Effect of Ethylene Glycol on the Interaction of Different Myosin Subfragment 1·Nucleotide Complexes with Actin

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Received November 17, 1988; Revised Manuscript Received April 12, 1989

ABSTRACT: To elucidate the structure of the cross-bridge intermediates in the actomyosin ATPase cycle, several laboratories have added both ethylene glycol and AMP-PNP to muscle fibers. These studies suggested that ethylene glycol shifts the structure of myosin·AMP-PNP toward the weak-binding conformation, i.e., toward the structure of myosin·ATP. Since only the weak-binding conformation of myosin subfragment 1 (S-1) binds with no apparent cooperativity to the troponin–tropomyosin–actin complex (regulated actin), we used this as a probe to examine the conformation of various S-1·nucleotide complexes in ethylene glycol. Our results show that ethylene glycol markedly weakens the binding strength of S-1, S-1·ADP, and S-1·AMP-PNP to actin but has almost no effect on the binding strength of S-1·ATP. As in muscle fibers, at 40% ethylene glycol, the binding strength of S-1·AMP-PNP to actin becomes very similar to the binding strength of S-1·ATP. In the presence of troponin–tropomyosin, the binding of S-1·AMP-PNP to actin shows no apparent cooperativity in 40% ethylene glycol. Therefore, our results confirm that ethylene glycol shifts the structure of the myosin·AMP-PNP toward the weak-binding conformation. However, our results also suggest that ethylene glycol has a direct effect on the regulated actin complex. This is shown by the fact that ethylene glycol markedly increases the cooperative binding of S-1·ADP to regulated actin both in the presence and in the absence of Ca^{2+}. In addition, in an effect which is fully reversible, ethylene glycol markedly decreases the extent to which regulated actin activates the S-1 ATPase activity in the presence of Ca^{2+}. Therefore, in addition to affecting the conformation of S-1, ethylene glycol also appears to affect the conformation of the troponin–tropomyosin–actin complex, possibly by shifting the regulated actin units to the turned-off form.

During muscle contraction, the myosin cross-bridge is thought to oscillate between two main conformations as ATP is hydrolyzed. In the strong-binding conformation, which occurs in the absence of ATP, the isolated myosin cross-bridge, myosin subfragment 1 (S-1),[1] binds very tightly to actin at about a 45° angle (Reedy et al., 1965; Moore et al., 1970). In the weak-binding conformation, which occurs in the presence of ATP, S-1 binds very weakly to actin at an angle postulated to be about 90° (Eisenberg & Hill, 1985). These two conformations also differ in that troponin–tropomyosin markedly inhibits the binding of S-1 to actin when the S-1 is in the strong-binding conformation but does not significantly affect the binding of S-1 when it is in the weak-binding conformation. Since the weak-binding conformation only occurs transiently during the cross-bridge cycle, many laboratories have looked for ways of stabilizing S-1 in the weak-binding conformation, that is, of making long-lived analogues of this S-1 conformation.

One of the first candidates for an analogue of the weak-binding conformation was S-1 with bound AMP-PNP (Yount et al., 1971), but further study showed that, in fact, S-1·AMP-PNP was not such an analogue. S-1·AMP-PNP binds more strongly to actin than S-1·ATP, and it binds cooperatively to regulated actin (Greene, 1982). In addition, the structure of the cross-bridge in the presence of AMP-PNP is very different from that obtained in the presence of ATP (Reedy et al., 1983, 1987). More recently, other candidates for the weak-binding conformation of S-1 have been developed, including S-1 which has been modified with pPDM (Chalovich et al., 1983; Greene et al., 1986), S-1 with bound ADP and vanadate (Goodno, 1979; Goodno & Taylor, 1982), and S-1 with bound AMP-PNP in the presence of ethylene glycol (Clarke et al., 1980; Marston & Tregear, 1984; Tregear et al., 1984). In the case of the latter analogue, Tregear and co-workers have made several observations suggesting that S-1·AMP-PNP in the presence of ethylene glycol resembles S-1·ATP. First, they found that, in glycerinated muscle fibers, the rigor-like appearance of insect flight muscle in the presence of AMP-PNP becomes relaxed-like in the presence of ethylene glycol and, in addition, the fibers become much less stiff (Clarke et al., 1980; Tregear et al., 1984). Second, they found that, in vitro, ethylene glycol weakens the binding of S-1·AMP-PNP to actin about 100-fold, which makes the affinity of S-1·AMP-PNP for actin in ethylene glycol comparable to the affinity of S-1·ATP for actin in aqueous solution (Marston & Tregear, 1984).

