Effect of ionic strength on skinned rabbit psoas fibers in the presence of magnesium pyrophosphate

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ABSTRACT The effect of ionic strength on the kinetics of myosin cross-bridges in the presence of the ATP analogue PP_i has been examined. It was found that increasing ionic strength from moderate values ($\mu \sim 100$ mM) to high values ($\mu \sim 200$ mM) has three effects. It causes a big decrease in the half time for the force decay after a small stretch, it causes a significant decrease in the sigmoidicity of the nucleotide analogue concentration dependence of the "apparent rate constant" of force decay after a small stretch, and it causes a big decrease in the range of rate constants necessary to describe the multiexponential force decay. It causes the last of these by causing a much larger increase in the slowest rate constants of the decay than in the fastest rate constants. The results suggest that whereas the behavior of cross-bridges in the presence of ATP is well-described by the simple independent-head equilibrium cross-bridge model of Schoenberg (1985. *Biophys. J.* 48:467–475), cross-bridges in the presence of the ATP analogue PP_i require the more complicated double-headed equilibrium cross-bridge model of Anderson and Schoenberg (1987. *Biophys. J.* 52:1077–1082) to describe their behavior.

INTRODUCTION

Recent experiments using skinned rabbit psoas fibers have shed light on the effect of ionic strength on the strength of binding of myosin cross-bridges to actin in the presence of ATP analogues (Brenner et al., 1986; Pate and Cooke, 1988; Fajer et al., 1988), but little is known about the effect of ionic strength on cross-bridge kinetics under these conditions. The expected effects of ionic strength in a muscle fiber are unclear. The simple single-headed equilibrium cross-bridge model of Schoenberg (1985) predicts that ionic strength should affect the number of cross-bridges attached but should not affect the kinetics of the force response. This type of behavior was seen for the myosin·ATP cross-bridge as ionic strength was varied (Schoenberg, 1988). The doubled-headed equilibrium cross-bridge model of Anderson and Schoenberg (1987) suggests that the cross-bridge with ATP analogue bound, because it presumably is bound to actin with two heads (Pate and Cooke, 1988; Fajer et al., 1988), should exhibit more complicated behavior than the cross-bridge with ATP bound.

To learn about the kinetics of cross-bridges with ATP analogue bound, the present work examines the force decay after a small stretch as both [$MgPP_i$] and ionic strength are varied. It is found that the nucleotide analogue concentration dependence of the rate constant for force decay after a small stretch is rather sigmoidal at low ionic strength, but not at high ionic strength. It is also found that the half-time for force decay decreases more than 30-fold in going from low to high ionic strength. This effect on the half time is due mainly to an effect upon the slowest rate constants in the multiexponential decay. The results are explained well in terms of the model of Anderson and Schoenberg (1987) and Schoenberg (1991).

METHODS

Fiber preparation

The protocols for preparing the skinned rabbit psoas fibers used in these experiments have been described previously in Schoenberg and Eisenberg (1985) and Schoenberg (1988). In brief, the sarcolemma of a single rabbit psoas fiber is made permeable to bathing medium in a manner similar to that described by Eastwood et al. (1979). The fiber is isolated and then mounted in a 3-ml bath at 5°C between a displacement generator and force transducer. It is put into rigor before going into the first experimental solution.

Experimental solutions and procedures

After being put in the appropriate experimental solution, the fiber was allowed to equilibrate for ~15 min in each solution before being stretched 2 nm/half-sarcomere two to four times. Table I gives the composition of the three stock solutions from which the experimental solutions were made. Solutions used in the experiments with varying nucleotide analogue concentration were made from appropriate mixtures of solutions 1 and 2 and those with varying ionic strength were made from appropriate mixtures of solutions 1 and 3. Thus, the solutions with varying nucleotide had a total ionic strength of ~215 mM, an excess Mg of 2 mM, and PP_i concentrations ranging from 0 to 4 mM. The [$MgPP_i$] was calculated as described previously (Anderson

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TABLE 1 **Millimolar concentrations of solution constituents**

Solution	Na_4PP_i	MgAcetate	KCl	EGTA	Imidazole
Solution 1	4	6	175	3	10
Solution 2	0	2	195	3	10
Solution 3	4	6	0	3	10

All solutions were pH 7.0 ± 0.1 at 5°C and contained 0.5 mM DTT.

and Schoenberg, 1987) and ranged from 0 to 3 mM. The solutions in experiments with varying ionic strength all had 4 mM Na_4PP_i and 6 mM MgAcetate, with the total ionic strength ranging from 40 to 215 mM.

