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The Reversibility of Adenosine Triphosphate Cleavage by Myosin

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For the simplest kinetic model the reverse rate constants (k_{-1} and k_{-2}) associated with ATP binding and cleavage on purified heavy meromyosin and heavy meromyosin subfragment 1 from rabbit skeletal muscle in the presence of 5mM-$MgCl_2$, 50mM-KCl and 20mM-Tris–HCl buffer at pH 8.0 and 22°C are: $k_{-1} < 0.02 s^{-1}$ and $k_{-1} = 16 s^{-1}$. Apparently, higher values of k_{-1} and k_{-2} are found with less-purified protein preparations. The values of k_{-1} and k_{-2} satisfy conditions required by previous ^{18}O-incorporation studies of $H_2{}^{18}O$ into the P_i moiety on ATP hydrolysis and suggest that the cleavage step does involve hydrolysis of ATP or formation of an adduct between ATP and water. The equilibrium constant for the cleavage step at the myosin active site is 9. If the cycle of events during muscle contraction is described by the model proposed by Lymn & Taylor (1971), the fact that there is only a small negative standard free-energy change for the cleavage step is advantageous for efficient chemical to mechanical energy exchange during muscle contraction.

The elucidation of the kinetic pathway of the Mg^{2+}-dependent ATPase* of myosin is important on account of its relevance to those states of muscle where the myosin heads are dissociated from the actin filaments. Such states exist in relaxed muscle and also occur transiently during contraction. Previous kinetic studies on the myosin ATPase (Trentham *et al.*, 1972) have resulted in the proposal of the following scheme (eqn. 1). M represents myosin or its proteolytic subfragments, and the starred forms are intermediates with increased protein fluorescence compared with free myosin (Bagshaw *et al.*, 1972). The following values have been ascribed to the forward rate constants: k_{+1}, $2.4 \times 10^6 M^{-1} \cdot s^{-1}$; k_{+2} $160 s^{-1}$ (Lymn & Taylor, 1970, 1971); k_{+3}, $0.04 s^{-1}$; k_{+4}, probably rapid ($>10 s^{-1}$); k_{+5}, $2.3 s^{-1}$ (Trentham *et al.*, 1972). The measurements to determine these rate constants were made at pH 8 and room temperature which are the conditions used in the experiments described here, although some differences are to be expected because of the different KCl and buffer concentrations. The values of k_{+2} and k_{+3} were deduced from a kinetic model which assumed $k_{-2} \ll k_{+2}$. It is important to evaluate the reverse rate constants because they have bearing on the steady-state concentration of each intermediate during ATPase activity and these concentrations need to be known for a wide range of studies on relaxed muscle. Further the more complete our understanding of the elementary steps of the Mg^{2+}-dependent ATPase, the more specifically the effects of actin and other muscle proteins on myosin can be determined.

The experiments described below estimate, or at least set limits for the reverse rate constants k_{-1} and k_{-2}. The value of k_{-2} can be evaluated since $k_{+2}+k_{-2}$ is known from the observed rate of transient ADP or P_i formation at high ATP concentration (Lymn & Taylor, 1971; Bagshaw *et al.*, 1972) and k_{-2}/k_{+2}, the equilibrium constant of the transformation

$$M^*ATP \rightleftharpoons M^*ADP \cdot P_i$$

can be measured as follows. If [γ-^{32}P]ATP or [2-^{3}H]ATP is mixed with a large molar excess of myosin and the reaction is quenched at a time τ when the initial binding is complete (controlled by k_{+1}) but before product release occurs (controlled by k_{+3}), ATP will be predominantly equilibrated between M*ATP and M*ADP·P_i, so that product analysis after quenching will give the ratio k_{-2}/k_{+2}. The value of k_{-1} can be evaluated by treating the equilibrium mixture of radioactive M*ATP and M*ADP·P_i with a large excess of non-radioactive ATP and allowing sufficient time for the radioactive substrate

$$M + ATP \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} M^*ATP \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} M^*ADP \cdot P_i \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} MADP \cdot P_i \underset{k_{-4}}{\overset{k_{+4}}{\rightleftharpoons}} MADP + P_i \underset{k_{-5}}{\overset{k_{+5}}{\rightleftharpoons}} M + ADP \quad (1)$$

