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Detergent Modification of Myosin Function and Structure in Solution†

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ABSTRACT: Rabbit skeletal muscle myosin and myosin subfragment 1 (S1) MgATPase activities were increased 2-3-fold by the addition of a variety of molecules that contained single straight saturated 12-16-carbon chains. The nonionic detergent dodecyl nonaoxyethylene ether ($C_{12}E_9$) increased the activity of S1 to 50% of maximum at a free $C_{12}E_9$ concentration of 27 ± 9 μM. The activation was reversible and was not due to chemical modification of S1 amino acid side chains. The V_{max} for actin-activated S1 MgATPase activity was increased 3-fold by $C_{12}E_9$. The apparent association constant for S1 binding to pure F-actin was reduced 3-fold by $C_{12}E_9$. The [$C_{12}E_9$] dependencies of the increase in S1 and acto-S1 MgATPase activities and of the decrease in acto-S1 binding were equal, within experimental uncertainty, suggesting that a single detergent-induced S1 conformational change is sufficient to explain the results. The stoichiometry of $C_{12}E_9$ bound to S1 in the S1-$C_{12}E_9$ complex was estimated, by the S1 concentration dependence of the $C_{12}E_9$ activation midpoint and by the light-scattering increase when S1 and detergent were mixed, to be 7 and 57 $C_{12}E_9$ molecules per S1, respectively. The results are discussed in relation to possible structural aspects of the mechanism of action for S1 and acto-S1 MgATPase activities.

Actin binding accelerates the rate of MgATP hydrolysis by myosin in solution by 1 or 2 orders of magnitude, depending on the conditions. Presumably, actin activation is a manifestation of the mechanism of energy transduction by which actomyosin in muscle uses energy from MgATP binding and hydrolysis to produce force. By investigating the activation of myosin, by actin or by other molecules, one hopes to gain insight into the transduction mechanism. Activation by organic molecules received attention a few decades ago but has not been studied much recently. Aliphatic alcohols with from one to four carbons were shown to increase the rate of myosin or myosin subfragment 1 (S1)[1] activity (Laidler & Ethier, 1952; Ebashi & Ebashi, 1959; Kay & Brahms, 1963; Stone & Prevost, 1973), as were some small ethers (Laidler & Ethier, 1952; Brahms & Kay, 1962; Tonomura et al., 1963; Yasui & Watanabe, 1965). The increase in the rates of CaATP, MgATP, or KATP hydrolysis by myosin or myosin subfragment 1 (S1) is from 1.2- to 5-fold for these small water-miscible organic molecules.

In general, the more hydrophobic molecules are more potent activators. For the one- through four-carbon alcohols, the longer and less branched the hydrocarbon chain, the lower the concentration at which it is effective at increasing the rate of hydrolysis (Ebashi & Ebashi, 1959). However, in all cases, relatively high concentrations (0.1-10 M) of the cosolvent were required for maximum activation, which was typically followed by irreversible inhibition at higher concentrations. The data were interpreted in terms of modifier-induced conformational changes of myosin (Rainford et al., 1964), although evidence for myosin secondary structural changes was not obtained. For example, dioxane at about 1 M increases the CaATPase activity of myosin 1.4-fold, but no structural changes were detected by optical rotary dispersion (Tonomura et al., 1963).

Reported here are the results from recent measurements on the effects of somewhat larger organic molecules on the MgATPase activity of myosin and actomyosin. In particular, the nonionic detergents of the alkyl polyoxyethylene ether type (C_XE_Y), and related structures, were found to increase myosin, S1, and acto-S1 MgATPase activities 2-3-fold, and to reduce the affinity of S1 for F-actin. This effect on activity is different from those reported recently for other amphipathic molecules which are inhibitory (Toste & Cooke, 1979; Davies, 1980) or benign (Reisler & Liu, 1981; Borejdo, 1983) with regard to activity. It appears that most of the activation observed in the present case is due to the long unbranched saturated hydrocarbon portion of the detergent. As the alcohol results in the literature suggest, the activation of S1 by these longer chain hydrocarbon structures is observed at much lower concentrations than were observed for the shorter chain molecules. S1 is activated by $C_{12}E_9$ and related structures in the 20-70

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[1] Abbreviations: S1, myosin subfragment 1; ATP, adenosine 5′-triphosphate; C_XE_Y, *n*-alkyl polyoxyethylene ether with *X* carbons in the alkyl chain and *Y* ethylene glycol units in the polyoxyethylene chain.

