THE APPARENT RATES OF CROSSBRIDGE ATTACHMENT AND DETACHMENT ESTIMATED FROM ATPase ACTIVITY IN INSECT FLIGHT MUSCLE

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ABSTRACT The ATPase activity of single fibers of small fiber bundles (one to three fibers) of insect flight muscle was measured when fibers were repetitively released and restretched by 1.5% of their initial length. The ATPase activity increased with increasing duration of release-restretch pulses applied at a constant repetition frequency, reaching a maximum at a duration of ~20 ms. For a given duration, the average ATPase activity also increased with increasing frequency of applied length changes and reached a maximum (200% of the isometric ATPase) at a frequency of ~50 Hz. The data could be fitted to a two-state model in which the apparent rate of crossbridge detachment is enhanced when the crossbridges are mechanically released. Estimates of the apparent rates of attachment and detachment in the isometrically contracting state and of the enhanced detachment rate of unloaded crossbridges were derived from fits to the two-state model. After short pulses of releasing and restretching the fiber the force was low and increased after the restretch in a roughly exponential manner to the initial level. The rate at which force increased after a release-restretch pulse was similar to the sum of the apparent attachment and detachment rates for the isometrically contracting muscle derived from the ATPase activity measurements.

INTRODUCTION

It is now generally accepted that force production in muscle results from the formation of mechanically strained, elastic crossbridges that are formed between the actin and the myosin filaments. The cyclic making and breaking of these crossbridges causes the actin and myosin filaments to slide past each other, enabling the muscle to shorten against an external load (H. E. Huxley, 1969). The first mechanical contraction model for muscle based on these considerations was proposed by A. F. Huxley in 1957 (Huxley, 1957) and distinguishes between an attached, force-generating state and a detached non–force-generating state of the crossbridge.

In subsequent years a considerable number of different biochemical states have been identified which are thought to correspond to specific mechanically attached or detached states (for review see Taylor, 1979 and Eisenberg and Greene, 1980). It also became clear from further mechanical measurements that a more complex multiple-state model was required to account for the rapid tension responses of muscle to sudden changes of load or length (see A. F. Huxley, 1974 and Eisenberg and Hill, 1985). However, despite the shortcomings of the original two-state model in describing all aspects of the transient response, it is nevertheless able to describe elegantly some of the fundamental features of the contracting muscle such as the force-velocity relation and the enhanced energy consumption of the shortening muscle.

It is the detachment rate of released crossbridges in A.F. Huxley's model which specifically affects the force-velocity relationship and the energy consumption during shortening. Clearly either detachment rate or attachment rate, or both, must increase to account for the activated ATPase producing the "extra heat of shortening," and Huxley's choice of detachment rate was later shown to be appropriate since stiffness (thought to represent the number of crossbridges attached; A. F. Huxley and Simmons, 1971) is reduced during shortening (Julian and Sollins, 1975; Tsuchiya et al., 1982) Thus the detachment rate must increase in relation to the attachment rate.

It has been shown in insect flight muscle that the "apparent rate" of crossbridge detachment is enhanced when the crossbridges are mechanically released as postulated by the Huxley model (A. F. Huxley, 1957; Ford et al., 1985). The evidence comes from experiments in which the rate of stiffness decay was measured after varying amplitudes of rapid release of stretch-activated fibers; the more the crossbridge elasticities are discharged, the faster the decay in stiffness (Güth et al., 1981).

Since this preparation so clearly demonstrates one of the central postulates of Huxley's model, we went on to measure the influence of rapidly discharging the cross-bridges (i.e., rapid length releases) on the ATPase activity. In such experiments it is technically impossible to measure the ATP splitting after a single release, thus the fibers were repetitively released and restretched with varying pulse durations and frequencies and the change in the average ATPase activity measured.

Interpreted on the basis of a two-state model these data yield estimates of the apparent rates of attachment and detachment in isometric contraction, and of the enhanced detachment rate of released crossbridges.

METHODS

Preparation

The dorsal longitudinal muscle of the tropical waterburg *Lethocerus indicus* was extracted in a 50% glycerol solution at pH 7.0 (Jewell and Rüegg, 1966) while still attached to the thorax. After extracting for 24 h, the muscle was stored at −20°C in 50% glycerol solution containing a 2 mM EGTA at pH 7. From this preparation single fibers or small-fiber bundles could be easily prepared.