Since the studies of Marston and Tregear (1984) indicate that ethylene glycol causes S-1·AMP-PNP to resemble S-1·ATP in its affinity for actin, we wanted to test whether ethylene glycol also causes S-1·AMP-PNP to resemble S-1·ATP in its binding to regulated actin, that is, whether S-1·AMP-PNP in the presence of ethylene glycol also shows no cooperativity in its binding to regulated actin. We find that, in agreement with the suggestion of Tregear, there is no ap-

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[1] Abbreviations: S-1, subfragment 1 of myosin; acto·S-1, complex of actin with S-1; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Ap_5A, P^1,P^5-di(adenosine-5′) pentaphosphate; AMP-PNP, adenyl-5′-yl imidodiphosphate; pPDM, *N*,*N*′-*p*-phenylenedimaleimide; regulated actin, troponin–tropomyosin–actin complex.

Table I: Effect of Ethylene Glycol on Binding of S-1·Nucleotide to Actin (μ = 30 mM, 25 °C)[a]

nucleotide	binding constants (M^{-1})			extent of weakening	
	aqueous[b]	40% ethylene glycol	50% ethylene glycol	40% ethylene glycol	50% ethylene glycol
none	3×10^8	1×10^7[c]	2×10^6	30[c]	150
ADP	1×10^7	1×10^6	2×10^5	10	50
PP_i	6×10^5	8×10^4	3×10^4	11	20
AMP-PNP	6×10^5	3×10^4	8×10^3	20	75
ATP	6×10^3	7×10^3	6×10^3	1	1

[a] Conditions are given in the legend of Figure 1. [b] The binding constants in aqueous solution are from those given in Chalovich et al. (1983), corrected for ionic strength according to Greene et al. (1983). [c] Due to the strong binding under this condition, the binding constant was measured at 60 mM ionic strength in the presence of 2 mM $MgCl_2$ and then corrected for ionic strength according to Greene et al. (1983).

parent cooperativity in the binding of S-1·AMP-PNP to regulated actin in 40% ethylene glycol either in the presence or in the absence of Ca^{2+}. However, when we tested the effect of 40% ethylene glycol on the binding of S-1·ADP to regulated actin we found surprisingly that there was a marked increase in the cooperative response compared to that observed in aqueous solution both in the presence and in the absence of Ca^{2+}. These results cannot be explained by an effect of ethylene glycol on the conformation of S-1. Rather, they imply that ethylene glycol is directly affecting the troponin–tropomyosin complex, by increasing the fraction of regulated actin units in the turned-off form. Therefore, although our data support the suggestion of Tregear and co-workers that ethylene glycol causes the conformation of S-1·AMP-PNP to resemble that of S-1·ATP, ethylene glycol also appears to directly affect the conformation of the regulated actin.

Materials and Methods

Proteins. Rabbit skeletal myosin, S-1, and actin were prepared as described by Stein et al. (1978). The troponin–tropomyosin complex was prepared according to Eisenberg and Kielley (1974). The molecular weights used for S-1, actin, and troponin–tropomyosin complex were 120000, 42000, and 150000, respectively. Protein concentrations were determined by UV absorption and the following extinction coefficients: 750 cm^2/g at 280 nm for S-1, 1150 cm^2/g at 280 nm for F-actin, and 380 cm^2/g at 278 nm for the troponin–tropomyosin complex. The rabbit myosin was labeled with [^{14}C]-iodoacetamide (Greene & Eisenberg, 1980), resulting in 1 ± 0.1 mol of iodoacetamide incorporated/mol of S-1.

