TENSION DEVELOPMENT OF GLYCERINATED INSECT MUSCLE FIBRES AS A MEASURE OF THE CONFORMATIONAL STATE OF THE MYOSIN

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The allosteric model of Monod, Wyman and Changeux (1965) predicts that it should be possible to determine directly the fraction of an allosteric protein in one particular state. In the context of muscle biophysics the concept of conformational changes accompanying the transition between two or more states of the actomyosin complex is particularly appropriate, for such changes may be the cause of the mechanical phenomena.

Evidence has recently been presented that insect actomyosin ATPase is an allosteric protein (Chaplain, 1966 a & b, 1967) which can exist in three states. (1) A catalytically inactive state with high affinity for the substrate, which is favoured by Mg^{2+}. The affinity of myosin for actin in this state is very low, the apparent association constant being 1.8×10^{2} at 30^{o} and 0.1 ionic strength. (2) In presence of low substrate concentrations or upon binding of the ligands Ca^{2+} or inorganic phosphate the ATPase activity becomes strongly activated. The ATPase activity of glycerinated insect fibrillar muscle fibres is also increased if the fibres are stretched by 1 - 3% (cf. Ruegg and Tregear, 1965). (3) In presence of ADP the actomyosin complex undergoes a transition to a second inactive state which exhibits decreased affinity for the substrate, together with an increase in the affinity of the myosin for actin ($K_{app.}$ 9×10^{19} at 30^{o} and 0.1 ionic strength). Conditions which favour the third state lead to superprecipitation in actomyosin gels or tension development of muscle fibres. The three states have been defined as the relaxed or R-state, the active or A-state and the contracted or C-state. Light scattering studies on actomyosin gels and changes in the kinetics of ADP inhibition in presence of high concentrations of ADP, above 1.5 mM, have suggested that under these conditions the equilibrium is shifted from the C-state directly to the R-state. The relationship between the three states is shown in Fig. 1.

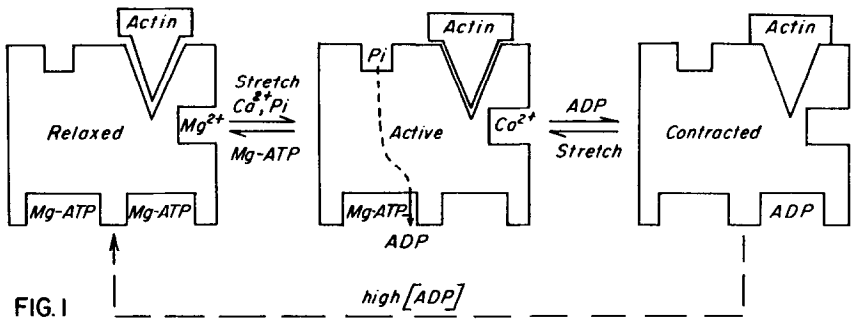

FIG. 1

The present investigation is concerned with the effect of ADP on the tension of muscle fibres. In terms of the allosteric model by Monod et al. (1965) tension developed by glycerinated muscle fibres should be a "function of the conformational state" of the protein.

Materials and Methods

Glycerination procedure: Dorsal longitudinal muscles of the giant water bug Lethocerus cordofanus have been glycerinated in situ as described earlier (Abbott and Chaplain, 1966). Experimental material was prepared by treating bundles of about 20 glycerinated fibres for 18 hrs at 0-2° with the detergent Tween 80 (1% Tween 80 in 50 mM K-phosphate buffer, pH 7.5, containing 5 mM $MgCl_2$). This treatment removes the bulk of the non-myofibrillar cell constituents and facilitates diffusion of ATP and ADP in and out of the core of the fibres, without affecting the mechanical properties (Abbott and Chaplain, 1966).

Tension measurements: Bundles of 10-12 fibres were attached between two glass rods, glued to the glass 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 mounted on a micromanipulator movement. A free fibre length of 5 mm was used. The temperature of the baths was thermostatically controlled at 20°. After removal of the glycerol by immersing the fibres in the rigor solution, the fibres were transferred to the relaxing solution. The fibres were extended by 1% from the slack length in the relaxing solution by moving the force transducer. They were transferred to the respective activating solution (the pCa is given in the figure) and immersed in a series of baths containing the same activating solution supplemented with increasing concentrations of ADP.

Solutions: The rigor solution containing 65 mM KCl, 5 mM $MgCl_2$, 2 mM EGTA and 20 mM Tris-Cl buffer (pH 7.2); relaxing solution: the same plus 5 mM ATP; activating solution: CaEGTA was added to the relaxing solution to obtain a stabilized $[Ca^{2+}]$ as des-

cribed by Portzehl et al. (1964). The relaxing and activating solutions contained additionally small quantities of oligomycin (150 μg/ml) to inhibit any non-myofibrillar ATPase remaining after detergent treatment. Oligomycin does not affect the actomyosin ATPase (Abbott and Chaplain, 1966).

Results and Discussion

If increasing concentrations of ADP are added to an activating solution co-operative tension increases can be observed (Fig. 2). The co-operativity with respect to ADP is more marked at lower Ca^{2+} concentrations. The tension decreases again at higher ADP levels. Studies on the kinetics of ATPase inhibition by ADP and of the effects of ADP on light scattering intensity on actomyosin gels have suggested that ADP can bind both to the contracted and to the relaxed state, although the affinity of the ligand for the R-state as deduced from the change of slope in the Hill plot may be one order of magnitude lower (Chaplain, 1967). A kinetic analysis of data obtained for substrate inhibition has provided evidence that the active myosin unit is a trimer (Chaplain, 1966a). Considering tension as a function of the C-state, the measured tension comes from the C_0, C_1, C_2 and C_3 states (the subscripts denote the complexes involving 0, 1, 2 and 3 ADP molecules). As the A- and the R-state do not contribute significantly to tension development, tension is a function of $\Sigma C/\Sigma C + \Sigma A + \Sigma R$ where $\Sigma C = C_0 (1 + ADP/K_C)^3$, using the mathematical formulations of Monod et al. (1965). If we assume that ADP can also bind to the R-state, and that there exists an equilibrium between the C_3 and the R_{3ADP} state, with an equilibrium constant k then $\Sigma C = C_0(1 + \alpha)^3 - C_0 k\alpha^3$.