* Abbreviation: ATPase, adenosine triphosphatase.

and products to dissociate from the protein. The ratio of radioactive substrate to products is then $k_{-1}k_{-2}/k_{+2}k_{+3}$, from which k_{-1} can be calculated. In practice the design of our quenched flow apparatus limits the time-ranges in which we can quench solutions with adequate mixing to 3–50ms and greater than 2s. This means M*ATP concentration has to be measured when the step controlled by k_{+3} has progressed significantly and then the concentration at τ has to be calculated by extrapolation.

Both measurements can be carried out with simple apparatus on a variety of myosin systems, although rapid-reaction equipment is necessary for control experiments and estimating the forward rate constants.

However, the evaluation of k_{-1} and k_{-2} from the experimental data presumes a specific model (eqn. 1). Recent experiments (C. R. Bagshaw & D. R. Trentham, unpublished work) suggest that a second binary complex of myosin and ATP can be characterized. In any new scheme the conclusions about reversibility presented here will still hold, but the values for k_{+1}, k_{+2} and k_{-2} will have to be equated with more complex functions of rate constants because of the extra step involved.

Experimental

Proteins

Subfragment 1 and heavy meromyosin were prepared from myosin extracted from rabbit skeletal muscle essentially as described by Lowey *et al.* (1969). Both proteins were purified by ion-exchange chromatography on DEAE-cellulose. Concentrations of subfragment 1 are quoted as μM-sites on the basis of molecular weight 115000 and $E_{280}^{1\%} = 7.9\,cm^{-1}$. Concentrations of heavy meromyosin are quoted as μM-subfragment 1 heads on the basis of molecular weight 340000 (equiv. wt. 170000) and $E_{280}^{1\%} = 6.47\,cm^{-1}$ (Young *et al.*, 1965). Small corrections for light-scattering were made by measuring the extinction between 320 and 400nm and extrapolating back to 280nm. The proteins were used within 48h of column elution, and contained 0.1mM-dithiothreitol.

Reagents

[γ-^{32}P]ATP was prepared by the method of Glynn & Chappell (1964) and [2-^{3}H]ATP was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). Unlabelled ATP was purchased from C. F. Boehringer und Soehne, G.m.b.H., Mannheim, Germany. ATP concentrations were determined either spectrophotometrically from $\epsilon = 1.54 \times 10^4$ litre·mol^{-1}·cm^{-1} at 259nm, or, for very dilute [2-^{3}H]ATP solutions, from the manufacturer's specifications. All other reagents were A.R. grade where possible. Double-glass-distilled water was used throughout.

Spectroscopic and rapid-reaction equipment

The extinctions of protein and ATP solutions were measured with a Zeiss PMQII spectrophotometer and the u.v. spectra checked with a Perkin–Elmer 402 spectrophotometer. The fluorescence stopped-flow apparatus, built by Professor H. Gutfreund and Dr. D. W. Yates, has been described previously (Bagshaw *et al.*, 1972). The protein was excited by light at 300nm and the emitted light between 335 and 375nm analysed. The quench flow apparatus has been described by Gutfreund (1969), although it was used mainly as a rapid-mixing device, the quenching solution being added manually from a syringe.

Estimation of k_{-2} and k_{-1}

Experiments were carried out at room temperature (21°±2°C). Subfragment 1 or heavy meromyosin was made up to a concentration of 20–50μM-sites in 5mM-$MgCl_2$–50mM-KCl–20mM-Tris buffer adjusted to pH8.0 with HCl. A single turnover of ATP hydrolysis was examined in the fluorescence stopped-flow machine by pushing against 5μM-ATP, and from the trace the values of τ and the turnover rate were ascertained.

