Ca\textsuperscript{2+}-controlled Conformational States of the Ca\textsuperscript{2+} Transport Enzyme of Sarcoplasmic Reticulum*

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The fluorescent reagent, S-mercuroc N-dansyl-cysteine, reacts specifically with thiols of the purified Ca\textsuperscript{2+}-ATPase of the sarcoplasmic reticulum, producing an increase of fluorescence intensity at 500 nm ($\lambda_{ex} = 355$ nm). The reaction is stoichiometric, and the increase of the fluorescence intensity is proportional to the number of blocked thiols. Twelve reactive thiols per 10\textsuperscript{5} daltons of ATPase peptide fall into roughly three classes. Blocking of the most reactive thiol entails little inhibition of enzyme activity. Blocking of the five thiols reacting next (intermediate class) results in almost complete inhibition of both phosphorylated intermediate formation and ATP hydrolysis. The second order rate constants of the reaction of thiols have been determined by stopped flow studies. The most reactive thiol and the six least reactive thiols can each be treated as a single class with respect to the rate constant; five thiols of intermediate reactivity appear to have different rate constants ($k_2, k_3, \ldots k_6$). Of these constants, $k_1$, corresponding to the most reactive thiol, does not change with [Ca\textsuperscript{2+}]. Upon increasing [Ca\textsuperscript{2+}] from $10^{-6}$ to $10^{-4}$ M, $k_2$ increases and $k_{12}$ decreases; the changes roughly parallel the activation of ATPase activity and the Ca\textsuperscript{2+} binding to the high affinity $\alpha$ sites (Ikemoto, N. (1975) J. Biol. Chem. 250, 7219-7224). Upon further increase of [Ca\textsuperscript{2+}] $k_2$ decreases and $k_{12}$ increases, in parallel with the inhibition of ATPase activity and with the Ca\textsuperscript{2+} binding to the low affinity $\gamma$ sites.

Important functions of the sarcoplasmic reticulum, e.g. the formation of the phosphorylated enzyme intermediate (1-5), hydrolysis of ATP (6, 7), and the rate of Ca\textsuperscript{2+} uptake (8, 9), are all activated in the same range of the Ca\textsuperscript{2+} concentration (half-activation $1 \times 10^{-6}$ M). In contrast, higher concentrations of Ca\textsuperscript{2+} (1-5 mm), which presumably prevail inside the sarcoplasmic reticulum in the steady state of Ca\textsuperscript{2+} transport (10, 11), inhibit both Ca\textsuperscript{2+} uptake and ATP hydrolysis (12), but not formation of phoshoenzyme (4, 13).

Our recent studies of the SR\textsuperscript{1} (14, 15) have permitted assignment of various roles in the regulation of enzyme activity to several types of Ca\textsuperscript{2+} binding sites located on the purified ATPase enzyme. Binding of Ca\textsuperscript{2+} to the high affinity $\alpha$ sites ($K_a = 3 \times 10^5$ M\textsuperscript{-1}) activates the enzyme reaction, while the binding to the low affinity $\gamma$ sites ($K_a = 1 \times 10^7$ M\textsuperscript{-1}) inhibits it. Recent reports have suggested that Ca\textsuperscript{2+} binding to the high affinity sites would induce some changes of conformation of the SR ATPase. According to the work of Murphy (16, 17) both the thiol reactivity with Nbs\textsubscript{2} and the intrinsic tryptophan fluorescence (cf. Dupont, Ref. 18) are increased by Ca\textsuperscript{2+} (half-activation $1 \times 10^{-5}$ M). Champel et al. (19, 20) have shown that the mobility of a spin-labeled derivative of iodoacetamide attached to the SR membrane is increased in the range of Ca\textsuperscript{2+} concentration in which the activation of phosphorylation takes place, and that in the same [Ca\textsuperscript{2+}] range thiol reactivity with reagent decreases.

This study was initiated in attempts to reveal details of the mechanism by which the binding of Ca\textsuperscript{2+} to the high affinity $\alpha$ sites and to the low affinity $\gamma$ sites produce activation and inhibition of the enzyme, respectively. The use of fluorescent thiol reagent, S-mercuric N-dansyl-cysteine (Dns-Cys-Hg) (21), in the stopped flow studies on the purified ATPase of SR, has permitted us to distinguish several classes of thiols whose kinetic constants vary with [Ca\textsuperscript{2+}]. The results shown here suggest that the [Ca\textsuperscript{2+}]-dependent changes of the thiol reactivity reflect changes in the enzyme conformation controlled by the Ca\textsuperscript{2+} binding, which may have an important bearing on the mechanism by which the enzyme activity is regulated by [Ca\textsuperscript{2+}].