Binding Studies. In the binding studies, EGTA and Ca^{2+} were added prior to the addition of the proteins which were then added in the order of actin, S-1, and the troponin–tropomyosin complex. Although the troponin–tropomyosin was routinely added last to ensure that the S-1 actually dissociated from actin in the turned-off region of the binding isotherm, the same results were obtained regardless of the order of addition. In these experiments, ethylene glycol was always added last very slowly with constant mixing. Due to the viscosity of the ethylene glycol, the correct amount was obtained by weighing the solution. The troponin–tropomyosin complex was typically added to actin at a 2:7 molar ratio. Ap_5A (~250 μM) was always added to inhibit myokinase activity. The solution was stirred for several minutes and then allowed to remain for 30 min at 25 °C before centrifugation. The binding experiments were conducted either in the preparative ultracentrifuge (Beckman L2-65B) or in a Beckman Airfuge. In the former case, 3 mL of a total of 4 mL of solution was centrifuged for 1 h at 80000*g* at 25 °C. In the latter case, 0.6 mL of a total of 1 mL of solution was centrifuged for 20 min at 178000*g* at 25 °C. The same results were obtained with the preparative ultracentrifuge and the Airfuge. All binding studies used [^{14}C]iodoacetamide-modified S-1.

The solution prior to centrifugation and the supernatant after centrifugation were assayed for radioactivity in a Beckman LS-250 liquid scintillation counter to determine the total and free S-1 concentrations, respectively. In this method, the free S-1 concentration does not change during centrifugation because the actin and acto·S-1, being in the same filament, sediment at the same rate. Controls, run under the same conditions as the binding experiments, showed that in the absence of actin, 93% of the S-1 remained in the supernatant after centrifugation while in the presence of regulated actin and Ca^{2+}, >95% of the S-1 bound to actin.

ATPase Assays. These measurements were performed with the use of an automatic pH stat (Eisenberg & Moos, 1967), with all rates being corrected for the rate of S-1 alone. Unmodified S-1 was used in all of the ATPase studies. Regulated actin used in the ATPase studies was prepared by mixing actin with a 100% excess (2 mol/7 mol of actin) of native tropomyosin and dialyzing overnight at 4 °C against 3 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM EGTA, and 4 mM imidazole, pH 7.0.

Treatment of Data. To determine the equilibrium constant between the turned-off and turned-on forms of the tropomyosin–actin units in the regulated actin filament, it is necessary to determine, from the binding isotherm, the group cooperativity term (L') and the interaction cooperativity term (Y). Mathematically, L' is given by the equation:

$$L' = (1 + K_{\text{turned-on}}[\text{S-1}]')^7/(1 + K_{\text{turned-off}}[\text{S-1}]')^7$$

where [S-1]′ is the free S-1 concentration at which half of the tropomyosin–actin units are in the turned-on form, and $K_{\text{turned-on}}$ and $K_{\text{turned-off}}$ are the binding constants of S-1 to the turned-on and turned-off forms, respectively. $K_{\text{turned-off}}$ and $K_{\text{turned-on}}$ are determined from the binding data at low levels of θ ($\theta < 0.05$) and high levels of θ ($\theta > 0.5$), respectively, where θ is the number of moles of S-1 bound per mole of F-actin monomer. Y is independent of L' and is determined by adjusting its value until the theoretical curve has the same slope as the experimental points in the neighborhood of [S-1]′ [see Hill et al. (1980) for equations].

Chemicals. ADP and Ap_5A were from P-L Biochemicals, and AMP-PNP was from Sigma. The purity of the AMP-PNP and ADP was analyzed by both poly(ethylenimine)–cellulose chromatography in 0.75 M KH_2PO_4 (pH 3.4) and phosphate analysis. The ADP was >95% pure, and the AMP-PNP was >92% pure. The [^{14}C]iodoacetamide was from Amersham/Searle. Different ethylene glycol preparations (Fisher, Sigma, Aldrich) were used, and these all gave the same results as the electronic grade from Ashland Aemad Co.