The same stock protein solution was then mixed with [γ-^{32}P]ATP or [2-^{3}H]ATP (0.05–5μM syringe concentration) in the quench flow apparatus, and ejected into a glass vial. After intervals between 2 and 60s, timed with a stop watch, an equal volume (3.2ml) of quenching solution, containing 7% (w/v) perchloric acid, 1mM-carrier ATP and 1mM-carrier P_i at 0°C, was added manually from a syringe. The mixture was immediately transferred to a bench-centrifuge tube containing 1.6ml of cold 4M-sodium acetate buffer to raise the pH to 3.4, and the protein precipitate was spun down for 1min. Portions (20μl) of the supernatant were applied to a polyethyleneimine-cellulose thin-layer chromatogram and developed in 0.75M-KH_2PO_4 buffer adjusted to pH3.4 with HCl. The position of the labelled ATP and ADP (where applicable) spots were determined by inclusion of marker ATP and ADP. $^{32}P_i$ runs at the salt front as is readily seen under u.v. light (it also runs coincident with marker AMP). The radioactive bands were cut out and eluted for several hours with 1ml of 1M-HCl, and 0.5ml of the eluates were mixed with 15ml of scintillation fluid (containing 3 litres of toluene, 2 litres of 2-methoxyethanol, 400g of naphthalene and 30g of 5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxo-3,4-diazole (Koch–Light, Colnbrook, Bucks., U.K.) and counted for radioactivity in a Nuclear–Chicago Unilux II counter. The ATP and product counts were corrected for background, then the labelled ATP was expressed as a percentage of the total isotope content for each assay. Time-zero points were obtained during the course of the experi-

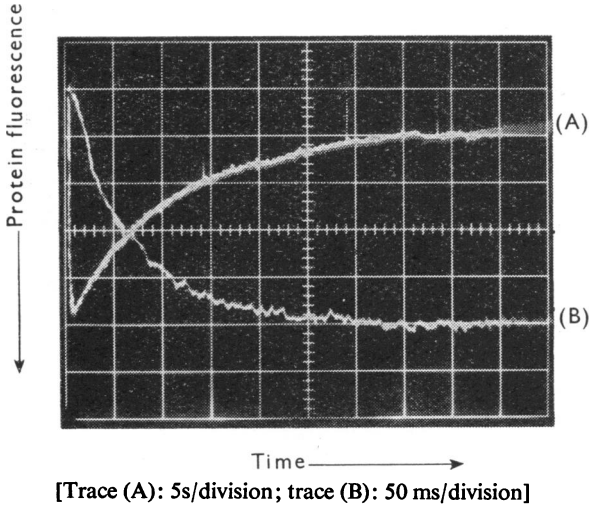
ment by mixing the protein with the quenching solution before addition of labelled ATP. The assay counts could then be corrected for $^{32}P_i$ or [2-^{3}H]ADP contamination originally present in the stock labelled ATP and that, which arose during $HClO_4$ treatment.

A similar technique was employed for estimating k_{-1}. Here 0.5ml of 10mM-non-radioactive ATP was added after 2s to the stirred mixture of labelled ATP and protein, which had been ejected from the quench flow apparatus. After quenching with $HClO_4$ the products were analysed as above. As a check on the efficiency of the non-radioactive 'chase' a control experiment was carried out by adding the unlabelled ATP before the labelled ATP. After a single turnover very little of the labelled ATP should be hydrolysed.

Results

Fluorescence stopped-flow experiments

When excess of subfragment 1 was mixed with ATP the reaction profile showed two distinct exponential phases (Fig. 1) as noted previously (Trentham *et al.*, 1972; Bagshaw *et al.*, 1972). The maximum protein fluorescence is reached at 300ms after mixing reactants and this gives a value for τ, the time at which the binding equilibrium controlled by k_{+1} is established. The rate of the slow phase was not affected by increasing subfragment 1 concentration supporting previous findings that the experiments were carried out at a subfragment concentration well above the K_m value for ATP ($<10^{-7}$M; Lymn & Taylor, 1970). Therefore according to eqn. (1) the observed rate of decomposition of the high-fluorescent forms of the protein ($0.10s^{-1}$) equals $k_{+2}k_{+3}/(k_{+2}+k_{-2})$. It is conceivable this should be replaced by a more complex function involving k_{-3} and k_{+4} as well. However, this possibility would not affect subsequent arguments about the extent of reversibility of the cleavage step and is unlikely in view of the lack of effect of P_i on myosin ATPase kinetics (Trentham *et al.*, 1972). A single turnover is more than 99% complete in 60s. Similar traces were obtained with heavy meromyosin.