Mechanical Measurements

One to three fibers were mounted between a semiconductor type force transducer (type AE 801, Aksjeselskapet Mikro-Elektronikk, Norway) and a feedback controlled lengthstep generator. For detailed information see Güth and Wojciechowski (1986). At the start of an experiment fibers were released to slack length, zero force, and than stretched by 2%. Length steps of 150 μm could be applied within 0.8 ms.

ATPase Activity Determinations

The ATPase activity and the mechanical performance of the fiber could be measured simultaneously. The fibers were incubated in a narrow chamber which could be perfused by different incubation solutions. The ATPase activity was determined using a NADH coupled optical assay method. In the assay the ADP produced by the actomyosin ATPase is rephosphorylated by PEP (phosphoenol pyruvate) in the presence of pyruvate kinase. In an additional enzymatic reaction the pyruvate produced is reduced to lactate in the presence of lactate dehydrogenase and in the latter reaction NADH is reduced to NAD. NADH fluoresces at 470 nm (excitation at 340 nm) whereas NAD does not. The fluorescence intensity was observed with a Zeiss microscope photometer. When the perfusion of the chamber was stopped the NADH in the chamber decreased in proportion to the production of ADP, i.e., in proportion to the ATPase activity of the fiber bundle. Starting the perfusion again renews the incubation solution, and a new ATPase activity determination can be performed. An original chart record of the NADH fluorescence (*top trace*) and the tension, measured simultaneously, (*middle trace*) is shown in Fig. 1. For details of the ATPase activity measurements see Güth and Wojciechowski (1986).

Solutions

After the fibers were mounted they were washed for ~5 min in relaxing solution: for the experiments reported in Figs. 1, 3, and 4 the relaxing solution contained 40 mM imidazole, 15 mM ATP, 5 mM PEP, 4 mM EGTA, 17 mM $MgCl_2$, 10 mM NaN_3 at pH6.7. For the experiments reported in Figs. 2 and 5 the solutions contained 20 mM imidazole, 8.5 mM ATP, 15 mM PEP, 5 mM EGTA, 9.33 mM $MgCl_2$ at pH 6.7. The ionic strength was adjusted by KCl to 150 mM. Then the fibre bundle was immersed into contraction solution, which contained the same ingredients as the relaxing solution but additionally the same amount of $CaCl_2$ as EGTA. All solutions contained pyruvate kinase (150 U/ml for experiments reported in Figs. 1, 3, and 4 and 100 U/ml for the experiments reported in the Figs. 2 and 5), 140 U/ml lactate dehydrogenase, 0.2 mM P1, P5di(adenosin-5'-) pentaphosphate (as a myokinase inhibitor) and NADH (1.2 mM for the experiments reported in Figs. 1, 3, and 4; 0.6 for the experiments reported in Figs. 2 and 5). The different NADH concentrations are correlated with a change in the size of the chamber in use. Details of the control experiments designed to test the efficacy of the enzymatic system are given in Güth and Wojciechowski (1986).

RESULTS

Mechanical Response to Release-Restretch Pulses

It has been shown that releases of ~1.5% are sufficient to discharge the tension held by attached crossbridges in isometrically contracting muscle (Kuhn et al., 1979). We also reported (Güth et al., 1981) that in insect flight muscle those fully released crossbridges detach very rapidly; i.e., when fibers are released by 1.5% of the initial length the detachment rate constant, measured from the rate of stiffness decay, is 320 s^{-1} at 10°C. The experiments presently reported were performed at 20°C and thus the elevated detachment rate is likely to be even higher but, as yet, we have no information on its temperature sensitivity. Fig. 2 shows the form of the tension transient produced by a 1.5% release and subsequent restretch to the initial length. There is a large drop in the tension simultaneous with the release which does not recover as long as the muscle stays released. The comparatively slow drop in tension seen after the release is small and its origin is unclear but we believe, for reasons given below, that most of the crossbridges have detached by this time. When the muscle is restretched the force increases (after a viscoelastic peak) with a roughly exponential time course to the