Results

Table I shows the weakening effect of 40% and 50% ethylene glycol on the binding of various S-1·nucleotide com-

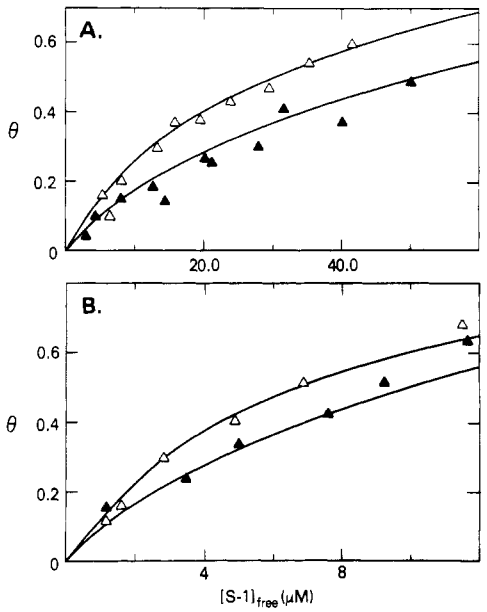

FIGURE 1: Binding of S-1·AMP-PNP (A) or S-1·PP_i (B) to regulated actin in 40% ethylene glycol at μ = 30 mM. Conditions are 25 °C, 10 mM KCl, 5 mM $MgCl_2$, 10 mM imidazole, 3 mM AMP-PNP or 3 mM PP_i, 1 mM dithiothreitol, 1 mM EGTA or 0.5 mM $CaCl_2$, and 40% ethylene glycol. In (A), varying concentrations of S-1 (1–70 μM) were added to 25 μM regulated actin in the presence (open triangles) or absence (closed triangles) of Ca^{2+}. The theoretical curves are for independent binding of S-1 to actin with binding constants of 3.5×10^4 and 2×10^4 M^{-1} for the data obtained in the presence and absence of Ca^{2+}, respectively. In (B), varying concentrations of S-1 (1–20 μM) were added to 20 μM regulated actin in the presence (open triangles) or absence (closed triangles) of Ca^{2+}. The theoretical curves are for independent binding of S-1 to actin with binding constants of 1.5×10^5 and 1×10^5 M^{-1} for the data obtained in the presence and absence of Ca^{2+}, respectively. θ is the fraction of actin sites occupied with S-1.

plexes to actin. As expected, the weakening effect was greater at 50% than at 40% ethylene glycol, but the same general trend was observed with both solutions (see columns 5 and 6). In agreement with the results of Marston & Tregear (1984), we find that ethylene glycol markedly weakens the binding of S-1 alone to actin. Ethylene glycol also weakens the binding of S-1·ADP, S-1·AMP-PNP, and S-1·PP_i to actin, and with S-1·AMP-PNP, the binding is weakened to the point where it becomes very similar to the binding of S-1·ATP to actin in aqueous solution. Interestingly, however, we find that ethylene glycol has almost no effect on the binding of S-1·ATP to actin. Therefore, it appears that ethylene glycol can weaken the binding of S-1·nucleotide complexes to actin until the strength of S-1 binding approaches the strength of binding of S-1·ATP to actin in aqueous solution but it cannot weaken the binding beyond this point.

The absence of cooperativity in the binding of S-1 to regulated actin has also been used as a criteria of the weak-binding conformation. We next used this criteria to probe the conformation of S-1·AMP-PNP in ethylene glycol. As shown in Figure 1A, the binding of S-1·AMP-PNP to regulated actin in 40% ethylene glycol shows no apparent cooperativity either in the presence or in the absence of calcium. These results are in agreement with the suggestion of Tregear and coworkers that S-1·AMP-PNP in the presence of ethylene glycol is an analogue of the weak-binding conformation (Tregear et al., 1984). The slight Ca^{2+} sensitivity observed in the binding indicates that S-1·AMP-PNP in ethylene glycol binds about 2-fold stronger to the turned-on form than to the turned-off form of regulated actin just as we have shown for S-1·ATP and pPDM·S-1 in aqueous solution (Chalovich et al., 1983).