[Trace (A): 5s/division; trace (B): 50 ms/division]

Fig. 1. *Stopped-flow spectrophotometric record of protein fluorescence during a single turnover of the subfragment* 1 *ATPase*

One syringe contained 15μM-subfragment 1 (reaction-chamber concentration) and the other 2.5μM-ATP (reaction-chamber concentration). Both syringes contained 5mM-$MgCl_2$–50mM-KCl–20mM-Tris buffer adjusted to pH8.0 with HCl. For trace (A) the time-scale is 5s/division; for trace (B) 50ms/division.

Measurement of k_{-2}

$k_{+2}+k_{-2}$ ($160s^{-1}$; Lymn & Taylor, 1971, Fig. 3) is rapid compared with the enhanced fluorescence decay rate, $k_{+2}k_{+3}/(k_{+2}+k_{-2})$ ($=0.10s^{-1}$), so that the equilibrium between M*ATP and M*ADP·P_i will be maintained throughout their decay to the low-fluorescent forms of myosin. This means that both M*ATP and M*ADP·P_i will decay exponentially at a rate of $0.10s^{-1}$. The results of Table 1 show first that there is a significant amount of ATP present when all the nucleotide is bound (at time τ, 300ms) and that it decays at a rate comparable with the fluorescence decay rate (Fig. 1). By using this

Table 1. *Concentration of free ATP+M*ATP during a single turnover of subfragment* 1 *ATPase*

The experiment was carried out with 15μM-subfragment 1 (reaction-chamber concentrations) and 2.5μM-[γ-^{32}P]ATP in 5mM-$MgCl_2$–50mM-KCl–20mM-Tris buffer adjusted to pH8.0 with HCl as described in the Experimental section. The 37ms time-point was obtained by using the quench flow apparatus in the normal manner. Each value is an average of duplicate assays which agreed within 2% and has been corrected for the decomposition of ATP during the quenching procedure which was 4% of the ATP present at the time of quenching. The subfragment 1 preparation was the same as used in the fluorescence stopped-flow experiment (Fig. 1) and this quenching experiment was completed within 5h of obtaining the stopped-flow traces.

Quenching time (s)	% labelled ATP present
0	100
0.037	51
2.0	7
5.0	4
60	0.3

exponential decay rate ($0.10 s^{-1}$) and the ATP concentration at 2s, the ATP concentration can be calculated at time τ by extrapolation and equals 10% of the total nucleotide. It follows that $k_{-2}/k_{+2} = 10/90 = 0.11$, $k_{+2} = 144 s^{-1}$ and $k_{-2} = 16 s^{-1}$.

As a control that the rapid cleavage step occurred almost concomitant with the fluorescent change, the ATP concentration was measured after 37ms and found to be 51% of the total nucleotide, so that about 50% of the myosin should be in its high-fluorescence form at 37ms, which is consistent with the result shown by the amplitude of rapid phase of Fig. 1.

Measurement of k_{-1}

Eqn. (2) represents the kinetics of the dissociation of radioactive nucleotide from myosin after mixing the protein with unlabelled ATP. In the scheme, M*ATP+M*ADP·P_i decays exponentially to ATP,

$$M+ATP \xleftarrow[k_{-1}]{} M^*ATP \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} M^*ADP\cdot P_i \xrightarrow{k_{+3}} \xrightarrow{k_{+4},\ k_{+5}} M+ADP+P_i \qquad (2)$$

ADP and P_i with a rate constant of $k_{+2}k_{+3}/(k_{+2}+k_{-2})+k_{-1}k_{-2}/(k_{+2}+k_{-2})$ and the product ratio of ATP to ADP or P_i is $k_{-1}k_{-2}/k_{+2}k_{+3}$.