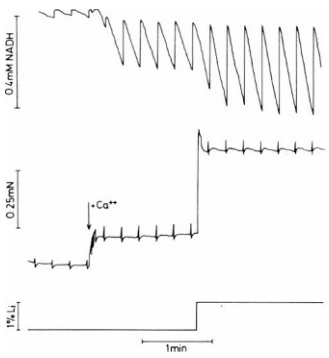

FIGURE 1 Experimental protocol of the ATPase measurement. The upper trace shows the signal of the NADH fluorescence, the lower trace the simultaneously measured isometric force. Within the short time interval during which the incubation solution is renewed the measured NADH concentration, i.e., the fluorescence intensity, increases rapidly. After the perfusion is stopped the fluorescence intensity decreases again because of the decreasing NADH concentration. The slope of the fluorescence intensity decrease after the perfusion has stopped is proportional to the ATPase activity of the investigated muscle fiber within the chamber. The arrow marks the time when the fiber bundle (three fibers) was activated by adding Ca. (pCa 4.3) to the incubation solution. Note that the slope of the NADH intensity signal, i.e., the ATPase activity, increases when the Ca concentration is increased, but the stretch (*lowest panel*) performed after the fiber bundle is CA-activated further increases the ATPase activity and the generated force (*middle trace*) even more.

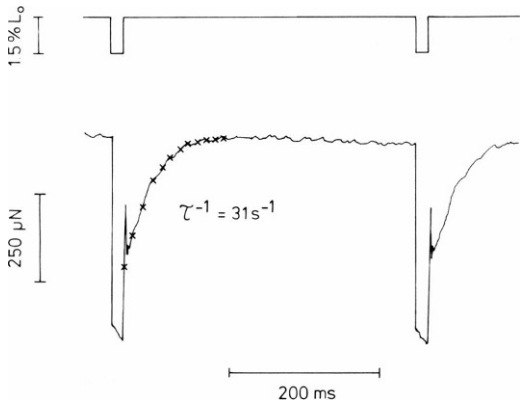

FIGURE 2 Force transient of repetitively released and restretched insect flight muscle fibers (two fibers). The upper trace shows the muscle length, the lower trace the corresponding force transient. The duration of the release-restretch pulse is 15 ms. The crosses correspond to the best exponential fit (rate constant given alongside curve). The amplitude of the length change was 1.5% of the initial length. Temperature, 22°C.

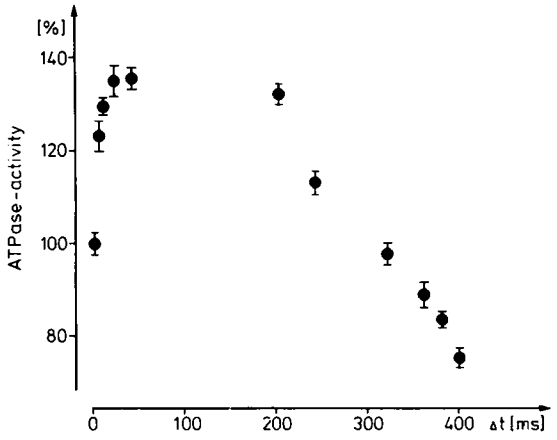

FIGURE 4 ATPase activity plotted versus the duration of release in the release-restretch cycle (abscissa). The amplitude of the length changes was 1.5% of the initial length. The repetition frequency was 2.5 s^{-1}. The error bars refer to ±SD of the mean over six subsequent ATPase determinations from the same preparation. Temperature, 22°C.

level before the release (within the so-called delayed tension rise). The best fit to an exponential is shown in the figure (*crosses*) together with its rate constant. Averaged over 32 fits to data from six different fibers, a rate of 28.6 ± 2 s^{-1} (SE) was obtained.

Dependence of ATPase Activity on the Duration of the Release-Restretch Pulse

In contrast to the rapid detachment of mechanically unloaded crossbridges, in the isometrically contracting state, i.e., when the crossbridges are under mechanical stress, the "apparent rate" of crossbridge detachment may be the rate-limiting step in the cycle. If so, then one would predict that the ATPase activity would increased with an increase in the detachment rate. By releasing the muscle fiber the crossbridges that are attached at the moment of the release will be mechanically discharged and their detachment rate therefore greatly accelerated. Consequently, the average cycle time of this population of crossbridges will be shortened, thus causing an increased ATPase activity.