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μM range, about 10^5-fold lower than the range observed for the smaller alcohols. The affinity of S1 for actin is reduced in the same [$C_{12}E_9$] range. For the detergent $C_{12}E_9$, activation is concurrent with the formation of an S1–$C_{12}E_9$ complex involving several $C_{12}E_9$ molecules. The effects of $C_{12}E_9$ on S1 structure and function are discussed in the context of an S1 that contains tertiary structural subdomains.

Materials and Methods

Chemicals were reagent grade, or better. Enzymes for the coupled assay were from Sigma. The detergents were from Sigma Chemical or Nikko Chemicals. Detergents were dissolved in 10 mM MOPS, heated at 100 °C for 1 h, then cooled to 25 °C, and adjusted to the appropriate pH before use. Egg phosphatidylcholine was from Avanti. Biobeads (SM-2) were from Bio-Rad and were used as described by Holloway (1973) to remove $C_{12}E_9$ from samples. Myosin was prepared from rabbit skeletal muscle (Nauss et al., 1969) and used for the preparation of S1 by the action of α-chymotrypsin (Weeds & Taylor, 1975). S1 was purified by ammonium sulfate fractionation followed by ion-exchange chromatography by using DE-52 resin and a linear NaCl gradient eluent. Trypsinolysis of S1 was done as described by Mornet et al. (1979b). F-Actin was isolated from rabbit skeletal muscle acetone powder (Feuer et al., 1948) and purified by the method of Spudich and Watt (1970).

The rate of the production of ADP from ATP was measured by using the coupled assay of Imamura et al. (1966) and monitoring the disappearance of NADH at 340 nm, or by using the S1 tryptophan fluorescence technique of Bachouchi et al. (1986). The two methods gave the same values for the steady-state rate for S1, within experimental error. At 22 °C in pH 7.0 buffer containing 5–10 mM K^+, 5 mM $MgCl_2$, and 0.10 mM ATP, the S1 MgATPase activity at steady state averaged for six S1 preparations was 0.041 ± 0.015 s^{-1}.

Light-scattering measurements were made by using a Perkin-Elmer MPF-44B fluorescence spectrophotometer for 90 °C detection of scattered 350-nm incident light. All solutions were filtered through Millipore 0.3-μm pore-size cellulose acetate/cellulose nitrate filters to remove particles. By using S1 tryptophan fluorescence or MgATPase activity to assay for protein, it was determined that less than 3% of the protein was removed by filtering in the presence or absence of $C_{12}E_9$ if the filters were pretreated with S1 solutions.

The apparent association constants for S1 binding to pure F-actin in the presence of varying amounts of $C_{12}E_9$ were determined at 13 °C by the method of Marston and Weber (1975) using S1 that had Cys-515 modified by *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine (Duke et al., 1976) instead of iodoacetamide. After fluorescent modification, the S1 was dialyzed to remove free probe and then centrifuged for 1 h at 80000*g* before use. $C_{12}E_9$ did not affect the fluorescence intensity at 480 nm of the probe on S1 when irradiated at 342 nm.

Results

Activation of MgATPase Activity. The addition of the nonionic detergent $C_{12}E_9$ to S1 in solution increased the rate of MgATPase hydrolysis. At 23 °C, the increase was (2.5 ± 0.3)-fold with the midpoint of the transition near 30 μM added $C_{12}E_9$ (see Figure 1 for a typical titration). For [$C_{12}E_9$] up to 0.10 M, activation was obtained within 1 min and was then unchanged by incubation for up to 4 h. Loss of S1 MgATPase activity due to incubation with $C_{12}E_9$ was not observed for incubation times as long as 12 h. Control measurements indicated that $C_{12}E_9$ did not affect the assay used to detect