Solutions for a given experiment were applied in random order, with one exception. The solution with 4 mM PP_i and 175 mM KCl, which tended to irreversibly decrease the fiber's stiffness, was almost always applied last.

Data processing

The 2 to 4 force recordings obtained from the fiber stretches in a given solution were digitized by a Nicolet model 4094 Digital Processing Oscilloscope (Nicolet Instr. Corp., Madison, WI), transmitted to a SUN workstation (model 3/260; Sun Microsystems, Sun Valley, CA) via an RS-232 interface, averaged together, and then analyzed for the half time of force decay as described previously. To examine whether the experimental data was compatible with Michaelis-Menten theory, an apparent experimental "rate constant for force decay" was defined and calculated as 0.69 divided by the half time of the decay. This was done even though the experimental decay was multiexponential and more properly described by a combination of rate constants (Schoenberg and Eisenberg, 1985).

RESULTS

Rate constant of force decay at high ionic strength as a function of $MgPP_i$

Fig. 1 *A* shows the apparent rate constant for force decay as a function of $MgPP_i$ concentration at ionic strength 215 mM. The error bars, when larger than the symbols, show ±1 S.E.M. For comparison, Fig. 1 *B* shows concentration dependence data previously obtained at an ionic strength of 110 mM by Anderson and Schoenberg (1987).

Previously, Anderson and Schoenberg derived an analytic equation to describe the concentration dependence of the rate constant of force decay, r. That equation was

$$r/r_{max} = \frac{[N]^n}{(k_d + [N])^n}, \quad (1)$$

where $[N]$ is nucleotide or nucleotide analogue concentration, r_{max} is the rate constant at saturating concentration, k_d is the dissociation constant for nucleotide or analogue binding and n is the equivalent of the cooperativity coefficient in the A. V. Hill formulation of cooperativity. The solid lines in both *A* and *B* show the best least squares fit of the analytic equation to the data. The best fit to the 110-mM data, as reported previously, gives an n value of 1.6 ± 0.2. The best fit to the data obtained at 215 mM ionic strength gives an n value significantly less than

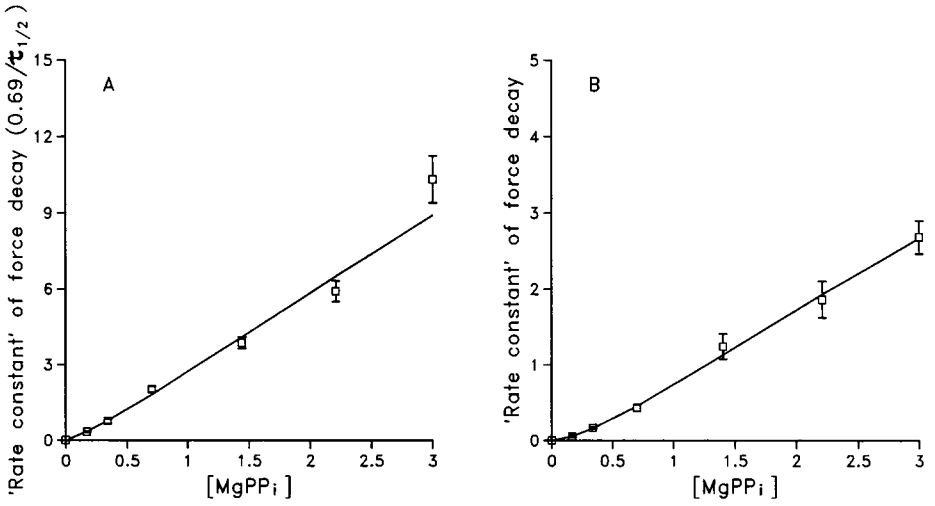

FIGURE 1 Relationship between the 'rate constant' of force decay ($0.69/\tau_{1/2}$) and the concentration of nucleotide analogue ($MgPP_i$) in skinned rabbit psoas fibers at 5°C. *A*, at high ionic strength (μ = 215 mM). *B*, at moderate ionic strength (μ = 110 mM). Solid lines show the best fit of Eq. 1 to the data. In *B*, the fit gave a cooperativity parameter, n, of 1.6 ± 0.2. In *A* it gave 1.2 ± 0.1, reflecting the less sigmoidal nature of the data at higher ionic strength.

that, $n = 1.2 \pm 0.1$. This means, as seen directly from Fig. 1, that the nucleotide analogue concentration dependence relationship of the rate constant of force decay is significantly less sigmoidal at 215 mM than it is at 110 mM. It comes close to being described by the Michaelis-Menten equation ($n = 1$), but is still slightly sigmoidal.