If, however, the time during which the muscle is released is too short to allow all mechanically discharged crossbridges to dissociate from the actin filament, those remaining crossbridges will be restrained again when the muscle fiber is restretched. For the restrained crossbridges detachment is no longer accelerated and, consequently, the turnover time for them will be the same as for undisturbed crossbridges in the isometrically contracting state. Thus each release-restretch cycle produces a shorter turnover time for only those crossbridges that are actually dissociated after the release. Therefore, one would expect that

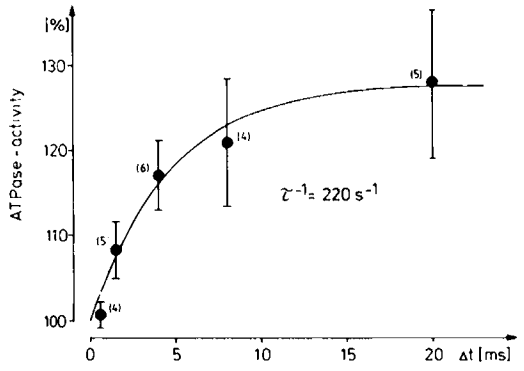

FIGURE 3 ATPase activity versus duration of release in the release-restretch cycle. The amplitude of the length changes was 1.5% of the initial length. The repetition frequency of the length changes was 2.5 s^{-1}. The ATPase activity is normalized to 100% in the isometrically contracting state after the ATPase activity of the relaxed muscle (pCa 8) was subtracted. The error bars correspond to ±SE. The data were obtained from nine different fibers. The numbers alongside each data point refer to the number of measurements over which it was averaged. Temperature, 22°C.

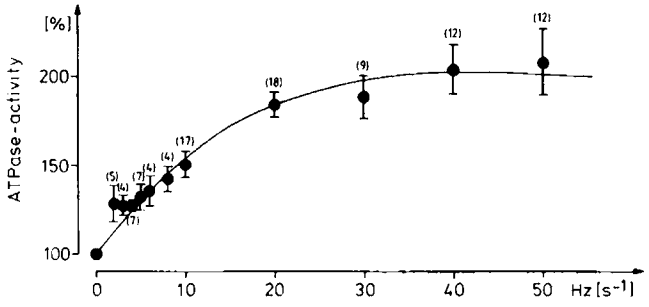

FIGURE 5 ATPase activity versus repetition frequency of release-restretch pulses. The amplitude of the length changes was 1.5% of the initial length. The ATPase activity is normalized to 100% in the isometrically contracting state after the ATPase activity of the relaxed muscle (pCa 8) was subtracted. The data were obtained from 18 fiber bundles (three fibers). The numbers alongside each data point refer to the number of ATPase determinations at the particular frequency. The error bars correspond to ±SE. The solid line is a fit to the data from Eq. 5. Temperature, 22°C.

the average ATPase activity of the fiber, which is assumed to be proportional to the average cycle frequency of the crossbridges, would increase with increasing duration of each release pulse in the train of pulses applied to the muscle fiber. Moreover, an exponential fit to the increasing ATPase activity should provide the rate constant of the accelerated detachment of the mechanically released crossbridges. In Fig. 3 the duration of the imposed release is plotted versus the average ATPase activity (measured ATPase activity = average ATPase activity). The ATPase activity was normalized with respect to its value in the isometrically contracting state, i.e., without applying length change pulses to the fiber. The frequency at which the pulses were applied was 2.5 Hz. It can be seen from Fig. 2 that this frequency was low enough to allow time for the steady state force to redevelop between releases. Fig. 3 shows that, as predicted, the ATPase activity indeed increased with increasing duration of the release pulses.

The solid line in Fig. 3 represents the best exponential fit to the data and has a rate constant of 220 s^{-1}. This value is rather lower than expected from previous estimates of the accelerated detachment rate (see above) but, nevertheless, the experiment successfully demonstrates a dependence of ATPase activity on the release duration and shows that at 20 ms the effect saturates.