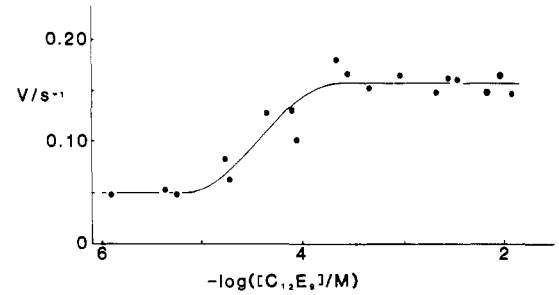

FIGURE 1: Detergent activation of S1 MgATPase activity. The steady-state MgATPase activity of 0.84 μM S1 in 10 mM MOPS (pH 7.0), 5 mM KCl, 5 mM $MgCl_2$, 100 μM ATP, 0.45 mM NADH, 2.2 mM phosphoenolpyruvate, 0.01 mg/mL pyruvate kinase, and 0.02 mg/mL lactate dehydrogenase at 22–23 °C was determined by monitoring the linear decrease in absorbance at 340 nm due to the loss of NADH (Imamura et al., 1966). Aliquots of concentrated (0.17 M) $C_{12}E_9$ in 10 mM MOPS, pH 7.0, were added to obtain increasing [$C_{12}E_9$] in the assay. The added [$C_{12}E_9$] at 50% maximum activation is 30 μM.

Table I: Activation of Myosin MgATPase by $C_{12}E_9$[a]

enzyme	[KCl] (mM)	activation
myosin	5	2.2 ± 0.3
myosin	500	2.9 ± 0.4
S1	5	2.5 ± 0.2
S1	500	2.3 ± 0.2
trypsinized S1	5	2.4 ± 0.4
trypsinized S1	500	2.4 ± 0.4

[a] The ratio of MgATPase activity with and without 1 mM $C_{12}E_9$ is given for assays done under the conditions given in Figure 1. Each activation value represents 4–10 determinations. S1 values here are for a mixture of S1A1 and S1A2 before or after purification by ion-exchange chromatography (see text). Varying [ATP] between 0.050 and 1.00 mM had no measurable effect on the degree of activation.

the production of ADP, in agreement with the results of Murphy et al. (1982). The original S1 activity was restored when the detergent was removed. The ratio of activities for S1 plus 2×10^{-4} M $C_{12}E_9$ to S1 alone was reduced from 2.6 ± 0.3 to 1.1 ± 0.2 by treatment of the S1–$C_{12}E_9$ sample with Biobeads SM-2, using the method of Holloway (1973).

Quantitatively similar changes of increased MgATPase activity were observed for myosin, S1, and trypsinized S1 (Table I), indicating that the S1 result was not due to proteolytic modification of myosin activity. Varying the [KCl] between 5 and 500 mM, or the [ATP] between 0.050 and 1.00 mM, did not change the degree of activation. The ratio of MgATPase activities ±$C_{12}E_9$ remained near 2.3 for S1 in 100 mM KCl and 0.10 mM ATP when the temperature was varied between 7 and 37 °C (Figure 2). The results suggest that $C_{12}E_9$ induces a reversible S1 conformational change for conditions that include a substantial range of ionic strength, temperature, and substrate concentration.

Mechanism of Activation. Several types of experiments suggested that the reversible increase of S1 activity was due to physical rather than chemical interactions of $C_{12}E_9$ and S1. As a test for the possibility that some impurity might be causing activation by modifying cysteine (Kielly & Bradley, 1956), lysine (Kubo et al., 1960; Fabian & Muhlrad, 1968), or arginine (Mornet et al., 1979a) side chains, stock solutions of $C_{12}E_9$ were preincubated with 0.50 mM dithiothreitol, L-lysine, or L-arginine for 4–6 h at 25 °C before the $C_{12}E$ was used. In all cases, the pretreated $C_{12}E_9$ was as effective as untreated $C_{12}E_9$ at increasing S1 activity. These results are consistent with the absence of any inhibition of S1 activity when it is incubated for long times (up to 4 h) in the presence of 10 mM $C_{12}E_9$, and confirm the Biobead SM-2 result (see

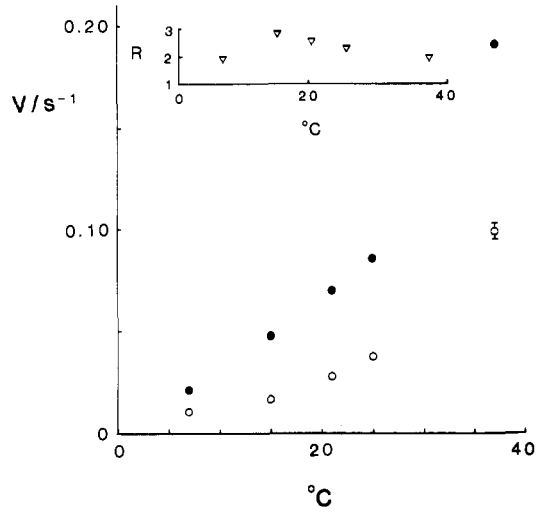

FIGURE 2: Temperature dependence of $C_{12}E_9$ activation of S1 MgATPase. The MgATPase activity (V) of S1 is shown for conditions with (●) and without (○) 1 mM $C_{12}E_9$ at temperatures between 7 and 37 °C. The ratio (R) of $V(+C_{12}E_9)/V(-C_{12}E_9)$ is shown for each temperature in the inset (▽). See Figure 1 for solution components.

