before the addition of ATP the initial E_{350} values for *A*, *B*, *C* and *D* were 0·55, 0·51, 0·41 and 0·44 respectively. *A*, CaCl$_2$ (2·5 mM); *B*, MgCl$_2$ (2·5 mM); *C*, MgCl$_2$ (0·05 mM); *D*, no additions.

The pattern of turbidity changes observed after the addition of ATP to the acto-H-meromyosin system was also determined by the Mg^{2+} concentration. In the absence of added bivalent metal ion the E_{350} began to rise slowly immediately after the addition of ATP and continued in a steady fashion until it reached the stationary value (Fig. 4). With magnesium chloride concentrations comparable with those of the original ATP concentrations (2·5–5·0mM) the E_{350} remained low and constant after the addition of ATP and changed rapidly to reach the higher stationary value only when most of the ATP had been converted into ADP (Figs. 1 and 4). With low Mg^{2+} concentrations, e.g. 0·05–0·1mM, the pattern of the turbidity changes was intermediate between these two extremes (Fig. 4).

Systems containing calcium chloride. When Ca^{2+} was the added bivalent activator in the acto-H-meromyosin system certain characteristics of the ATP hydrolysis and the time-course of the turbidity and viscosity changes were different from those obtained with Mg^{2+}. First, at low ionic strength immediately after the instantaneous fall that occurred on the addition of ATP to the system at low ionic strength, both turbidity and relative viscosity began to rise slowly again in parallel with the steady rate of hydrolysis of ATP. The steady increase in turbidity and viscosity continued until the liberation of inorganic phosphate levelled off (Fig. 5). This was in sharp contrast with the behaviour of the system in the presence of magnesium chloride, when the viscosity and turbidity remained at a low level until just before most of the ATP was hydrolysed and then changed rapidly (see Fig. 1). Secondly, for given ATP and Ca^{2+} concentrations the time required for the E_{350} to return to the plateau value was much less affected by ionic strength. Fig. 6 illustrates that, although the duration of dissociation of acto-H-meromyosin after the addition of ATP was shortest at about 0·1M-potassium chloride, the time was not very different from those obtained at other potassium chloride concentrations over the range 0–0·5M.

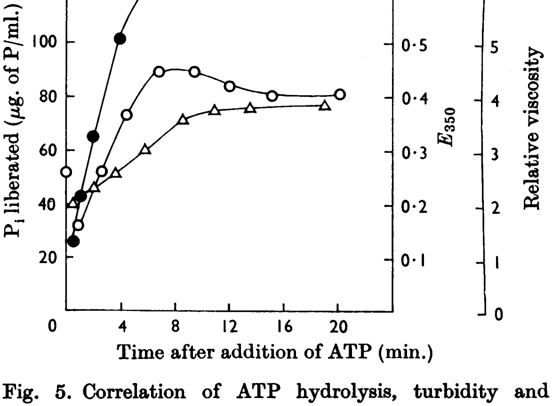

Fig. 5. Correlation of ATP hydrolysis, turbidity and viscosity changes after the addition of ATP to acto-H-meromyosin in the presence of Ca^{2+}. To 13ml. of acto-H-meromyosin solution containing H-meromyosin (1·5mg./ml.), F-actin (0·75mg./ml.), tris–HCl buffer, pH7·6 (54·2mM), and $CaCl_2$ (2mM), 1·3ml. of 50mM-tris–ATP was added at zero time. The procedure was otherwise as given for Fig. 1. Before the addition of ATP the E_{350} value was 0·85. ●, Inorganic phosphate liberated; ○, relative viscosity; △, E_{350}.