Insect flight muscle has the particular feature of having an activated tension and ATPase activity when it is stretched. The applied stretch required to maximally activate the ATPase is only two or three times larger than the amplitude of the releases administered to the muscle in the above experiments (Rüegg and Stumpf, 1969). It is therefore important to check whether the extra ATPase activity which is obtained when short release-restretch pulses are administered to the stretch-activated fiber is due to the release performed in the release-restretch cycle or to the stretch. If it was the stretch rather than the release which caused the effect, one might expect that stretches of short duration would have the same effect. Fig. 4 shows an experiment in which we extended the duration of the releases, applied at a constant frequency of 2.5 Hz (repetition period 400 ms), so that short stretches were created in the same experiment. The duration of the release is plotted on the abscissa and the corresponding ATPase activity on the ordinate. The ATPase activity was normalized with respect to the value in the stretched state in the absence of length changes. (The ATPase activity of the relaxed muscle [pCa 8] was subtracted before the normalization.) As before, the amplitude of the releases and the restretches was 1.5% of the initial length. The ATPase activity at "zero duration" of release corresponds to a sustained stretched state of the fiber (left side of the diagram). At this point the ATPase activity is higher than it is at 400ms duration, at which point release and repetition periods are equivalent and the muscle is therefore continuously "released" (75% ATPase activity, Fig. 4).

As already shown in Fig. 3, releases of short duration (<20 ms) increase the ATPase activity maximally. However, further increasing the duration of the release phase causes the ATPase activity to decrease again (see also Bruell et al., 1973).

Dependence of the ATPase Activity on the Frequency of Release-Restretch Pulses

All the length changes reported so far were applied at the same repetition frequency (2.5 Hz) while varying the duration of the released state compared with that of the stretched state of the muscle. In contrast, in the following experiments the duration of the release was held constant and the repetition frequency was varied. The ATPase activity of the muscle obtained at different repetition frequences of the release-restretch pulses is shown in Fig. 5. The ATPase activity is normalized to 100% in the stretched state (after the ATPase activity of the relaxed muscle [pCa 8] was subtracted). The figure shows that increasing the repetition frequency above 2.5 Hz considerably enhances the ATPase activity which reaches a plateau at a frequency of 50 Hz. The latter finding is different from the finding of Steiger and Rüegg (1969), who oscillated insect flight muscle fibers sinusoidally and found a maximum extra ATPase activity at the frequency of the maximum mechanical power output. The difference might be due to the different wave forms of the length changes; if the length change is sinusoidal then crossbridges will attach at all lengths intermediate between the maximum and the minimum. Consequently, at any one time, the attached population of crossbridges will have a wide distribution of strains. In contrast, the application of short-duration releases forces the bridges to detach rapidly but the released state does not last long enough to allow a significant number of crossbridges to attach (see also Discussion). Therefore the average strain of the crossbridges is probably distinctly different on application of short release-restretch pulses from that on application of sinusoidal length changes.

DISCUSSION

The ascending limb of the ATPase activity versus release duration curve of Fig. 4 corresponds to the increase in ATPase with pulse duration shown in Fig. 3 and described in the Results section. The descending limb of the curve, where ATPase activity falls with further increases in pulse duration, may be described in the following way. After each release-restretch pulse crossbridges are formed with a certain net reattachment rate (actually equal to the sum $f + g$ in the two-state model as described by Thorson and White, 1969). If the restretch is followed too quickly by the next release (as in Fig. 4 at the longer pulse durations) so that the duration of the stretch is short compared with the time constant of net crossbridge formation, then the number formed will be restricted. Since only those bridges attached at the time of the next release will detach at the elevated rate and contribute to the enhancement of the

ATPase rate, then the extra ATPase activity measured on repetitively releasing and stretching the fibers will be expected to fall as the stretch duration shortens.

The above mechanism does not predict that the ATPase activity should fall below the initial value at longer release durations (*far right* of Fig. 4, cf. *far left*). However, this finding is expected since it is known that muscle length plays an important role in the activation of the ATPase activity in this preparation; stretch causing an activation of activity and release a depression (Rüegg and Stumpf, 1969).

The arguments used to account for the results of Fig. 4 can also be used to describe the form of the dependence of the ATPase activity upon the frequency of applied release-restretch pulses. As it is the average ATPase being measured, then, of course, this would be expected to rise in proportion to the pulse frequency, at least in the low frequency range. However, if the time interval between releases becomes comparable to the time constant of net crossbridge reattachment after the restretch $[1/(f+g)]$ then only a fraction of the bridges dissociated after the release will have time to reattach. Thus, fewer bridges are discharged by the next release and hence the ATPase activity is no longer proportional to the frequency.