Comparison of the results in Tables 1 and 2 shows that after mixing M*ATP with unlabelled ATP at 2s the product distribution at both 5s and 60s is the same as if no ATP had been added. This shows that $k_{-1} = 0$ because the product ratio of ATP to P_i is very small and the rate of decay of ATP is the same as in Table 1 and therefore equals $k_{+2}k_{+3}/(k_{+2}+k_{-2})$. In practice we conclude that $k_{-1} < 0.02 s^{-1}$ since this is the limit set by the experimental sensitivity.

Table 2 also shows in a control experiment that if ATP is mixed with protein before addition of radioactive ATP, then no radioactive ATP can bind. The fact that 96% rather than 99% of ATP remains (the expected efficiency of blocking the myosin site to [γ-^{32}P]ATP) is probably within the limits of experimental error since the same result was obtained if the concentration of unlabelled ATP was increased tenfold.

Identical results yielding $k_{-2} = 16 s^{-1}$ and $k_{-1} < 0.02 s^{-1}$ were found with heavy meromysin prepared by a tryptic digestion and purified on DEAE-cellulose.

These experiments were repeated about 20 times with a variety of modifications and controls and also working in 0.5 M-KCl and by using myosin. In particular we have looked for possible artifacts. The most likely cause of an artifact would be due to there being a heterogeneous population of protein molecules, as might arise for example because of thiol oxidation. In control experiments no significant difference in rate constants was found in myosin solutions prepared at the same time under identical conditions except for the presence of protecting EDTA and thiol reagent. Changing the quenching reagent to KOH and neutralizing with acetic acid to pH 3.4 for product chromatography gave the same values of k_{-2} and k_{-1}. However, the ratio of M*ATP to M*ADP·P_i rose significantly to 0.2 in a number of cases and this was generally paralleled by an increase in the apparent value of k_{-1} up to $0.05 s^{-1}$. These anomalous results were associated with aged or less-purified protein solutions. In no case did the ratio of M*ATP to M*ADP·P_i drop below 0.09 and this ratio was consistently reproduced by the purest protein samples.

In further control experiments k_{-2}/k_{+2} was measured and found to be 0.09 in each case when the subfragment concentration was 37 or 12 μM and the ATP concentration was 0.07 μM. This shows, as was indicated above, that the protein concentration was well above the K_m value for ATP and so all the nucleotide is bound at time τ. The ratio k_{-2}/k_{+2} was also unaltered if unlabelled nucleotide was omitted from the quenching solution, eliminating artifacts arising from binding of nucleotide to denatured protein.

Discussion

Although a significant value for k_{-2} can account for our results, other possibilities that would indicate

Table 2. *Effect of 'chasing' labelled M*ATP and M*ADP·P_i intermediates with non-radioactive ATP*

The conditions were as reported in Table 1. 'Chasing' involved the addition of unlabelled ATP to a concentration of 1.3mM.

Quenching time (s)	'Chasing' time (s)	% labelled ATP present
5.0	2.0	4.5
60	2.0	0.3
60	0	96

apparent reversibility of the cleavage step should be considered. The presence of a dead-end complex of ATP and myosin is ruled out because in the experiment to measure k_{-1} the radioactive ATP decays at about $0.10s^{-1}$ and yet does not dissociate into the ATP pool (Table 2). The possibility that any ATP is not bound to myosin at 2s after mixing is ruled out by the control experiments described. The myosin preparations used contain hardly any slow muscle as characterized by the light chains (A. G. Weeds, personal communication) and the association rate constants of ATP to fast and slow cat muscle myosin only differ by a factor of 3 (D. R. Trentham, unpublished results). A third possibility that the protein exists as a heterogeneous population containing 10% of a modified form is difficult to rule out absolutely. However, our experiments indicate that such a modified form, if it exists, does not arise from thiol oxidation or metal-ion inactivation. Moreover, the cleavage rate of this hypothetical modified form would equal the turnover rate of normal subfragment 1 which would be fortuitous (Fig. 1 and Table 1). In the experiments with aged or less-purified protein the increase in k_{-2} is generally paralleled by a rise in k_{-1} indicating reversible dead-end complex formation and/or heterogeneous protein populations in these samples.