As the ionic strength increased under otherwise identical conditions it was noted that the turbidity of the acto-H-meromyosin solution in the absence of ATP fell, and in consequence the fall in E_{350} on the addition of ATP was less at higher ionic strength (Fig. 7). This effect is similar to that reported for the viscosity of acto-H-meromyosin by Perry & Cotterill (1964), and was obtained whether Ca^{2+} or Mg^{2+} was present. Fig. 7 also illustrates that, with Ca^{2+} as bivalent activator, increasing the ionic strength altered the pattern of turbidity change so that at ionic strength greater than 0·2 it was similar

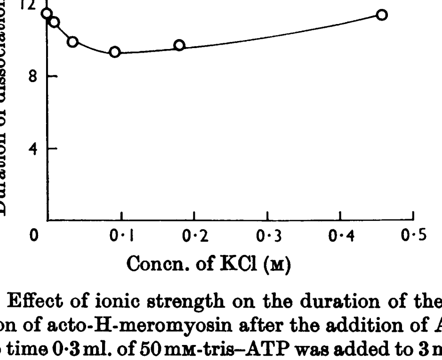

Fig. 6. Effect of ionic strength on the duration of the dissociation of acto-H-meromyosin after the addition of ATP. At zero time 0·3ml. of 50mM-tris–ATP was added to 3ml. of solution containing H-meromyosin (1·5mg./ml.), F-actin (0·75mg./ml.), $CaCl_2$ (5mM), 50mM-tris–HCl buffer, pH 7·6, and KCl to give the final concentrations indicated.

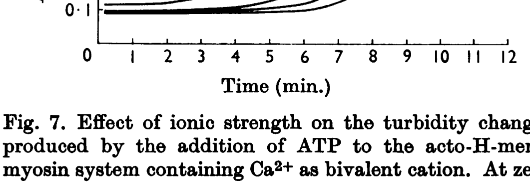

Fig. 7. Effect of ionic strength on the turbidity changes produced by the addition of ATP to the acto-H-meromyosin system containing Ca^{2+} as bivalent cation. At zero time 0·4ml. of 50mM-tris–ATP was added to 3·6ml. of the protein sample. The final concentrations were: H-meromyosin (1·5ml./ml.), F-actin (0·75mg./ml.), $CaCl_2$ (5mM) and tris–HCl buffer, pH7·6 (50mM). *A*, KCl (10mM); *B*, KCl (40mM); *C*, KCl (0·10M); *D*, KCl (0·20M).

to that obtained with Mg^{2+}, i.e. the return to the stationary value occurred more rapidly and took place during the last stages of ATP hydrolysis rather than changing slowly throughout the whole course of ATP hydrolysis.

Effects of ethylenedioxybis(ethyleneamino)tetra-acetic acid. The presence of 1 mM-ethylenedioxybis-(ethyleneamino)tetra-acetic acid [ethylene glycol bis(aminoethyl)-*NNN'N'*-tetra-acetic acid; EGTA] had little effect on the turbidity or viscosity response of a system containing acto-H-meromyosin, magnesium chloride (4 mM), tris–ATP (2·5 mM) and tris–hydrochloric acid buffer, pH 7·6 (25 mM). The rate of ATP hydrolysis was slightly decreased (by less than 20%) under these conditions, but if the ethylenedioxybis(ethyleneamino)tetra-acetic acid concentration was raised to 5 mM the inhibition did not further increase significantly. In its relative insensitivity to this chelating agent the Mg^{2+}-activated ATPase of acto-H-meromyosin resembled that of synthetic actomyosin rather than that of natural actomyosin (Perry & Grey, 1956*b*).

ITP as substrate. With Mg^{2+} as the bivalent activator, the addition of ITP to acto-H-meromyosin solutions brought about falls in viscosity and E_{350} similar to those obtained on the addition of ATP. The pattern of recovery of E_{350} was different, however, for with magnesium chloride and ITP both at 2·5 mM the E_{350} slowly rose immediately after the sharp fall on the addition of the triphosphate, finally reaching the stationary value. As with ATP, the stationary value reached when hydrolysis had ceased was less than the original E_{350} and the percentage recovery was usually greater the lower the ITP concentration used. The pattern in general was very similar to that obtained with Ca^{2+} and ATP (see Fig. 7), and likewise as the ionic strength of the system increased the recovery pattern changed to the type obtained with systems containing Mg^{2+} and ATP (cf. Fig. 4).