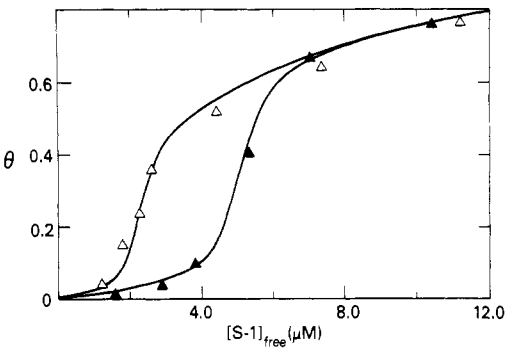

FIGURE 2: Binding of S-1·ADP to regulated actin in 40% ethylene glycol at μ = 0.12 M, 25 °C. Varying concentrations of S-1 (2–15 μM) were added to 4 μM regulated actin in the presence (open triangles) or absence (closed triangles) of Ca^{2+}. The conditions were the same as in Figure 1A except that 100 mM KCl was added and the nucleotide was ADP (3 mM). The theoretical curves were obtained with the model of Hill et al. using the following parameters: $K_{\text{turned-on}} = 3 \times 10^5$ M^{-1}; $K_{\text{turned-off}} = 1.5 \times 10^4$ M^{-1}; $L' = 350$, $Y = 20$ in the absence of Ca^{2+}; $L' = 22$, $Y = 20$ in the presence of Ca^{2+}.

Since PP_i and AMP-PNP similarly affect the binding of S-1 to regulated actin in aqueous solution (Williams & Greene, 1982), we next examined whether this is the case in the presence of ethylene glycol. Figure 1B shows that, like S-1·AMP-PNP, S-1·PP_i shows no apparent cooperativity in its binding to regulated actin in 40% ethylene glycol. Apparently, even though ethylene glycol does not weaken the binding of S-1·PP_i to actin as much as it weakens the binding of S-1·AMP-PNP, the conformation of S-1·PP_i in ethylene glycol is still quite similar to that of S-1·ATP in aqueous solution.

Although the lack of cooperativity observed with S-1·AMP-PNP and S-1·PP_i could be due to an effect of ethylene glycol on the conformation of S-1, as suggested by Tregear, it could also be due to a direct effect of ethylene glycol on the conformation of the regulated actin; i.e., regardless of the nucleotide bound to S-1, the binding of S-1 to regulated actin would show no apparent cooperativity in 40% ethylene glycol. To test this possibility, we examined the binding of S-1·ADP to regulated actin in 40% ethylene glycol both in the presence and in the absence of calcium. In Figure 2, the binding of S-1·ADP to regulated actin shows very pronounced cooperativity both in the presence and in the absence of calcium. This shows that 40% ethylene glycol did not eliminate the cooperative response of the regulated actin filament.

We fitted the binding data in Figure 2 to the cooperativity model of Hill et al. (1980) to provide us with the cooperativity parameters, L' and Y, which are only dependent on the properties of the regulated actin filament and not on the conformation of S-1. Although the data are relatively insensitive to the value of Y, which provides a measure of the interaction between adjacent tropomyosin molecules, the data in the presence of ethylene glycol could be fitted with a Y value of 20 in both the presence and absence of Ca^{2+}, the same value observed in aqueous solution. We next determined the value of L', the equilibrium constant between the turned-off and turned-on forms of regulated actin, which like Y is independent of the conformation of S-1. The results show that ethylene glycol did, in fact, affect the value of L' but rather than reducing the cooperativity ethylene glycol actually caused an increase in the cooperative response. In ethylene glycol, the values of L' are about 300 and 20 in the absence and presence of calcium, respectively compared to values of 33 and 2 observed in aqueous solution at 0.18 M ionic strength (Greene, 1982). Therefore, ethylene glycol causes an increase in the

