INDICATION FOR AN ALLOSTERIC EFFECT OF ADP ON ACTOMYOSIN GELS AND GLYCERINATED FIBRES FROM INSECT FIBRILLAR FLIGHT MUSCLE

R.A. Chaplain, R.H. Abbott and D.C.S. White

Department of Zoology, University of Oxford, Oxford, England

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Adenosinetriphosphatase activity of actomyosin and myosin isolated from insect flight muscles have been extensively studied by Maruyama (1958). The requirement of ATP for tension and oscillatory work in glycerinated fibres has recently been demonstrated by Jewell, Pringle and Rüegg (1964). The present paper describes a regulation of actomyosin interactions by ADP, as manifested by a co-operative inhibition of the Ca^{++}-activated ATPase and increased tension developed by glycerinated fibres in the presence of this nucleotide. A structural transition has also been observed in actomyosin gels in the presence of ADP.

Material and Methods

Dorsal longitudinal muscles of the giant water bug, Lethocerus cordofanus, have been glycerinated in situ, using the schedule given by Huxley (1963), and stored at $-18^{\circ}C$ for six months.

Mechanical experiments: The mechanical apparatus was similar to that used by Jewell, Pringle and Ruegg (1964) Bundles of about twelve fibres were attached between two glass rods with cellulose nitrate dissolved in acetone. The position of one of the rods was fixed and the other was connected to an RCA 5734 tension transducer. A free fibre length of 5 mm was used and the tension was recorded on a Devices two-channel recorder. The temperature was kept constant at $20^{\circ}C$.

Preparation of actomyosin: Actomyosin was prepared from the freshly excised indirect flight muscles of the water bugs using the procedure of Maruyama (1958), except that the myofibrils were separated prior to extraction by differential centrifugation of the homogenate at 600 g, 20 min. in an MSE centrifuge.

Other assays: Light scattering was monitored at 540mμ with an Eppendorf photometer equipped with a 90° light scattering accessory. ATPase activity was measured in activating solution, pCa 6.8 at $25^{\circ}C$, by following the amount of inorganic phosphate

released, using the method of Fiske and Subbarow. The final volume was 2.0 ml, and the actomyosin concentration was 1.08 mg/ml.

Solutions. Rigor solution: 20mM Tris-Cl, 5mM $MgCl_2$, 4mM ethylene glycol bis (β-amino ethyl ether) -N_1N^1 tetra-acetate (EGTA), 70 mMKCl, pH 7.1; relaxing solution: the same plus 5mM ATP; activating solution: $CaCl_2$ was added to the relaxing solution to obtain a stabilised [Ca^{++}] of 10^{-7} to 10^{-8}M (Portzehl, Caldwell and Ruegg, 1964).

Results

1. Aggregation and disaggregation of actomyosin gels: The change of light scattering intensity at 90° in the presence of ATP and ADP was investigated. The results are summarised in Table I.

Table I: Change of light scattering intensity with ATP and ADP
Standard solution: 0.6 M KCl, 1 mg actomyosin per ml, neutralised with 20mM Tris-Cl buffer to pH 7.1, 21°C

Assay System	Change to relative intensity
1 mM Mg ATP	- 10% ± 0.6% S.D.
1 mM Mg ATP + 40 mM urea	- 28% ± 1% S.D.
1 mM Mg ADP	+ 8% ± 0.5% S.D.

The drop in light scattering intensity upon addition of ATP, which becomes more marked in the presence of urea, indicates that ATP induces a structural change of the actomyosin complex, which differs from that occurring in presence of ADP.

2. Adenosine triphosphatase activity

(a) Inhibition by ADP: The ATP-splitting activity of isolated actomyosin and glycerinated fibre bundles from Lethocerus indirect flight muscle is activated by Ca^{++} ions (H.H. vom Brocke, in preparation). However, in contrast to rabbit actomyosin, the insect contractile protein fails to exhibit the Mg^{++} activated ATPase at low ionic strength (Abbott and Chaplain, 1965). As shown in Fig. 1, increasing concentrations of ADP inhibit the enzyme activity in such a fashion that between 0.75-1.25mM ADP the extent of inhibition increases much faster than the inhibitor concentration, suggesting a co-operative inhibitory effect by ADP. The ATPase of insect actomyosin in the absence of divalent cations and the myosin ATPase show typical competitive Michaelis-Menten kinetics for ADP-inhibition.