Irrespective of the pattern of E_{350} change after the addition of ITP inorganic phosphate was liberated at a steady rate, the precise value of which depended on the ionic conditions, from zero time until the E_{350} flattened off at the stationary value.

Recovery of viscosity and turbidity after the addition of ATP. Although the viscosity and light-scattering changes appeared to occur simultaneously after the addition of ATP when either Mg^{2+} or Ca^{2+} was the activating cation, there was a marked difference in the quantitative aspects of the response of these two properties. The viscosity returned to a value appreciably higher than that possessed by the systems before the addition of ATP at the same time as the E_{350} flattened out to the stationary value; the viscosity then slowly fell to the original value (Figs. 1 and 5). This viscometric behaviour was apparently not critically affected by ATP concentration as it was observed in the range 0·05–2 mM.

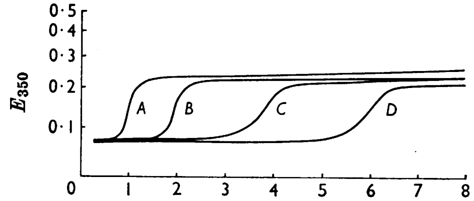

Fig. 8. Turbidity changes after the addition of various concentrations of ATP to acto-H-meromyosin solutions. At zero time 0·8 ml. of ATP was added to 7·2 ml. of solution. The final concentrations were: H-meromyosin (1·5 mg./ml.), F-actin (0·75 mg./ml.), tris–HCl buffer, pH 7·6 (50 mM), and $MgCl_2$ (2·5 mM). Before the addition of ATP the E_{350} values for *A*, *B*, *C* and *D* were 0·44, 0·42, 0·52 and 0·41 respectively. *A*, ATP (0·5 mM); *B*, ATP (1·0 mM); *C*, ATP (2·0 mM); *D*, ATP (3·0 mM).

In contrast, on no occasion was an overshoot observed during recovery of the E_{350}. On the contrary, when reassociation took place after the addition of higher ATP concentrations the E_{350} plateaued at values representing 40–50% of the fall obtained immediately on the addition of the triphosphate. This effect was observed with acto-H-meromyosin systems at low ionic strength and with actomyosin at 0·5 M-potassium chloride. In both systems, however, the percentage recovery of E_{350} depended on the concentration of ATP used to dissociate the complex, recovery being more nearly complete at low ATP concentrations, e.g. 0·1 mM (Fig. 8). This observation suggested that either the products of ATP hydrolysis or possibly ATP itself was responsible for the effect.

The addition of inorganic phosphate (1–5 mM) or AMP (0·05–2 mM) to acto-H-meromyosin systems produced no significant change in E_{350}, indicating that these substances were not preventing the recovery of E_{350}. On the other hand, ADP did produce a significant persistent decrease in E_{350} in such systems, the fall increasing with concentration. This effect was unlikely to be due to the trace (less than 1%) of ATP in the ADP preparations, which no doubt accounted for the transient marked fall in E_{350} that occurred immediately after the addition of ADP at higher concentrations. Immediately after this sharp initial fall the E_{350} rapidly rose to the stationary value, which depended on the ADP concentration.

As the acto-H-meromyosin had no detectable myokinase activity the ADP that accumulated in the system after hydrolysis of ATP would be expected to be in part responsible for the failure of the E_{350} to recover completely. Other factors appeared to be involved, however, for in no case did ADP depress the E_{350} to the stationary value obtained with the same concentration of ATP.

DISCUSSION

The effects of nucleoside triphosphates on the viscosity and light-scattering properties of solutions of acto-H-meromyosin at low ionic strength are very similar to those observed with actomyosin at the ionic strength (greater than 0·3) required to keep the latter complex in solution. These effects are classically interpreted to imply dissociation; nevertheless, the enzymic characteristics of H-meromyosin are modified by the presence of actin insofar as the system shows all the characteristics of Mg^{2+} activation previously reported for actomyosin itself and isolated myofibrils.