Table II: Hydrophobic Activation of S1 MgATPase

agent	activation	concn (M)
$C_{12}E_8$	2.3 ± 0.3	above 10^{-4}
$C_{12}E_9$	2.4 ± 0.2	above 10^{-4}
$C_{12}E_{23}$	2.2 ± 0.3	above 10^{-4}
Triton X-100	1.3 ± 0.2	above 10^{-4}
poly(ethylene glycol) (MW 400)	0.99 ± 0.2	up to 10^{-3}
dodecenol	1.5 ± 0.1	saturated
dodecenol + poly(ethylene glycol)	1.5 ± 0.2	saturated + 10^{-3}
myristic acid	1.5 ± 0.2	saturated
phosphatidylcholine	1.0 ± 0.1	saturated

[a] The ratio of MgATPase activities with and without various agents present is given (n is 4–10 in each case). Conditions are given in Figure 1.

above) suggesting that it is chemically unreactive $C_{12}E_9$ itself that activates S1 rather than some chemically reactive impurity.

It appears that it is the alkyl chain of $C_{12}E_9$ that is responsible for the activation. The results obtained using structural analogues of $C_{12}E_9$ to activate S1 are given in Table II. Varying the length of the poly(ethylene glycol) portion has a negligible effect as shown by comparing $C_{12}E_8$, $C_{12}E_9$, and $C_{12}E_{23}$. Eliminating the poly(ethylene glycol) portion or introducing a phenyl group between the alkyl and poly(ethylene glycol) moieties caused only a 40% reduction in activation (see dodecanol and Triton X-100 in Table II). In contrast, eliminating the aliphatic hydrocarbon chain eliminated the activation, at least in the millimolar concentration range [see MW 400 poly(ethylene glycol) in Table II]. Phosphatidylcholine did not activate, but myristic acid was as effective as dodecenol. Taken together, the results in Table II indicate that a single long-chain hydrophobic hydrocarbon structure is most effective at activating S1 and that the polyether portion of $C_{12}E_9$ contributes only if it is attached to the hydrocarbon portion.

The relative effectiveness of the 12-carbon alkyl chain of $C_{12}E_9$ in comparison to that of shorter straight-chain alkyl groups can be estimated by comparing the concentrations required to activate S1 to the chain length of the activator. Published data for the concentration dependence of the increase of myosin and S1 ATPase activities for methanol, ethanol, 2-chloroethanol, 1-propanol, and 1-butanol (Ebashi & Ebashi, 1959; Laidler & Ethier, 1952; Brahms & Kay,

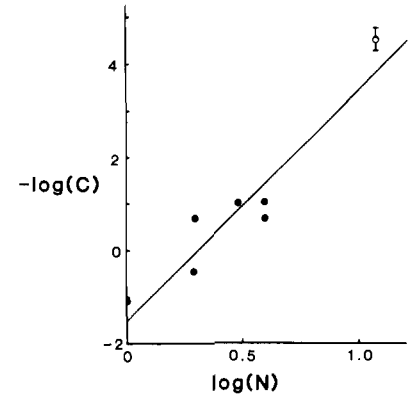

FIGURE 3: ATPase activation by organic molecules. The concentration (C) of methanol, ethanol, 2-chloroethanol, 1-propanol, 1-butanol, and $C_{12}E_9$ that produces 50% of their total activation of myosin or S1 activity is plotted versus the number of carbons (N) in the hydrocarbon chain, as a log–log plot. The $C_{12}E_9$ datum is from this study. The other data are from published work (Laidler & Ethier, 1952; Ebashi & Ebashi, 1959; Kay & Brahms, 1963; Stone & Prevost, 1973) and are given without experimental uncertainties.