Rate constant of force decay as a function of ionic strength

Fig. 2 shows how the apparent rate constant of force decay after a small stretch in the presence of 3 mM $MgPP_i$ varies with ionic strength. As ionic strength increases from 40 to 215 mM, the decay half time, and thus the apparent "rate constant" of force decay, changes more than 30-fold. In solution, the strength of binding of rabbit skeletal myosin subfragment-1 to actin is tremendously sensitive to ionic strength (Greene et al., 1983) whereas the detachment rate constant is not (Marston, 1982; Konrad and Goody, 1982). Accordingly, the Schoenberg (1985) model of single-headed cross-bridge equilibrium behavior would predict very little change in the rate constant of force decay as a function of ionic strength. In contrast, the model of double-headed cross-bridge equilibrium behavior of Anderson and Schoenberg (1987) and Schoenberg (1991) does predict the experimentally observed big dependence of force decay rate constant on ionic strength.

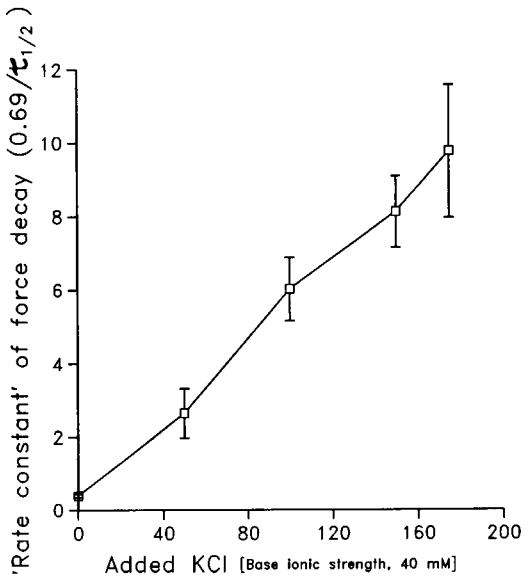

FIGURE 2 Effect of increasing ionic strength on the 'rate constant' of force decay after a 2-nm/half-sarcomere stretch of skinned rabbit psoas fibers in $MgPP_i$ solution at 5°C. By analogy with the monoexponential decay case, the 'rate constant' for decay is calculated as $0.69/\tau_{1/2}$, where $\tau_{1/2}$ is the half time for the decay. The date (±S.E.M.) summarize results from experiments on five fibers. The base solution, to which KCl was added, had an ionic strength of ~40 mM and contained 3 mM EGTA, 6 mM $MgCl_2$, 4 mM Na_2PP_i, and 10 mM imidazole, pH 7.0.

Comparison between the rate constant of force decay at high ionic strength and the detachment rate constant of myosin subfragment-1 in solution

The rate constant of force decay after a small stretch has generally been reported to be significantly less than the corresponding detachment rate constant of myosin subfragment-1 from actin in solution (Clarke and Tregear, 1982; Tozeren and Schoenberg, 1986). At the highest ionic strength used in the current experiments, the apparent rate constant of force decay, 10 s^{-1}, is considerably higher than that measured previously in experiments at lower ionic strength. It is therefore of interest to compare this number to the corresponding detachment rate constant of myosin subfragment-1 in solution.

We can estimate the detachment rate constant of myosin subfragment-1 from actin in solution under the conditions of our experiment as follows. Using unregulated actin and conditions of similar Mg^{2+}, PP_i, and anion as our experiments (1 mM Mg^{2+}, 0.5–2 mM PP_i, 0.2 M KCl), Biosca and Eisenberg (1990) showed a value for the detachment rate constant of myosin subfragment-1 from unregulated actin at 4 mM PP_i of 120 s^{-1}. Because in the presence of 4 mM PP_i (3 mM $MgPP_i$) the binding constant of subfragment-1 to regulated actin is three times stronger than it is to unregulated actin (Greene and Eisenberg, 1980), and because this difference in binding constant is probably attributable almost solely to differences in the detachment rate constant (Geeves and Halsall, 1986), this suggests that the detachment rate constant for myosin subfragment-1 detachment from *regulated* actin (as exists in the fiber) is ~40 s^{-1}. Thus, the rate constant of force decay in the fiber at high ionic strength (10 s^{-1}) differs by less than a factor of 5 from the detachment rate constant of myosin subfragment-1 in solution.