In any event the results show that in steady-state kinetic studies of the myosin ATPase a significant fraction of the steady-state complex is the binary complex of myosin and ATP. This suggests that at least a fraction of the refractory state described by Eisenberg *et al.* (1972) is this binary complex. It also may account in part for the 0.8:1 rather than 1:1 stoicheiometry between the transient phosphate and heavy meromyosin subfragment 1 site concentrations in the transient kinetic studies of ATP cleavage of Lymn & Taylor (1970) (Bagshaw *et al.*, 1972).

The reversible cleavage step indicated by our results is particularly interesting when taken in conjunction with the work of Sartorelli *et al.* (1966). They showed that after mixing myosin with excess of ATP at 0°C and quenching after 30s into $HClO_4$ containing $H_2{}^{18}O$, there was no incorporation of ^{18}O into P_i. The major species leading to P_i production at the time of quenching would be $M^*ADP \cdot P_i$ according to eqn. (1) indicating the intermediate is hydrolysed ATP or an adduct of ATP and water such as might exist with a pentacoordinate γ-P atom and not some form of anhydride.

They further showed that if the experiment was repeated except that $H_2{}^{18}O$ was in the original myosin solution but not in the quenching solution, then approximately three water oxygen atoms were introduced into the P_i. This has two implications. First, the step in which ^{18}O is introduced into ATP must be rapidly reversible relative to the rate at which P_i is released into the medium, which is controlled by the step $M^*ADP \cdot P_i \rightarrow MADP \cdot P_i$ which is rate limiting (Trentham *et al.*, 1972) and secondly, there must be a rearrangement of the moiety which contains the incorporated ^{18}O on the protein otherwise the same ^{18}O would be abstracted in the reversal of the ^{18}O-incorporation step by the principle of microscopic reversibility. The rapid reversibility condition is satisfied by the rate constants reported here for k_{+2} and k_{-2} and the species undergoing the rearrangement can be identified as $M^*ADP \cdot P_i$. (The rearrangement should not be confused with the transition to $MADP \cdot P_i$ controlled by k_{+3}.)

If the proposed rearrangement of the protein-bound moiety is also rapid compared with the cleavage step, then one might expect ^{18}O exchange on all four oxygen atoms of P_i both in the experiments of Sartorelli *et al.* (1966) and in other experiments in which a large concentration of ATP was hydrolysed (Levy & Koshland, 1959). In practice the measured ^{18}O incorporation is more nearly equal to three oxygen atoms per phosphorus atom in most cases for which this reaction has been analysed. If this value of three has mechanistic significance it implies that one oxygen atom of the protein-bound moiety is restrained from exchange. A simple postulate consistent with this restriction is that $M^*ADP \cdot P_i$ does represent hydrolysed ATP and that the phosphate moiety is bound to the protein through an oxygen atom leaving the other three oxygen atoms free to rotate round the fixed P–O bond. However, the value for ^{18}O incorporation has been reported to be as high as four oxygen atoms incorporated per phosphorus atom (Yount & Koshland, 1963; Swanson & Yount, 1966) so that an unequivocal determination of the extent of oxygen exchange is required before any models on the structural nature of $M^*ADP \cdot P_i$ can be substantiated.

It is interesting that when Levy & Koshland (1959) investigated ^{18}O exchange in P_i produced from actomyosin-catalysed ATP hydrolysis, the total water oxygen incorporation dropped to about two oxygen atoms per ATP hydrolysed. Actomyosin ATPase has a turnover rate ($\sim 10s^{-1}$) (Lowey *et al.*, 1969) comparable with k_{-2} so that the decreased oxygen exchange is to be expected provided actin does not greatly modify the kinetics of the cleavage step as has been indicated by the results of Lymn & Taylor (1971), whose scheme (eqns. 3, 4 and 5) is described below. Although Levy & Koshland's (1959) results did indicate that actin influences the rate of formation of the intermediate capable of ^{18}O exchange if four oxygen atoms are exchangeable, their results were consistent with no influence of actin on the cleavage step if only three oxygen atoms are exchangeable (or if their measured value of three was low). A kinetic analysis of the influence of actin on the ^{18}O-exchange reaction is given in their paper.