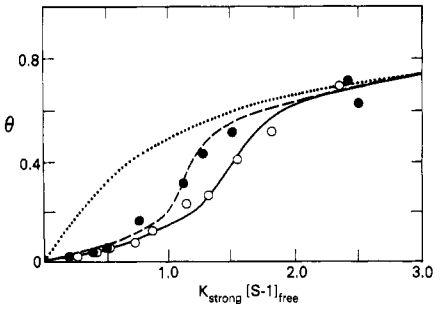

FIGURE 3: Binding of S-1·AMP-PNP to regulated actin in 20% and 30% ethylene glycol at μ = 30 mM in the absence of Ca^{2+}. Conditions are identical with those of Figure 1, except that 20% (closed circles) or 30% (open circles) ethylene glycol was used. In the binding studies conducted in 20% ethylene glycol, 0.5–15.0 μM S-1 was added to 6 μM regulated actin. In the binding studies conducted in 30% ethylene glycol, 2–25 μM S-1 was added to 30 μM regulated actin. The binding data were normalized on this one plot by plotting the data as θ vs $K_{turned\text{-}on}[S\text{-}1]_{free}$ where $K_{turned\text{-}on}$ was 4.5×10^5 and 1.5×10^5 M^{-1} for the data obtained in 20% and 30% ethylene glycol, respectively. The theoretical curves through the data were obtained by fitting the data to the model of Hill et al. using the following parameters: $K_{turned\text{-}on}/K_{turned\text{-}off} = 7$, $Y = 20$, and either $L' = 70$ for the dashed theoretical curve through the 20% ethylene glycol data or $L' = 150$ for the solid theoretical curve through the ethylene glycol data. The dotted theoretical curve is the curve for independent binding of S-1 to actin, which was used in fitting the data in Figure 1 for the binding of S-1·AMP-PNP to regulated actin in 40% ethylene glycol.

fraction of tropomyosin–actin units in the turned-off form. Since this would increase rather than decrease the observed cooperativity, the lack of cooperativity observed in the binding of S-1·AMP-PNP and S-1·PP_i to regulated actin in 40% ethylene glycol is not due to a direct effect of ethylene glycol on the regulated actin filament.

Further evidence that ethylene glycol has a direct effect on regulated actin, as well as an effect on the conformation of S-1, comes from the data shown in Figure 3. We previously showed that S-1·AMP-PNP binds with pronounced cooperativity to regulated actin in the absence of Ca^{2+} in aqueous solution (Greene, 1982), while Figure 1 shows that in 40% ethylene glycol, there is no apparent cooperativity in S-1·AMP-PNP binding to regulated actin. If ethylene glycol only affected the conformation of S-1, we might expect to see a gradual decrease in the cooperative binding of S-1·AMP-PNP to regulated actin at intermediate percentages of ethylene glycol. Figure 3 shows that as the percentage of ethylene glycol is raised from 20% to 30%, the value of L' is increased. Specifically, the value of L' is 70 in 20% ethylene glycol, the same as in aqueous solution at low ionic strength (Greene, 1982), while L' is 150 in 30% ethylene glycol. Presumably this is due to a direct effect of the ethylene glycol on the regulated actin, the same effect which we showed in Figure 2, where the magnitude of L' increased as the percentage of ethylene glycol was increased. Then, at 40% ethylene glycol, there is an abrupt loss in the cooperative binding of S-1·AMP-PNP to regulated actin with the data now falling along the dotted binding isotherm for independent binding of S-1 to actin (see Figure 1). Evidently, the direct effect of ethylene glycol on the conformation of S-1 becomes the dominant effect.