Detailed comparison between the data of Fig. 2 and behavior predicted by the double-headed cross-bridge model of Anderson and Schoenberg

Anderson and Schoenberg (1987) and Schoenberg (1991) presented a model of equilibrium cross-bridge behavior which was meant to describe muscle behavior when strongly-binding cross-bridges bind to actin with two heads. This model should be appropriate for the cross-

bridge with $MgPP_i$ at the nucleotide binding site (Pate and Cooke, 1988). The essence of the model of Anderson and Schoenberg is that so long as at least one head of a cross-bridge remains bound to actin, when the other head detaches from actin and subsequently reattaches, it cannot reattach in a position of lesser strain. With this model, only head detachment of a cross-bridge bound with one head leads to a reduction in the force supported by the cross-bridges. Thus, the rate constant of force decay after a small stretch depends not only upon the cross-bridge head detachment rate constant, but on the relative number of cross-bridges bound by one or two heads. In mammalian skeletal muscle, the rate constant of head detachment should be relatively insensitive to ionic strength (Konrad and Goody, 1982; Marston, 1982) but the relative number of singly and doubly-bound cross-bridges, which depends upon the equilibrium binding constant of the cross-bridge heads (Manuck et al., 1986), should be very sensitive. With increasing ionic strength, there should be a decrease in the head binding constant, an increase in the number of cross-bridges attached by only one head, and a large acceleration in the rate constant of force decay after a small stretch.

If we wish to have a precise comparison of how the data of Fig. 2 agree with the double-headed model of Anderson and Schoenberg (1987), we need to replot the data of Fig. 2 in such a way that it can be compared with the theoretical curves of Fig. 5 in Schoenberg (1991). To do this, we must not only know the corresponding detachment rate constant of myosin subfragment-1 from regulated actin in solution (calculated above), but we also need to estimate the strength of binding of the cross-bridge head to actin in the fiber at each of the ionic strengths studied. Because this is hard to measure directly, it is necessary to rely upon the approach outlined in Brenner et al. (1986) which uses the concept of effective actin concentration and estimates the fiber binding constants from the solution binding constants. The value for the solution binding constant of subfragment-1 to regulated actin at 5°C at ionic strength 35 mM was taken from Brenner et al. (1986). Values for the solution binding constants at the other ionic strengths studied were calculated by using the variation of binding constant with ionic strength reported in Greene et al. (1983). Fig. 3 shows the data of Fig. 2 replotted on top of the theoretical curves from Fig. 5 of Schoenberg, 1991, assuming that the effective actin concentration is 0.3 (○), 1 (△), or 3 (□) mM. The solid and dashed curves give the theoretical variation in the rate constant of force decay versus head binding constant assuming binding of the second head does or does not add to overall cross-bridge stiffness. It is clear that if the binding constant in the fiber is at all similar to the values

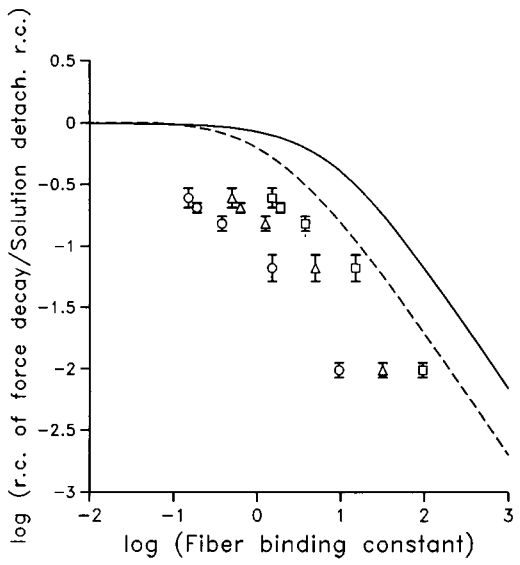

FIGURE 3 Comparison between theory and experiment as ionic strength is varied between 40 and 215 mM in the presence of 3 mM $MgPP_i$. The theoretical curves are from Schoenberg (1991). The symbols (○, △, □) show the data of Fig. 2 replotted assuming the proportionality constant between binding in the fiber and in solution is 0.3, 1 or 3 mM (Brenner et al., 1986; Pate and Cooke, 1988; Fajer et al., 1988). The solution detachment rate constant, as explained in the text, was estimated as 40 s^{-1}. Note that at all ionic strengths, the measured fiber rate constants differ by less than a factor of 5 from those predicted by the double-headed cross-bridge model.