**EXPERIMENTAL PROCEDURES**

**Preparation of the Purified Enzyme—**Fragmented SR was prepared from rabbit skeletal fast-twitch (white) muscles (22). The Ca\textsuperscript{2+}-ATPase was purified from the fragmented SR solubilized with Triton X-100 using the method described previously (15) except that 7.2 mm $\beta$-mercaptoethanol was used rather than dithiothreitol during solubilization of the membrane. The column-purified ATPase was sedimented at 78,500 x $g$ for 60 min, and homogenized in 0.3 M sucrose, 20 mM Tris/maleate (pH 7.0), final protein concentration 10 mg/ml. The homogenate was lyophilized to remove the last trace of $\beta$-mercaptoethanol, and stored in a freezer. Before use, the lyophilized preparation was homogenized in deionized water of the same volume as the original solution in a tight fitting glass homogenizer at 0°C. During storage for 2 months at least 90% of the ATPase activity remained.

**Preparation of Dns-Cys-Hg—**Dns-Cys-Hg was prepared according to the method of Leavis and Lehrer (21) with the modification that HgCl and HCl rather than HgNO\textsubscript{3} and HNO\textsubscript{3} were used in the reaction with N-dansyl-cysteine in the presence of 10 mm HCl. Dns-Cys-Hg solutions, 1.0 to 2.5 mm in 10 mm HCl, were stored at 4°C. We noticed that these solutions became turbid but cleared up upon dropwise addition of 1 M Tris base. Complete dissolution of Dns-Cys-Hg aggregates was essential for the thiol-specific reactivity of the reagent; without this procedure Dns-Cys-Hg aggregates seemed to be bound nonspecifically in hydrophobic domains of the enzyme preparation.
RESULTS

Reaction of Dns-Cys-Hg with Thiols—Upon addition of Dns-Cys-Hg that shows the emission maximum at 555 nm (λ_max = 335 nm) to the purified Ca^{2+}^-ATPase of SR there is an increase in fluorescence with a shift of the emission maximum to 500 nm. Fig. 1 depicts the results of adding increasing amounts of the reagent at [Ca^{2+}] = 1.5 \times 10^{-8} M; the emission spectra were recorded after the emission intensity became maximal (30 s to 3 min). Upon addition of dithiothreitol a spectrum characterized by an emission maximum at 555 nm and having a much lower fluorescence intensity, which is basically identical with that of free Dns-Cys-Hg at the same concentration with neither protein nor dithiothreitol, is recorded. This suggests that dithiothreitol causes detachment of the probe from the enzyme.

The fluorescence intensity of the probe reacted with the enzyme increases in proportion to the amount of added probe up to 12 mol/10^6 g. Upon further additions the increase of the fluorescence intensity becomes small and roughly parallels the curve for the free probe. The number of free thiols that had not reacted with Dns-Cys-Hg was determined by Nbsn in the presence of SDS (Fig. 2). With no Dns-Cys-Hg but with SDS present 16 thiols are titrated per 10^6 daltons protein. The number of free thiols decreases linearly with the amount of probe added and levels off at 4 mol of thiols/10^6 g enzyme. In the native conformation of the enzyme, namely in the absence of SDS, approximately 12 thiols are titrated with Nbsn. These results indicate that the 12 thiols are reactive with Dns-Cys-Hg and react stoichiometrically with the reagent.

The same type of experiment shown in Figs. 1 and 2 was
carried out also at a lower and a higher concentration of Ca$^{2+}$, namely $10^{-9}$ and $10^{-2}$ M. Regardless of $[Ca^{2+}]$ the number of reactive thiols was 12 and the reaction was stoichiometric.

Furthermore, the high fluorescence intensity at 500 nm characteristic of the protein-attached Dns-Cys-Hg is proportional to the number of thiols blocked by Dns-Cys-Hg. This is shown in Fig. 2, where the emission intensity at 500 nm in Fig. 1 was plotted against the amount of Dns-Cys-Hg added. The number of blocked thiols was calculated from the F/Fo values determined from the oscilloscope trace (see text).

Fig. 2. The relation between the increase of fluorescence intensity and the number of thiols blocked with Dns-Cys-Hg. The emission intensity at 500 nm in Fig. 1 was plotted against the amount of Dns-Cys-Hg added. For the determination of the extent of thiol blockage by Dns-Cys-Hg, a 1-ml sample treated with various amounts of Dns-Cys-Hg as described in the legend to Fig. 1 was incubated with a 1-ml solution containing 2 mM EDTA, 0.32% SDS, 0.4 mM Nbsl, 200 mM Tris-HCl, pH 8.0. After incubation for 60 min at 22°C, the enzyme was treated with 20 mM dithiothreitol.

Fig. 3. Inhibition of ATPase activity and phosphoenzyme formation by Dns-Cys-Hg. Various amounts of Dns-Cys-Hg, in the ratios indicated on the abscissa, reacted with 10 nM purified SR Ca$^{2+}$-ATPase in a 1-ml solution of 0.3 M sucrose, 0.1 M KCl, 5 mM MgCl$_2$, 1 mM EGTA, 0.9 mM CaCl$_2$ (free $[Ca^{2+}] = 1.5 \times 10^{-5}$ M), 0.1 M Tris/maleate, pH 7.0, for 10 min at 22°C. For the assay of ATPase activity, a 0.1-ml fraction of the Dns-Cys-Hg/ATP mixture was diluted with a 1.0-ml solution containing 0.1 M KCl, 5 mM MgCl$_2$, 5 mM ATP, 1 mM EGTA, 0.9 mM CaCl$_2$, 0.1 M Tris/maleate, pH 7.0 at 22°C. After incubation for 2 min, the reaction was stopped by adding 6.7% trichloroacetic acid, and the liberated P, was determined according to the method of Fiske and SubbaRow (28).