All of these results are consistent with the suggestion of Tregear and co-workers (Tregear et al., 1984) that ethylene glycol shifts S-1·AMP-PNP into the weak-binding conformation. However, the results also suggest that ethylene glycol has a direct effect on the regulated actin, increasing the fraction of tropomyosin–actin units in the turned-off form. We further examined this effect by studying the effect of ethylene glycol on the regulated acto·S-1 ATPase activity. If ethylene

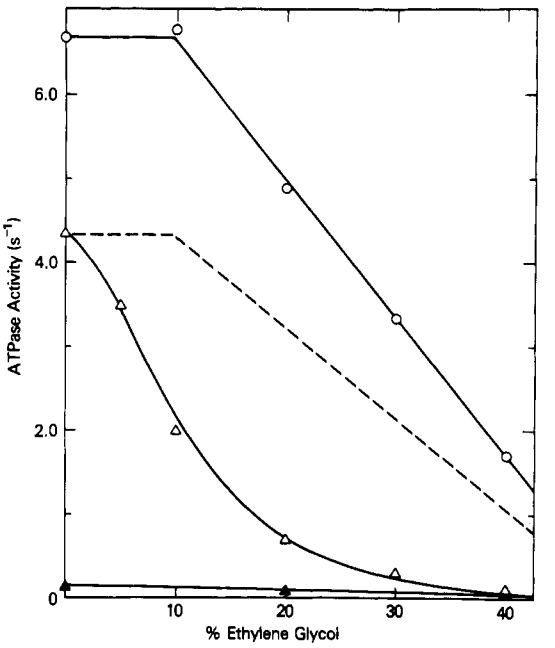

FIGURE 4: Effect of varying percentages of ethylene glycol on the acto·S-1 ATPase activity in the presence and absence of regulatory protein at μ = 15 mM, 25 °C. Conditions are 3 mM imidazole (pH 7.0), 3 mM $MgCl_2$, 1 mM ATP, 1 mM dithiothreitol, and 1 mM EGTA or 0.5 mM $CaCl_2$. The actin concentration was 10 μM, and the S-1 concentration was varied between 0.1 and 6.0 μM, depending on the rate. These rates were obtained with unregulated actin (open circles), regulated actin in the presence of Ca^{2+} (open triangles), and regulated actin in the absence of Ca^{2+} (closed triangles). The dashed line, in the presence of Ca^{2+}, assumed that ethylene glycol affected regulated acto·S-1 ATPase activity in the presence of Ca^{2+} the same as it does unregulated acto·S-1 ATPase activity.

glycol is causing an increase in the fraction of tropomyosin–actin units in the turned-off form in the presence of calcium, then the ATPase activity of regulated acto·S-1 should be inhibited in the presence of Ca^{2+}, just as it is in the absence of Ca^{2+}.

We first examined the effect of increasing the percentage of ethylene glycol on the unregulated and regulated acto·S-1 ATPase activity. In agreement with the results of Travers and Hillaire (1979), the open circles in Figure 4 show that increasing the percentage of ethylene glycol causes a decrease in the ATPase activity of unregulated acto·S-1. On this basis, the dashed curve is the theoretical plot predicted if ethylene glycol reduced the regulated acto·S-1 ATPase activity in Ca^{2+} by the same proportion as it reduces the unregulated acto·S-1 ATPase activity. However, comparison of this theoretical plot with the actual data we obtained using regulated actin (open triangles) shows that, in fact, ethylene glycol reduces the regulated acto·S-1 ATPase activity in the presence of Ca^{2+} much more than it reduces the unregulated acto·S-1 ATPase activity. In fact, at 40% ethylene glycol, there is no difference in the regulated acto·S-1 ATPase rates in the presence and absence of Ca^{2+}. It should be pointed out that this cannot be due to the inability of Ca^{2+} to bind to troponin–tropomyosin because we do detect Ca^{2+} sensitivity in the binding studies in 40% ethylene glycol (Figure 2). Furthermore, the effect of ethylene glycol is reversible; dilution of the 40% ethylene glycol returns the ATPase rate to its original value (data not shown). Therefore, our ATPase data provide further evidence that ethylene glycol is having a direct effect on the regulated actin, increasing the number of regulated actin units in the turned-off form, particularly in the presence of Ca^{2+}.