predicted from the reported effective actin concentrations (Brenner et al., 1986; Pate and Cooke, 1988; Fajer et al., 1988), the apparent rate constants of force decay measured in the fiber for the entire range of ionic strengths studied differ by less than a factor of 5 from those predicted by the double-headed cross-bridge model. The 30-fold decrease in the half time for force decay going from low to high ionic strength is precisely the magnitude of effect predicted.

Effect of ionic strength on the time course of the force decay

We have seen that an increase in ionic strength leads to a decrease in the half time of force decay after a stretch in the presence of $MgPP_i$. Although we have so far discussed the data only in terms of the pseudo first order rate constant, $r = 0.69/\tau_{1/2}$, it is known that with ATP analogues the decay of force after stretch is multiexponential. Thus, the decrease in half time for force decay with increasing ionic strength could be due to a equal increase in all of the decay rate constants or only some of them. In the first case the plot of force versus log of time after stretch will simply shift to the left along the

logarithmic axis; in the latter case there will be a distinct change in shape of the force – log time curve. Fig. 4 shows that as ionic strength is changed, there is indeed a dramatic change in the shape of the force – log time plot. Fitting the force decay curves to two exponentials reveals that in going from low to higher ionic strength the faster of the rate constants in the two-rate constant fit increases only ~8 fold, but the slower rate constant increases >80-fold. Although fitting the data to more than two exponentials (Schoenberg and Eisenberg, 1985) would change these exact numbers somewhat, the result is none-the-less clear: the decay curve at higher ionic strength, because of a very large increase in the slower of the rate constants, is described by a narrower range of rate constants. At higher ionic strength, the decay curve is steeper and the decay occurs over fewer decades in time.

DISCUSSION

This paper examines the effect of ionic strength on cross-bridge behavior in the presence of $MgPP_i$. Increasing ionic strength from moderate values ($\mu \sim 100$ mM) to high values ($\mu \sim 200$ mM) has three very clear effects. It causes a significant decrease in the sigmoidicity of the nucleotide analogue concentration dependence of the "apparent rate constant" of force decay after a small stretch, it causes a very big increase in the magnitude of this rate constant (i.e., a big decrease in the half time of the force decay), and finally, it causes a big decrease in the range of rate constants necessary to describe the multiexponential force decay. These results suggest that in the presence of the ATP analogue PP_i, ionic strength affects not only the number of cross-bridges attached, but the kinetics of the force response as well. This result is somewhat different from the case for ATP itself where it has been shown that in a relaxed muscle fiber ionic strength affects the number of myosin·ATP cross-bridges attached, but it does not have a big effect on the kinetics of the force response (Schoenberg, 1988).