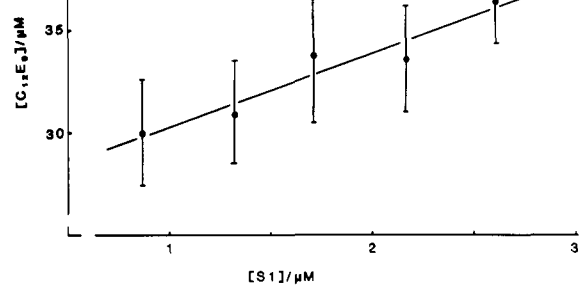

FIGURE 4: S1 concentration dependence of $C_{12}E_9$ activation. The added $[C_{12}E_9]$ that produced 50% maximum activation of S1 steady-state MgATPase activity (see Figure 1) was determined for increasing [S1]. The line is fitted to the data by using the equation given in the text. Three to eight measurements were made for each [S1]. The intercept indicates that free $[C_{12}E_9]$ for activation is 27 μM. The slope is 3.6 mol of $C_{12}E_9$/mol of S1 for 50% activation.

1962; Yasui & Watanabe, 1965; Stone & Prevost, 1973) along with the midpoint obtained from Figure 1 for $C_{12}E_9$ were used to compare the concentration (C) required for 50% activation of S1 to the number (N) of carbons in the chain (Figure 3). The correlation is clear. The longer the chain, the more effective it is at activation. The r value equals 0.95. These data suggest that the present activation is due to hydrophobic interactions of $C_{12}E_9$ with S1, in agreement with the results in Table II.

Stoichiometry of the S1–$C_{12}E_9$ Complex. There are no convenient means to measure directly the free concentration of $C_{12}E_9$ in solution, and the activation in Figure 1 is shown as a function of total $[C_{12}E_9]$. However, it is possible to estimate the free $[C_{12}E_9]$ for activation and its stoichiometry of binding by determining the dependence of total $[C_{12}E_9]$ required for 50% activation on the [S1], using eq 1 where

$$[C_{12}E_9]_{total} = [C_{12}E_9]_{free} + n[S1(C_{12}E_9)_n] \quad (1)$$

$[S1(C_{12}E_9)_n]$ is approximated as $[S1]_{total}$ and n is the number of bound $C_{12}E_9$ molecules per S1 for 50% activation. This procedure has been used to measure the stoichiometry of $C_{12}E_9$ binding to muscle sarcoplasmic reticulum CaATPase to obtain a value within 17% of values obtained by other independent methods (Anderson et al., 1984; Highsmith & Cohen, 1987). Data for 0.86–2.62 μM S1 were fitted to the above expression