Finally, we checked whether the decrease in ATPase activity caused by ethylene glycol was due to a reduction in V_{max} or

K_{ATPase}, the apparent binding constant of S-1 to actin obtained from a double-reciprocal plot of ATPase activity vs actin concentration. Since our binding studies have shown that ethylene glycol does not affect the steady-state binding of S-1·ATP to actin (Table I), we would not expect ethylene glycol to affect K_{ATPase}. We performed this study in 25% ethylene glycol, since at higher percentages of ethylene glycol we could not obtain significant actin activation of the S-1 ATPase activity. Under these conditions, ethylene glycol caused more than a 10-fold decrease in V_{max}, reducing the rate from 25 to 2 s^{-1}, while causing less than a 2-fold decrease in K_{ATPase} from 4×10^4 to 3×10^4 M^{-1} (data not shown). Therefore, consistent with our binding studies, ethylene glycol does not significantly affect K_{ATPase}.

Discussion

In this study, we examined the effect of ethylene glycol on the interaction of S-1 with regulated actin and found that, in support of the suggestion of Tregear and co-workers, ethylene glycol causes the conformation of S-1·AMP-PNP to resemble that of S-1·ATP. In particular, we found that not only does ethylene glycol weaken the binding of S-1·AMP-PNP to actin but also, in the presence of 40% ethylene glycol, the binding of S-1·AMP-PNP to regulated actin in the absence of Ca^{2+} does not appear cooperative. This is in marked contrast to the highly cooperative binding of S-1·AMP-PNP to actin observed in aqueous solution and is very similar to the non-cooperative binding observed with S-1·ATP.

Interestingly, although ethylene glycol weakens the binding of the various S-1·nucleotide complexes to actin, their binding never becomes weaker than the binding of S-1·ATP to actin in aqueous solution. Consistent with this observation, we also found that, in contrast to the results obtained with S-1·ADP, S-1·AMP-PNP, and S-1·PP_i, ethylene glycol does not weaken the binding of S-1·ATP to actin. This suggests that ethylene glycol may not have a generalized weakening effect on S-1 binding but rather, in agreement with the model of Tregear and his co-workers, may shift the structure of S-1 toward the weak-binding conformation. On this basis, once S-1 is in the weak-binding conformation, ethylene glycol will have no further effect on it.

In addition to its effect on S-1, our results also suggest that ethylene glycol has a major effect on the conformation of regulated actin, causing an increase in the fraction of tropomyosin–actin units in the turned-off form, especially in the presence of Ca^{2+}. We observed this effect of ethylene glycol both in our binding studies and in our ATPase studies. In the latter studies, we found that, at 40% ethylene glycol, there is almost no difference in the actin-activated ATPase activity in the presence and absence of Ca^{2+}; in both cases, almost no actin activation of the S-1 ATPase activity occurs. These studies predict that if ethylene glycol were applied to skinned muscle fibers, it might keep the fibers relaxed even in the presence of Ca^{2+}, and, in fact, preliminary experiments by M. L. Anderson and M. Schoenberg suggest that single-skinned rabbit muscle fibers that are activated by Ca^{2+} do indeed become relaxed upon addition of ethylene glycol. It is not necessarily surprising that ethylene glycol has an effect on the troponin–tropomyosin complex. It is of course a nonspecific reagent and probably will affect the conformation of all of the proteins in the fiber to some extent.

The advantage of using the combination of ethylene glycol and AMP-PNP as an ATP analogue is that these compounds can be used in in situ studies to study the conformation of the weak-binding cross-bridge. On this basis, Tregear and co-workers (Clarke et al., 1980; Tregear et al., 1984) have done extensive mechanical and structural studies on fibers in the presence of ethylene glycol and AMP-PNP. The combination of AMP-PNP and ethylene glycol may prove more useful for physiological studies in situ than labeling the cross-bridges with pPDM, which may present problems of specificity. However, at the same time, this study has established that ethylene glycol not only affects the conformation of the myosin cross-bridge but also affects the conformation of the regulated actin. This is important to keep in mind when analyzing mechanical and structural studies carried out in ethylene glycol.

Registry No. ATPase, 9000-83-3; ethylene glycol, 107-21-1.

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