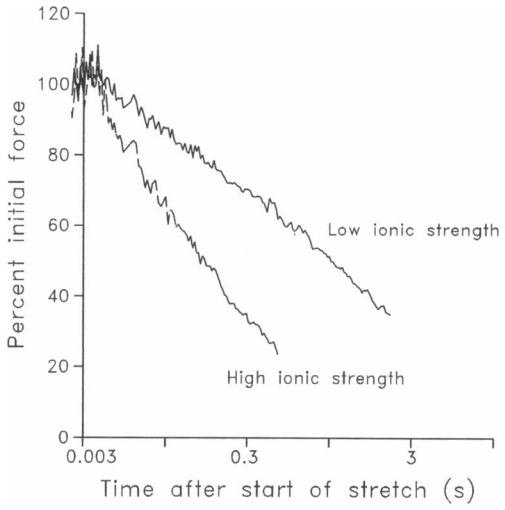

FIGURE 4 Original records of the force decay after stretch in low and high ionic strength 3 mM $MgPP_i$ solutions. Note that not only is the time for the force to decay to 50% of its initial value much longer in lower ionic strength solution, but the overall decay (say from 90 to 10%) covers many more decades in time at lower ionic strength. Fitting the decay curves to two exponentials ($F = A_1 \exp(-a_1 t) + A_2 \exp(-a_2 t)$) using a least squares method based upon Marquardt's compromise (Marquardt, 1963) yields $a_1 = 0.05\ s^{-1}$ and $a_2 = 4.9\ s^{-1}$ at low ionic strength and $a_1 = 4.5\ s^{-1}$ and $a_2 = 40\ s^{-1}$ at high ionic strength. A_1 and A_2 are approximately 60 and 40%, respectively, at both high and low ionic strength.

It is of interest to ask why, with regard to the properties we have been discussing, the cross-bridge with the ATP analogue PP_i behaves differently from the cross-bridge with ATP. ATP and PP_i have many properties in common. They both greatly weaken the affinity of myosin to actin by increasing the rate constant of myosin detachment from actin, they both have the ability to dissociate rigor cross-bridges, and they both reduce the tension of a rigor muscle to zero. On the other hand, some fundamental differences between ATP and PP_i cross-bridges are (*a*) that PP_i weakens the binding of myosin to actin much less than does ATP; (*b*) the PP_i cross-bridge generally binds with both heads simultaneously; and (*c*) the bound PP_i heads have a fixed orientation and presumably are immobile on the microsecond time scale (Pate and Cooke, 1988).

Considering the nature of the differences between ATP and PP_i cross-bridges it is noteworthy that the behavior of the PP_i cross-bridge is precisely as described by the double-headed equilibrium cross-bridge model of Anderson and Schoenberg (1987) and Schoenberg (1991). The agreement between theory and experiment shown in Fig. 3 is within an order of magnitude. This is remarkable considering the complexity of a muscle fiber and the simplifications in the Anderson and Schoenberg model.

The essence of the model of Anderson and Schoenberg is that cross-bridges can bind with two heads simultaneously and binding of one head affects the range of actin sites that the second head can bind to. The latter of these two assertions is the key feature since an earlier double-headed cross-bridge model not having this assumption (Tozeren and Schoenberg, 1986) does not explain the PP_i results.

Given that the PP_i cross-bridge requires the model of Anderson and Schoenberg to adequately describe its behavior, whereas the ATP cross-bridge is well de-

scribed by the simpler, single-headed, Schoenberg (1985) model, it is necessary to consider why this might be so. One possible explanation is that even if the myosin·ATP cross-bridge has both heads bound at very low ionic strength, the bound M·ATP heads may be much more flexible or mobile when attached than the M·PP_i ones. This is suggested by spin-label probe studies which show the attached M·ATP cross-bridge to be highly mobile and disordered whereas the attached M·PP_i or M·AMP-PNP cross-bridges are ordered and immobile (Svensson and Thomas, 1986; Berger et al., 1989; Fajer et al., 1988; Pate and Cooke, 1988). Thus, bound ATP cross-bridge heads may be flexible enough that binding of one head does not severely restrict or influence subsequent binding of the second head while the situation for the PP_i cross-bridge may be just the opposite. With PP_i or other ATP analogues, immobilization of the first head by binding may severely restrict binding opportunities for the second head.

Considering the above, the amount of motion of an attached head may, in fact, be the fundamental difference between so-called weakly-binding cross-bridges, such as the myosin·ATP cross-bridge, and so-called strongly-binding bridges. Under this definition, the PP_i, AMP-PNP, ADP, and rigor cross-bridges would all be classified as strongly-binding. The ATP and paraphenylenedimaleimide- and *N*-phenymaleimide-treated cross-bridges would be classified as weakly-binding. It seems at least plausible that the rapid motion of weakly-binding bridges when attached might also contribute to their fast detachment rate constants.

Another experimental fact that would seem to be compatible with the model of Anderson and Schoenberg (1987) is the finding that the increase in the apparent rate constant of force decay occurs largely because of a big effect on the slowest rate constants in the multiexponential decay. In light of the present work, it seems possible that the fastest rate constants in the force decay after stretch represent detachment of cross-bridges where either only one head is bound, or where the heads are bound to actin sites in a way such that the second head binds very weakly. Similarly, if the slowest rate constants in the multiexponential force decay are caused by cross-bridges having relatively tight binding of the second head, then the main way in which increasing ionic strength would increase the rate of force decay would be by causing acceleration, or disappearance, of these very slow rate constants. This is precisely what analysis of Fig. 4 indicates.