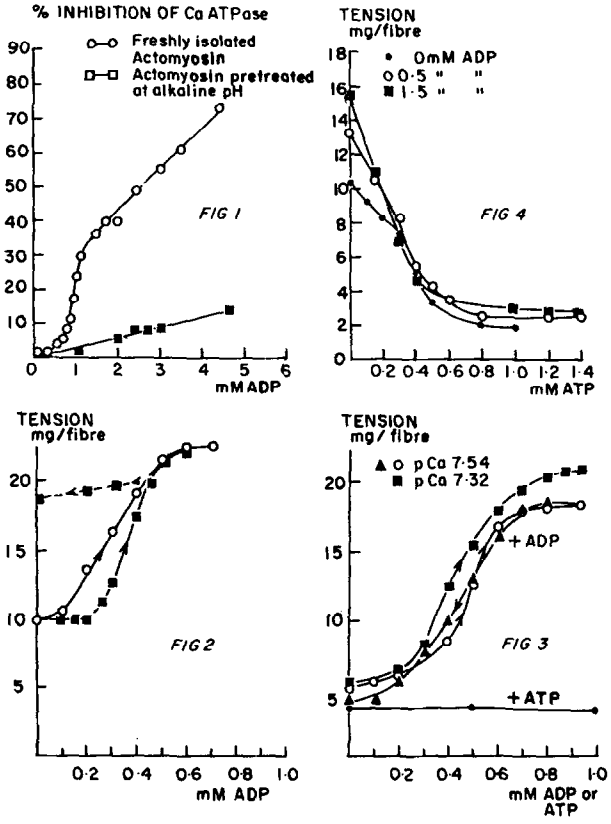

Figure legends: Fig. 1. ADP inhibition of Ca^{++}-stimulated ATPase.
Fig. 2 & 3. Tension changes induced by ADP in rigor solution (Fig 2), and by ADP or ATP in activating solution (Fig. 3).
Fig. 4. Absence of ADP effect under conditions of relaxation. Increasing concentrations of ATP were added to the rigor solution with different concentrations of ADP present.

(b) Effect of alkaline pH and urea: The Ca^{++}-stimulated ATPase shows a plateau of maximal activity over the range pH 8.4-9.1, with an activity 136% that at pH 7.1. At these high pH values a small competitive inhibition is obtained with ADP (Fig. 1). Similarly, addition of 40mM urea in the presence of Ca-ATP shows an activation of enzyme activity up to 145%.

3. Tension development of glycerinated fibres in the presence of ADP

(a) Addition of ADP to fibres in the rigor solution: As shown in Fig. 2, increasing ADP concentrations in the absence of ATP bring about a rise in tension in a sigmoid-shaped fashion. This effect is irreversible; the tension does not fall when the ADP concentration is lowered. The original state is restored by transferring the fibres

through the relaxing solution. When 0.7 M urea was added together with ADP, the tension increase was abolished; urea by itself did not change the tension. Withdrawal of the urea restored the sensitivity of the fibres to ADP. The same effects were observed on changing the pH from 7.1 to 8.7.

(b) Addition of ADP to fibres in the relaxing and activating solutions: Increasing ADP concentrations in the presence of Ca-ATP bring about a similar rise in tension to that produced in the rigor solution (Fig. 3). In this case the effect is fully reversible. This ADP effect in the presence of ATP occurs only when Ca^{++} ions are present. In the relaxing solution containing Mg-ATP alone, even a twofold excess of ADP has no effect on tension. The relaxation obtained as ATP is added to the rigor solution is also unaffected by ADP (Fig. 4). Increasing ATP concentration in the presence of Ca^{++} does not produce a change of tension (Fig. 3). Pretreatment of the fibres for 3 minutes in the rigor solution either at pH 8.7 or in the presence of 1M urea irreversibly abolishes the ADP effect in Ca-ATP solutions.

Discussion

Monod, Wyman and Changeux (1965) have recently suggested that allosteric control of enzyme activity involves co-operative interaction between substrate and controlling ligands as a result of an alteration of the molecular structure of the protein. Instances are listed where loss of allosteric control (desensitization) occurs by various treatments such as urea, mercurials and pH without impairing the enzyme activity. These two criteria are fulfilled in the actomyosin system studied. The inhibitory effect of ADP upon ATPase activity is co-operative as indicated by the sigmoid curve of inhibition of ATPase activity by increasing concentrations of ADP (Fig. 1). Desensitization by alkaline pH or urea increases the activity of the Ca^{++} stimulated actomyosin ATPase.

The mechanical experiments show that the allosteric control by ADP is reflected in the properties of the fibres; the tension generated in the presence of ADP also shows a co-operative effect.

The experiments show that the regulation by ADP requires conditions where interactions between the actin and myosin can take place; it occurs either in the presence of ATP + Ca^{++} or in the absence of ATP where the rigor state leads to cross-bridge formation between the A-filaments and the I-filaments (Reedy, Holmes & Tregear, 1965). ADP has no effect under conditions where the muscle fibres are relaxed and the two sets of

filaments no longer interact (Fig. 4). The fact that the increase of tension produced by ADP is irreversible in the rigor solution but reversible in the activating solution shows that ATP is necessary for this reversibility.

The molecular basis of this allosteric effect appears to be a strengthening by ADP of the subunit interaction in the actomyosin complex, as indicated by the increased scattering of light. Increased subunit interaction in aspartic transcarbamylase induced by the allosteric inhibitor cytidine triphosphate was deduced from the accelerated sedimentation of the enzyme-inhibitor complex by Gerhardt and Pardee (1963).

Further investigations are in progress on the nature of the interactions between the allosteric and substrate binding sites of the actomyosin complex.

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