In the remaining part of the discussion we use the two-state model shown in Fig. 6 to derive a formula to describe the ATPase activity as a function of the frequency at which release-restretch pulses are applied. In using the model to derive a fit to the data of Fig. 5 and, from that, the value of f and g, we require a value of the accelerated rate constant of detachment, G, and we make the assumption that this rate is much higher than the apparent rate constant of attachment, f. Thus during the 5-ms releases applied in the experiment of Fig. 5, crossbridges detach very rapidly and reattachment is negligible.

Let $D(t)$ represent the number of detached crossbridges at a given time "t." Making the above assumption, the number of detached bridges after the release and before the restretch is given by:

$$D(t) = N_0\,[1 - (1 - D_3)\exp(-G_t)];\ 0 \le t \le d_r, \quad (1)$$

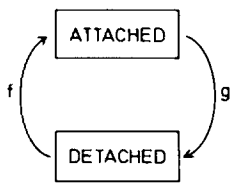

FIGURE 6 Illustrates the two states of the model, a detached non–force-generating state and an attached force-generating state. Of course this is a highly simplified scheme and each state represents a group of crossbridge states which occur in the cycle of ATP hydrolysis. Thus the so-called detached state may include a number of states of myosin which are weakly attached to actin but non–force generating. The apparent rate constant of exchange between these two groups of states under isometric conditions are represented by f and g, and, for simplicity, are referred to as the apparent rate constants of attachment and detachment, respectively. In this scheme the isometric ATPase activity is determined by the average rate of attachment of detached bridges, $d \times f = f \times g\ (f+g)$, where d is the number of detached bridges (White and Thorson, 1972; Brenner, 1986).

where N_0 is the total number of interacting crossbridges, D_3 the fraction of crossbridges detached at the moment of the release, and d_r the duration of the release.

After restretching the fiber the apparent rate of attachment (f) and detachment (g) may be of the same order of magnitude (consequently f cannot be neglected) and the number of detached crossbridges is thus given by:

$$D(\tau) = N_0\left\{\left(D_2 - \frac{g}{f+g}\right)\exp\left[-(f+g)\tau\right] + \frac{g}{f+g}\right\};\ 0 \le \tau \le d_s, \quad (2)$$

where τ_a represents the time after restretch and d_s the duration of the stretch. D_2 is the fraction of detached crossbridges just before the fiber is restretched. The muscle fiber is repetitively released and restretched. Therefore: $D(\tau_a = d_s)/N_0 = D_3 = D(t=0)/N$ and $D(\tau_a = 0)/N = D_2 = D(t = d_r)/N_0$. Therefore, D_2 from Eqs. 1 and 2, is:

$$D_2 = \frac{\exp(Gd_r) - 1 + g\{1 - \exp[-(f+g)d_s]\}/(f+g)}{\exp(Gd_r) - \exp[-(f+g)d_s]}. \quad (3)$$

In order to calculate the average ATPase activity of a complete release-restretch cycle we assume that during the period of time when the muscle is released, no crossbridges may attach. The corresponding underestimation of the average ATPase activity is presumably small since the insect flight muscle is deactivated for at least several milliseconds after the release. (This is apparent in Fig. 1, where no increase in force is observed during the time when the muscle is released.) Furthermore, the duration of the release is 5 ms for all frequencies and thus even for the highest frequency applied (50 Hz) the release duration is considerably smaller than the duration of the stretch. The average ATPase activity (A) is given by the average number of crossbridges attaching per unit time:

$$A = N_0 f \gamma \int_0^{d_s} D(\tau)d\tau = N_0\gamma\left(\left[\frac{D_2 f}{f+g} - \frac{f\cdot g}{(f+g)^2}\right]\cdot\{1 - \exp[-(f+g)d_s]\} + \frac{f\cdot g}{f+g}d_s\right), \quad (4)$$

where $\gamma = 1/(d_r + d_s)$ is the repetition frequency of the release-restretch pulses. In order to eliminate the unknown number of interacting crossbridges (N_0), the ATPase activity may be normalized to unity in the isometric contracting state:

$$\frac{A}{A_0} = \left(\frac{D_2\gamma}{g} - \frac{\gamma}{f+g}\right)\cdot\{1 - \exp[-(f+g)d_s]\} + \gamma d_s, \quad (5)$$

where $A_0 = N_0 \times f \times g/(f+g)$ represents the ATPase activity in the isometrically contracting state (see White and Thorson, 1973).