It has been fairly widely accepted that Mg^{2+}-activated ATPase is a characteristic of undissociated actomyosin (Szent-Györgyi, 1951; Hasselbach, 1952) and that when dissociation occurs Mg^{2+} will no longer activate the enzyme. Discussion by Hasselbach (1964) incorporates such ideas into an explanation of the low ATPase activity that is associated with relaxation in muscle. If our findings with acto-H-meromyosin can be extended to actomyosin, this explanation is untenable, for clearly, when the bond that is responsible for the high viscosity and light-scattering properties of the complex in solution is broken in the presence of ATP, the enzymic activity of myosin will still be strongly activated by Mg^{2+}. Some other mechanism is therefore required to explain the inhibition of the Mg^{2+}-activated ATPase that is associated with actomyosin in the presence of ATP and of relaxing factor. The fact that preparations of relaxing factor and chelating agents do not inhibit the ATPase of synthetic (Perry & Grey, 1956*a*,*b*) or trypsin-treated actomyosin systems unless an additional factor is present (Ebashi, 1963) is further evidence that the low Mg^{2+}-activated ATPase activity is not due to dissociation alone.

The nature of the interaction of actin with H-meromyosin that occurs in the presence of ATP, when viscosity and light-scattering measurements are interpreted as indicating dissociation, is not understood. It is clearly weak, may also involve one of the other components of the enzyme system and probably has some electrostatic character in view of the sensitivity of the Mg^{2+} activation to ionic strength. As has been discussed by Perry & Cotterill (1965), the evidence suggests that a centre on the actin molecule different from that responsible for the actin–myosin interaction, involving gross physical changes in viscosity and light-scattering, is involved in Mg^{2+} activation and interaction with the enzymic centre of myosin.

The differences in the E_{350} and viscosity changes that occur after the addition of ATP with Mg^{2+} on the one hand and Ca^{2+} on the other provide further evidence for the special role of Mg^{2+} in the interaction of actin and myosin. They suggest that at low ionic strength Mg^{2+} is much more effective in keeping the complex dissociated, and it would appear from the results with ITP that the 6-amino group of the purine ring is essential for this property.

A feature of the effect of ATP on the acto-H-meromyosin and actomyosin systems is the dependence of the extent of recovery of the turbidity change on the concentration of triphosphate used to bring about dissociation. The difference in response of the turbidity and viscosity values indicate, as would be expected, that the two parameters are reflections of different events at the molecular or micellar level. The light-scattering effects are partly explained by the apparent dissociating effect of the ADP produced in the enzymic reaction. Another contributing factor may be the persistence of small amounts of ATP in the systems with high initial concentrations of the triphosphate, owing to the inhibition of the ATPase by the high ADP concentrations produced in these systems.

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REFERENCES

Ebashi, S. (1963). *Nature, Lond.*, **200**, 1010.

Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.

Hasselbach, W. (1952). *Z. Naturf.* **7b**, 163.

Hasselbach, W. (1964). *Progr. Biophys. molec. Biol.* **14**, 167.

Leadbeater, L. & Perry, S. V. (1963). *Biochem. J.* **87**, 233.

Perry, S. V. (1955). In *Methods in Enzymology*, vol. 2, p. 582. Ed. Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Perry, S. V. & Cotterill, J. (1964). *Biochem. J.* **92**, 603.

Perry, S. V. & Cotterill, J. (1965). *Nature, Lond.*, **206**, 161.

Perry, S. V. & Grey, T. C. (1956*a*). *Biochem. J.* **64**, 184.

Perry, S. V. & Grey, T. C. (1956*b*). *Biochem. J.* **64**, 5P.

Schwartz, A., Bachelard, H. S. & McIlwain, H. (1962). *Biochem. J.* 628.

Straub, F. B. (1943). *Stud. Inst. med. Chem. Univ. Szeged*, 3, 23.

Szent-Györgyi, A. (1951). *Chemistry of Muscular Contraction*, 2nd ed., p. 56. New York: Academic Press Inc.

Szent-Györgyi, A. G. (1953). *Arch. Biochem. Biophys.* **42**, 305.