In further experiments in which myosin was mixed with ATP in the presence of $H_2{}^{18}O$ no ^{18}O was found in the ATP (Sartorelli *et al.*, 1966). This is expected in view of the small value found for k_{-1}. (The concentration of M*ATP that would have been expected to contain ^{18}O was less than 0.1% of the ATP concentration at the time of quenching.) The small value of k_{-1} is consistent with our previous studies which showed that $k_{-1} < k_2 + k_{-2}$ for ATP and $k_{-1} < 0.2 s^{-1}$ for ATP analogues modified in the triphosphate moiety (Bagshaw *et al.*, 1972).

It is interesting to relate these experimental results to the molecular model of muscular contraction outlined by Lymn & Taylor (1971) from the viewpoint of converting chemical free energy into mechanical work. Lymn & Taylor's (1971) scheme for actomyosin ATPase is summarized by eqns. (3), (4) and (5), where A represents actin and AM actomyosin:

$$AM + ATP \rightleftharpoons A + M^*ATP \quad (3)$$

$$M^*ATP \rightleftharpoons M^*ADP \cdot P_i \quad (4)$$

$$A + M^*ADP \cdot P_i \rightleftharpoons AM + ADP + P_i \quad (5)$$

Their results indicated that the kinetics of ATP cleavage (eqn. 4) were similar whether or not actin was present, so for the purpose of this discussion they will be treated as common steps in both the myosin and actomyosin ATPases. In their model mechanical work is obtained from the processes involving actomyosin and not therefore from the cleavage step (eqn. 4). The free energy, ΔG_c, liberated in the cleavage step either as heat or through an increase of entropy will not be available for conversion into mechanical work (temperature in muscle being effectively constant). It follows that for efficient conversion of chemical free energy into mechanical work the negative free-energy change of the cleavage step should be small relative to the total negative free-energy change derived from the chemical reactions. The standard free-energy change, ΔG_c^0, of the cleavage step equals $-5.5 kJ \cdot mol^{-1}$ calculated from the equilibrium constant ($=k_{+2}/k_{-2} = 9$) and

$$\Delta G_c = \Delta G_c^0 + RT \ln \frac{[M^*ADP \cdot P_i]}{[M^*ATP]}$$

The total free-energy change, ΔG_t, derived from chemical reactions during muscle contraction is difficult to evaluate precisely, but in muscle that has been treated with fluorodinitrobenzene ΔG_t is essentially the free-energy change derived from ATP hydrolysis and equals $-45 kJ \cdot mol^{-1}$ (Kushmerick, 1969). Present knowledge suggests that the steady-state concentration of M*ATP is larger than that of $M^*ADP \cdot P_i$ during actomyosin ATPase activity (Lymn & Taylor, 1971), so the term $RT \ln [M^*ADP \cdot P_i]/[M^*ATP]$ will be negative. The maximum efficiency (for negative values of ΔG_c) that can be derived from the chemical reactions is given by:

$$\frac{\Delta G_t - \Delta G_c}{\Delta G_t} = 1 - \frac{\Delta G_c^0 + RT \ln \frac{[M^*ADP \cdot P_i]}{[M^*ATP]}}{\Delta G_t}$$

and the advantage in ΔG_c^0 being much less negative than ΔG_t is apparent. This argument has a number of simplifications; in particular (1) the ratio of the concentrations of both free ADP and $M^*ADP \cdot P_i$ relative to free ATP and M*ATP respectively can be expected to increase when mechanical work is done which would make both ΔG_t and ΔG_c less negative (Wilkie, 1970; Taylor, 1973), (2) it is assumed that there is no interaction between neighbouring crossbridges and (3) the value of ΔG_c^0 for the situation *in vivo* has yet to be evaluated.

However, if the statement of the Lymn–Taylor (1971) model that actin is not associated with ATP cleavage is correct, it is desirable for there to be a relatively small negative standard free-energy change in this cleavage step. The fact that our results suggest this is so strengthens the case for this molecular model.

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