The data of Fig. 5 can be fitted with this function for a given value of G. There is some ambiguity in the determination of the detachment rate of the mechanically dis-

charged crossbridges (G). It is ~220 s^{-1} if calculated from Fig. 3 and using this value the best fit to the data in Fig. 5 is represented by the solid line. This yields values of 16.4 s^{-1} for f and 7.6 s^{-1} for g. If, however, for whatever reason this value for G is an underestimation and the value obtained from previous measurements of the rate of stiffness decay after releases (320 s^{-1} at 10°C) is more accurate, then a rate between 500 s^{-1} and 1,000 s^{-1} might be measured at 20°C. The best fits to Fig. 5 using these values were also calculated and were almost identical to the curve obtained using G = 220 s^{-1}, and the derived values of f were 22.9 s^{-1} (G = 500 s^{-1}) and 24.4 s^{-1} (G = 1,000 s^{-1}); and of g were 10.6 s^{-1} (G = 500 s^{-1}) and 11.5 s^{-1} (G = 1,000 s^{-1}). It is not possible, on the basis of a two-state model, to give an explanation of the discrepancy between the rate constants of G obtained by the different methods, but it should be noted that the scatter of the data shown in Fig. 3 is high and could be consistent with a faster rate constant.

The time course of the increase in force after the restretch might be interpreted as the time course of the net reattachment of the crossbridges and the rate constant of this process in the two-state model is given by the sum of the apparent attachment rate and the apparent detachment rate ($f + g$) (Thorson and White, 1969; Brenner 1986). Thus the above values of f and g determined from the ATPase data can be compared with the sum of f and g obtained independently from the rate of force redevelopment measured after the restretch. A rate of 31 s^{-1} is calculated from the time constant of the data shown in Fig. 2, and averaging over 32 fits to data obtained from six different fibers gave a value of 28.6 + 2 s^{-1} for $f + g$. These values are therefore in very good agreement with the sum of f and g derived from the fit of Eq. 5 to the data of Fig. 5. Similar rates of force redevelopment have been measured in rabbit psoas fibers by Brenner and Eisenberg (1986) but in their system they assume g to be neglegible and equate the force redevelopment rate with f only. This does not appear to be the case in insect flight muscle where the calculated isometric rates of f and g are of a similar order of magnitude, f being approximately double the value of g. However, in Brenner (1986) f and g have been calculated in rabbit muscle from the rates of force redevelopment after large releases in combination with the isometric ATPase activities measured at different MgATP concentrations. He found that f was threefold larger than g at hifg MgATP, which is in good agreement with the present findings in insect. In Huxley's model of 1957, which was used to fit data obtained from frog muscle, the attachment rate f was also found to be larger than the detachment rate g.

The fit of Eq. 5 to the data shown in Fig. 5 is excellent at higher frequencies but at low frequencies the ATPase activity seems to be significantly higher than predicted by the model. At low frequencies the phosphate concentration within the fiber might be lower than at high frequencies because of the higher ATPase activity at higher frequencies. Since phosphate is known to suppress the force (White and Thorson, 1972) and also (to a lower degree) the ATPase activity in rabbit psoas muscle (Kawai et al., 1986), the lower phosphate concentration at low frequencies may be the reason for the discrepancy between the measured and the predicted values at low frequencies.

Note that at around 50 Hz the fitted curve begins to decline. This tendency was also observed experimentally above 50 Hz (data not shown) and is due to the increasing significance of the 5-ms release duration as the release-restretch cycle period becomes shorter. Thus, as the frequency increases the fibers spend proportionately more time in the deactivated state, where it is assumed that no crossbridge reattachment occurs, and so the additional ATPase activity is depressed.

Recently it was stated that a two-state model with one apparent attachment and one apparent detachment rate is too simple and that crossbridge attachment is reversible. The evidence for this stems from experiments in which skinned muscle fibers are relaxed from the rigor state by light-induced liberation of ATP from "caged ATP" (Hibberd et al., 1985). In caged ATP experiments the effect of inorganic phosphate has been explained by allowing for crossbridge detachment to occur as a "backward" step in the crossbridge reaction cycle. In order to introduce this backward reaction into the two-state model, the apparent rate of crossbridge attachment must be reversible. However, including such a backward detachment step into the model described above does not affect our general conclusions, but the computed values for the reaction rate constants f and g will differ somewhat from those calculated here.

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