Although the data presented in this paper are satisfying in that they agree very well with the model of Anderson and Schoenberg (1987), it is nonetheless important to critically evaluate the data's validity. One important experimental finding is that the nucleotide analogue concentration dependence of the rate constant of force decay after a stretch has a sigmoidal shape rather than the shape of the Michaelis-Menten equation. In addition, this sigmoidicity is less at higher ionic strength. It is necessary to examine whether this sigmoidicity is a fundamental property of the cross-bridge with $MgPP_i$ at the nucleotide binding site or whether it could have been spuriously introduced in the data presentation.

One serious concern about the presentation of the data is the correct association constant for Mg^{2+} binding to PP_i^{-4}. This was discussed in Anderson and Schoenberg (1987), where it was concluded that one could avoid introducing spurious sigmoidicity into the data by using the largest possible correct value for this association constant. In Anderson and Schoenberg, 1987, and in the current work, the largest reported value for this association constant has been used, a procedure which, if anything, should reduce the sigmoidicity in the data.

Another concern is whether sigmoidicity could have been spuriously introduced into the data by using 0.69 divided by the half-time of the decay as a measure of the "rate constant" of the multiexponential decay. To rule out this possibility, a large number of constructed multiexponential curves where one or more rate constants varied in a Michaelis-Menten way with ligand concentration were examined using the same procedures as used on the real data. In no instance did the apparent rate constant for the decay calculated as 0.69 divided by the half time of the decay have a sigmoidal dependence on ligand concentration. From this it is concluded that calculating an effective rate constant from the half-time of the multiexponential decay will not introduce sigmoidity into the analogue concentration dependence of the force decay rate constants. This leaves us with the conclusion that the sigmoidicity seen in the concentration dependence data is real.

A criticism of the current work is that the previously published moderate ionic strength data that showed a highly sigmoidal concentration dependence curve were obtained with propionate as the major anion. The current high ionic strength data, which exhibit a much less sigmoidal concentration dependence curve, were obtained with chloride as the major anion. Thus, whereas the data are clearly compatible with the model of Anderson and Schoenberg (1987) which predicts a loss of sigmoidicity in the concentration dependence curve with increased ionic strength, they do not rule out the possibility that the loss of sigmoidicity is due not to the increase in ionic strength but to the switch from propionate to chloride.

The reason propionate was not used in the current work was that, because cross-bridges bind somewhat more tightly in the presence of propionate compared to

chloride (Schoenberg, 1988), it would have been necessary to go to rather high concentrations of propionate to cause the desired amount of cross-bridge dissociation. Because high concentrations of anion will dissolve and shorten thick filaments (Ishiwata et al., 1985), the use of high concentrations of propionate was deemed undesirable. However, in light of recent work of Andrews et al. (1989), showing that chloride begins to extract muscle proteins at significantly lower ionic concentrations than does propionate, it is not clear that using chloride in place of propionate in the current experiments was indeed best.

The current experiments show some evidence of filament dissociation, but only at the highest ionic strength studied. When a fiber is incubated in 3 mM $MgPP_i$ solution at ionic strength 40 mM, the stiffness is the same as that of a rigor fiber. Increasing the ionic strength to between 80 and 200 mM results in a rapid (within minutes) and reversible decrease in stiffness. This stiffness change was postulated previously to be due to cross-bridge dissociation without shortening or dissolving of filaments (Brenner et al., 1986) and the present results are consistent with this interpretation. However, when ionic strength is increased from 40 to 215 mM, the situation is slightly different. Here, there is again a rapid stiffness change, but the rapid decrease is followed by a much slower additional decrease. Even more importantly, returning to 40 mM ionic strength solution after incubation for a while at ionic strength 215 mM results in an incomplete increase of stiffness back to the rigor value. This small lack of reversibility at the highest ionic strength studied certainly suggests the possibility of some small amount of filament dissociation at ionic strength 215 mM.

In conclusion, taking all the above into consideration, the data of Anderson and Schoenberg (1987) and the present results clearly suggest that the double-headedness of the myosin cross-bridge is an important factor in determining the kinetics of strongly-binding cross-bridges.

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