Hence

$$\text{Tension} = \frac{C_0(1 + \alpha)^3 - C_0 k\alpha^3}{C_0(1 + \alpha)^3 + R_0(1 + d\alpha)^3 + A_0}$$

where $\alpha = ADP/K_C$ and $d\alpha = ADP/K_R$, $K_C = 2.5 \times 10^{-4}$ and $K_R = 3 \times 10^{-3}$ being the (evaluated) dissociation constants of ADP with the C- and R-state respectively. Let the equilibrium constants for the molecular transitions in absence of ADP be $L_1 = A_0/C_0$ and $L_2 = R_0/C_0$; an equation can then be written of the form

$$(1) \quad \text{Tension} = \frac{(1 + \alpha)^3 - k\alpha^3}{L_1 + L_2(1 + d\alpha)^3 + (1 + \alpha)^3}$$

The basic assumption has been made that when three ADP molecules are bound per myosin oligomer, the C-state is no longer stabilized by the ADP and the protein undergoes

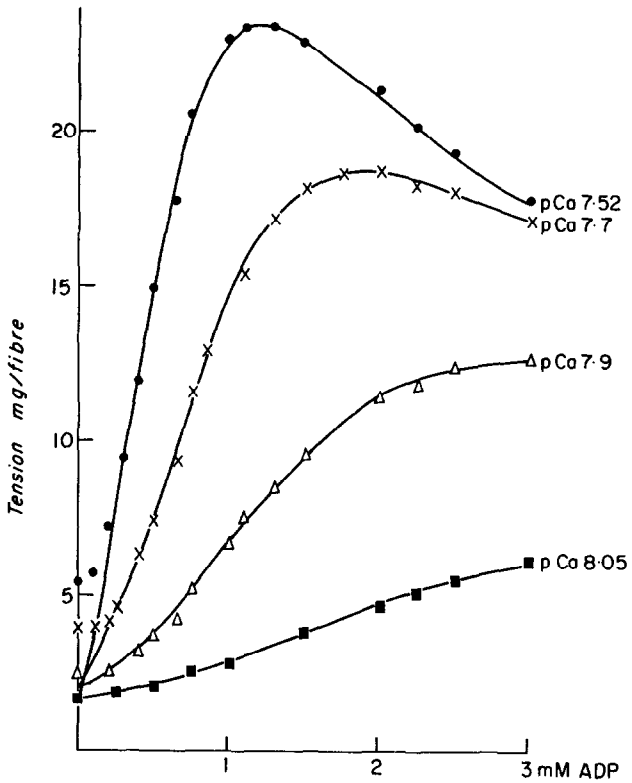

Fig. 2. Effect of ADP on tension development at different Ca^{2+} levels. The solid lines represent theoretical curves for equation (1) using the values for the allosteric equilibrium constants given below; the experimental points are super-imposed. At low levels of ADP the experimental points markedly deviate; this is to be expected since some ADP will be present within the fibres as a result of ATPase activity. The theoretical values of tension have been obtained by subtracting an elastic tension contribution of 1.4-2.0 mg/fibre and by assuming that the maximum fibre tension would be 70 mg, if all the myosin molecules are in the C-state. The allosteric constants involving the C-state vary with $[Ca^{2+}]$ as follows: pCa 8.05 $L_1 = 2400$, $L_2 = 530$; pCa 7.9 $L_1 = 560$, $L_2 = 110$; pCa 7.7 $L_1 = 164$, $L_2 = 28$; pCa 7.25 $L_1 = 37$, $L_2 = 20$.

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a transition from the C_3 to the R_{3ADP} state. The equilibrium constant for this transition was assumed to be 0.95 under the experimental conditions used. However, as the R-state exhibits a greater affinity for ATP ($K_R = 6 \times 10^{-6}$, Chaplain 1966a) than for ADP the three ADP molecules bound to the effector site will be replaced by ATP. Therefore the conditions required for the reverse transition from the R_{3ADP} state to the C_3 state will rarely occur and only the transition towards the R-state will be of importance.

As shown in Fig. 2 the tension changes as a function of [ADP] can be fitted by equation (1). The variation of the allosteric equilibrium constants L_1 and L_2 with $[Ca^{2+}]$ explains why the co-operativity of the ADP effect is more marked at lower Ca^{2+} levels, as more of the protein is initially in the R- and A-state. The relative proportion of the three states determines the maxima of tension development as a function of [ADP] at the different Ca^{2+} concentrations tested.

The effects of ADP on tension development may provide an important control mechanism in muscular contraction. As long as the intramyofibrillar ADP level remains low few myosin molecules will be in the C_3-state and the ADP produced as a result of ATP hydrolysis will stabilize a firm attachment between the myosin and the actin. However, as the ATPase activity increases at high $[Ca^{2+}]$ above a certain level the likelihood of a transition between the C_3 and R_{3ADP} states is greatly increased. The dynamic events leading to tension development under these conditions will no longer be fitted by "function of state" kinetics.

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