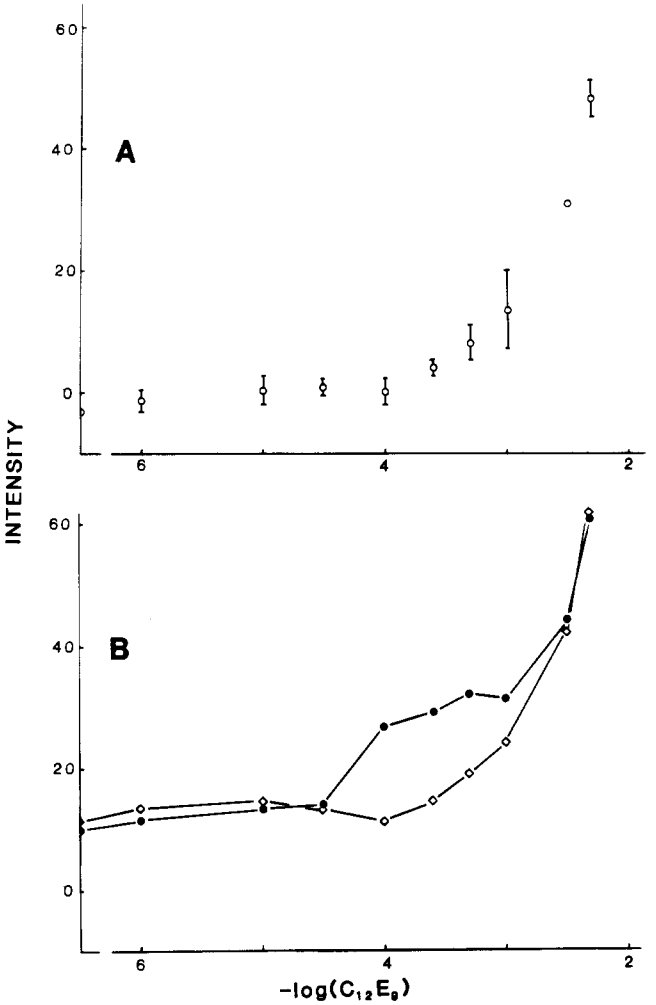

FIGURE 5: Light scattering from the S1–$C_{12}E_9$ complex. The relative intensity of scattered light is shown for solutions with increasing [$C_{12}E_9$] in the absence of S1 [(○) in panel A] and in presence of 1.71 μM S1 [(●) in panel B; standard error (not shown for graphical clarity) is ±2–3]. Also shown in panel B are the values from panel A, obtained without S1, which have the average scattered intensity from 1.71 μM S1 in the absence of $C_{12}E_9$ added (◇). The increase in the intensity of S1 in the presence of $C_{12}E_9$ (●) compared to the intensity of S1 alone plus $C_{12}E_9$ alone (◇) that is seen in panel B starting near 10^{-4} M $C_{12}E_9$ is assigned to the S1–$C_{12}E_9$ complex.

(Figure 4), and the values obtained for n and $[C_{12}E_9]_{free}$ were 3.6 and 27 μM, respectively. The uncertainty associated with these values is estimated to be ±30%. The r value for the fit was 0.960. These data suggest that seven molecules of $C_{12}E_9$ are bound in an S1–$C_{12}E_9$ complex when S1 is fully activated. The free [$C_{12}E_9$] = 27 μM for 50% activation is below the critical micelle concentration (cmc), 110 μM (Mukerjee & Mysels, 1971), which is consistent with micelles not being involved. However, the concentration of added $C_{12}E_9$ at full activation is nearer its cmc, and micelle formation cannot be excluded.

To obtain more information about the S1–$C_{12}E_9$ complex, the intensity of light scattering at 90° from a 350-nm incident beam was measured for solutions containing increasing amounts of $C_{12}E_9$ in the presence and absence of S1 (Figure 5). When S1 is absent (Figure 5A), the data are those predicted by the known characteristics of $C_{12}E_9$. Below the cmc, the increase in the intensity of scattered light is negligible for increasing [$C_{12}E_9$] because it remains monomeric. Above the cmc, large increases in intensity result from micelle formation. In Figure 5B, the data for $C_{12}E_9$ solutions containing 1.71 μM S1 are shown along with the data for $C_{12}E_9$ alone

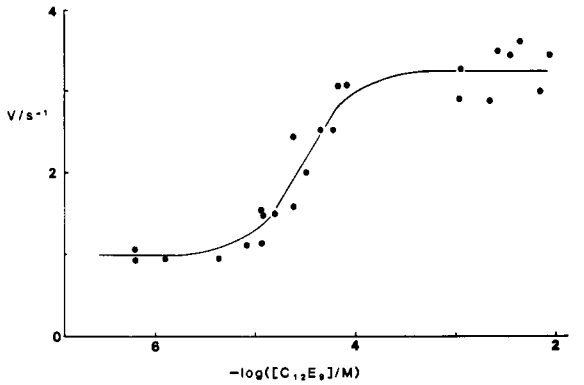

FIGURE 6: Detergent activation of acto–S1 MgATPase activity. With 10 μM actin present, $C_{12}E_9$ was added to the steady-state assay described in Figure 1. [S1] was 0.81 μM.

which has been adjusted for the scattering contribution of $C_{12}E_9$-free S1. The difference between the two curves shown in Figure 5B, beginning at about 50 μM $C_{12}E_9$, is attributed to scattering from the S1–$C_{12}E_9$ complex, which is subsequently overwhelmed by micelle scattering above 1 mM $C_{12}E_9$.

The undetected increase in the scattered light intensity for S1 in the presence of 30–50 μM added $C_{12}E_9$ in Figure 5B is consistent with the value of n = 3.6 $C_{12}E_9$ bound per S1 at 50% activation obtained from the data in Figure 4. Four bound $C_{12}E_9$ molecules would increase the mass of S1 by 2.0%, and assuming that scattered light intensity will vary as the square of the mass of the scatterer (Berne & Pecora, 1976), the S1–$C_{12}E_9$ complex would have about a 4% increased scattering intensity. The small predicted increase falls within experimental uncertainty. However, using this simple intensity dependence on the square of the mass relationship to estimate of the number of $C_{12}E_9$ molecules bound in the S1–$C_{12}E_9$ complex from the increase in scattering observed in the presence of 100 μM added $C_{12}E_9$, one obtains 57 bound $C_{12}E_9$ molecules from the observed 67% increase in intensity assuming the apparent increase is due to detergent binding to S1 (see Figure 5B). The observed scattering intensity is too small to be due to S1 dimer formation; but 57 molecules of $C_{12}E_9$ per S1 is much larger than 7, the value predicted from the analysis of the data in Figure 4. This quantitative discrepancy in the estimates of how many $C_{12}E_9$ molecules are in the S1–$C_{12}E_9$ complex will be discussed below, but all the data indicate a detergent–protein complex is being formed.

Actin Interactions with the S1–$C_{12}E_9$ Complex. The MgATPase activity of actin-activated S1 also was increased by the addition of $C_{12}E_9$ (Figure 6). The degree of activation and its [$C_{12}E_9$] dependence were similar to those obtained for S1 alone (Figure 1). The degree of acto–S1 activation in the presence of 1 mM added $C_{12}E_9$ was 2.8 ± 0.3 for [ATP] varied between 0.050 and 1.00 mM at 23 °C in solutions of 30 mM ionic strength. By varying the [actin], the $C_{12}E_9$ was shown to increase the V_{max} of the acto–S1 MgATPase activity (Figure 7).

The affinities of actin for S1 and for S1 MgADP were also modified by $C_{12}E_9$. The effects of increasing [$C_{12}E_9$] on the apparent association constants for actin binding to fluorescently labeled S1, in the presence and absence of 0.50 mM ADP at 12 °C, are shown in Figure 8. The solid lines were determined by using the midpoint obtained from the $C_{12}E_9$-induced increase in activity (Figure 1). The increase in activities (Figures 1 and 6) and the decrease in affinities (Figure 8) have [$C_{12}E_9$] dependencies that are consistent with a single transition of S1 between two conformations, which occurs when several $C_{12}E_9$ molecules bind S1 to form a complex. More complicated

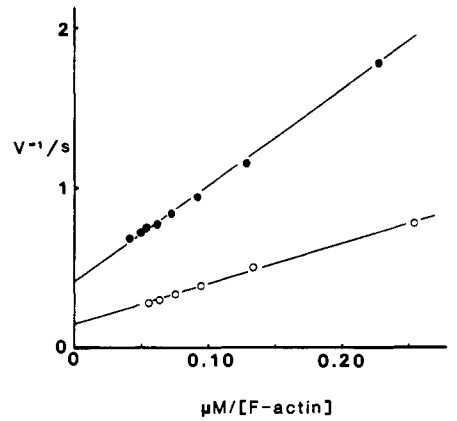

FIGURE 7: Actin activation of S1 MgATPase activity in the presence of $C_{12}E_9$. The effect of increasing [actin] on the activity of 0.74 μM S1 in the absence (●) and presence (○) of 1 mM $C_{12}E_9$ was determined as described in Figure 1.

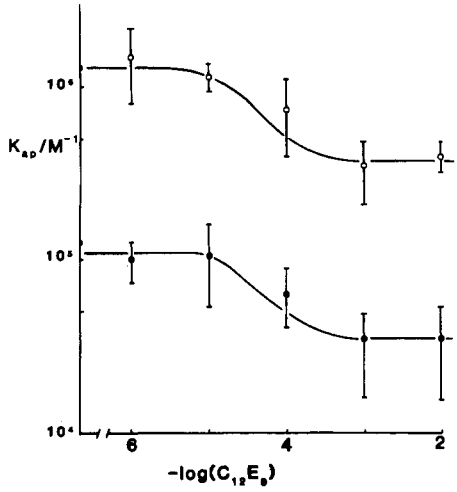

FIGURE 8: Actin binding to S1 and the S1–$C_{12}E_9$ complex. The dependencies on [$C_{12}E_9$] of actin binding to S1 ($K_{apparent}$) in the presence (●) and absence (○) of 1 mM MgADP are shown. The solid lines are for a transition in the acto-S1 apparent binding constant which occurs at the midpoint determined for $C_{12}E_9$ activation of MgATPase activity. The temperature was 12–15 °C. Solutions contained 0.4–1.0 μM S1, 0.6–2.0 μM actin, 100 mM KCl, 5 mM $MgCl_2$, and 10 mM MOPS, pH 7.0. S1 was 85–97% modified with a fluorescent reporter group (see Materials and Methods).

behavior may be occurring, but it is not required to explain the observations.

Discussion

All of the data are consistent with the increase in S1 activity being due to binding of $C_{12}E_9$ molecules to S1 to form a complex in which the conformation of S1 is changed. The striking features of the interaction of $C_{12}E_9$ with S1 are (1) the low [$C_{12}E_9$] (=27 μM) at which it is effective, (2) the readily detected complex of S1 and $C_{12}E_9$, and (3) the absence of S1 denaturation or inhibition even for prolonged exposure to high [$C_{12}E_9$]. A large number of investigations on covalent and noncovalent modifiers of myosin ATPase activity were analyzed in terms of a simple scheme consisting of three conformations of myosin, $\alpha \rightleftharpoons \beta \rightleftharpoons \gamma$ (Rainford et al., 1964). In this scheme, the β conformation has 2–5-fold higher activity than the α conformation, and the γ conformation is inhibited; increasing amounts of any modifier convert α to β and then β to γ. The action of $C_{12}E_9$ is consistent with $\alpha \rightleftharpoons \beta$, but the inhibited γ conformation does not occur, probably because the maximum $C_{12}E_9$ monomer concentration is kept low by micelle formation and because $C_{12}E_9$ does not covalently modify S1.

The results presented here indicate that a variety of hydrophobic molecules with linear saturated hydrocarbon moieties reversibly bind to myosin and to S1 and that binding enhances their rates of MgATP hydrolysis. The binding of the nonionic detergent $C_{12}E_9$ to S1 has an apparent association constant of $(2 \pm 1) \times 10^4$ M^{-1} and reduces the free energy of activation of MgATP hydrolysis by 0.5–0.7 kcal/mol under a variety of conditions. A quantitatively similar reduction in the free energy of activation for acto-S1 MgATPase hydrolysis is induced by $C_{12}E_9$ binding, which also weakens the standard free energy of acto-S1 binding by about 0.7 kcal/mol. These changes occur when an enzyme–detergent complex involving several $C_{12}E_9$ molecules is formed.

The stoichiometry of $C_{12}E_9$ binding to S1 was estimated by two methods. The dependency of the [$C_{12}E_9$] required for activation on the [S1] indicated seven $C_{12}E_9$ per S1 at full activation, while light-scattering intensity measurements indicated a much larger number. Although the light-scattering result in Figure 5 is a clear indication that a complex is formed, it is probably less accurate regarding stoichiometry in the present case. Solvation changes were ignored, and it was assumed that the optical polarizabilities of S1 and of the S1–$C_{12}E_9$ complex are identical, which may not be the case. The possibility that the S1–$C_{12}E_9$ complex reduces the $C_{12}E_9$ cmc cannot be rigorously excluded, either.

The location of the binding of the several $C_{12}E_9$ molecules could be either hydrophobic areas on the surface of S1 or interior hydrophobic regions. If the $C_{12}E_9$ molecules are binding on the surface, it is reasonable that changing the solvation of S1 would change its activity, although recent results on enzyme activity in anhydrous organic solvents suggest that replacing the layer of water nearest the enzyme surface with hydrophobic solvents is inhibitory rather than activating (Zaks & Klibanov, 1988). On the other hand, the known tendency of detergent molecules with long alkyl chains to penetrate into protein interiors makes it resonable to speculate that $C_{12}E_9$ would bind S1 interior hydrophobic regions, perhaps at hydrophobic interfaces between S1 subdomains.

It is well established that S1 is readily converted into three primary sequence fragments by proteolytic enzymes (Balint et al., 1978; Mornet et al., 1979b, 1985; Yamamoto & Sekine, 1979; Sutoh, 1982; Muhlrad & Hozumi, 1982; Applegate & Reisler, 1983; Hozumi, 1986; Hiratsuka, 1986; Chausspied et al., 1986; Vibert et al., 1986; Burke et al., 1987) which is often interpreted in terms of three corresponding tertiary structural subdomains with semiautonomous functions. For skeletal muscle S1 in solution, there is evidence for segmental motion from transient electrical berefringence measurements (Highsmith & Eden, 1986, 1987) and from fluorescence resonance energy transfer and anisotropy decay measurements (Dalby et al., 1983; Cheung et al., 1985; Aguirre et al., 1989). The segmental motions are presumed to be motions of subdomains, which may or may not correspond to the primary sequence fragments. Tertiary structural subdomains of any type would be likely to have hydrophobic interfaces in order to avoid being solvated. If these hydrophobic interfaces do exist, they are locations on S1 at which $C_{12}E_9$ alkyl chains would bind and be able to modify functions like MgATPase and actin binding by altering the subdomain–subdomain interactions without denaturing S1.

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