Fig. 4. Time course of the rapid reaction of thiols of the purified ATPase with Dns-Cys-Hg. A, reaction was started by mixing 2.32 nM purified ATPase with 5.2 nM Dns-Cys-Hg in 0.1 M KCl, 5 mM MgCl$_2$, 1 mM EGTA, 0.9 mM CaCl$_2$ (free $[Ca^{2+}] = 1.5 \times 10^{-5}$ M), 20 mM Tris/maleate, pH 7.0 at 22°C with the use of stopped flow apparatus. Increase in the fluorescence intensity was monitored as described under "Experimental Procedures." Two sets of time courses were recorded at different time scales: bottom trace, 50 ms/unit of time scale; top trace, 2 s/unit. B, stopped flow reaction of Dns-Cys-Hg with the enzyme was carried out in the same way as above except that various amounts of Dns-Cys-Hg were mixed with the enzyme. Numbers on each curve indicate mole of Dns-Cys-Hg added per mol of enzyme. The number of blocked thiols was calculated from the F/Fo values determined from the oscilloscope trace (see text).
to the number of blocked thiols. This excludes the possibility that the presence of EGTA which presumably has a high affinity for mercury might result in various degrees of dissociation between the mercury and cysteine moieties of Dns-Cys-Hg depending upon the proportion of EGTA and calcium.

**Effect of Thiol Blocking on the Enzymic Activity**—We have studied the effect of blocking of thiols on the phosphoenzyme formation and the rate of ATP hydrolysis at various Dns-Cys-Hg/enzyme ratios at 1.5 × 10⁻⁶ M Ca²⁺ (Fig. 3). It appears that blocking of the most reactive thiol, approximately one per 10⁴ daltons of ATPase protein, has little inhibitory effect on the enzyme activity. On blocking the next five thiols, almost complete inhibition of both the phosphoenzyme formation and ATP hydrolysis takes place. The type of experiment shown in Fig. 3 has also been carried out at 1 × 10⁻⁴ M Ca²⁺ and 1 × 10⁻⁵ M Ca²⁺. The results were basically the same as shown in Fig. 3.

**Kinetically Distinguishable Classes of Thiols**—The time course of the fluorescence increase during the Dns-Cys-Hg incorporation has been investigated by the stopped flow method. Fig. 4A shows an example of stopped flow trace of the experiment. As concluded from the data in Fig. 2, the reaction of Dns-Cys-Hg with the protein thiols is stoichiometric and the fluorescence intensity is proportional to the number of blocked thiols. Thus, applying the method of calculation outlined, if \( F_p \) is the maximum fluorescence for \( \mu g \) of Dns-Cys-Hg per 10⁵ g, \( x = F/F_p \), when \( F \) is the fluorescence intensity at the time \( t \).

Fig. 4B shows the time course of blocking of thiols with various amounts of added Dns-Cys-Hg. In the initial phase 0.5 to 1.0 thiol is rapidly blocked. In the subsequent phase, blocking proceeds rather slowly, and levels off when all added Dns-Cys-Hg is incorporated. This suggests that approximately one thiol per 10⁷ daltons of ATPase protein has considerably higher reactivity with Dns-Cys-Hg than the other 11 thiols. Presumably this is the thiol whose blocking has little effect on phosphoenzyme formation and ATP hydrolysis (cf. Fig. 3).

Fig. 5 shows plots of \((s - p)^{-1} \ln \{(1 - x/s)/(1 - x/p)\}\) versus \(t\) (cf. Equation 1) of the data shown in Fig. 4. As expected, the slope of the plots varies as a function of time. Following the initial steep phase, the slope (namely \( k \)) decreases rather rapidly and approaches the final slope. The final slope becomes smaller at larger values of the probe/enzyme ratio. However, at the probe/enzyme ratio ≥ 6, there is little or no further decrease in the final slope.

As discussed in "Method of Estimating Number and Kinetic Constants of Thiols," when the reagent has reacted with all thiols but the least reactive class, the final slope, namely \((df(t)/dt)\), is identical with the rate constant of the least reactive class. By this criterion, after blocking six thiols the remaining thiols, namely from the 7th to the 12th, can be treated as a single kinetic class, and the calculated rate constant defined as \(k_{7-12}\) is 1.2 × 10⁻³ M⁻¹ s⁻¹. The relation between the \(df(t)/dt\) value and the number of blocked thiols is shown in Table I. The \(df(t)/dt\) value when a certain number of thiols is blocked is basically the same regardless of the amount of probe mixed at the beginning of the reaction. However, as more thiols are blocked, \(df(t)/dt\) decreases sharply, and upon blocking approximately two thiols it reaches about the value of the final slope with \(p ≥ 6.0\). This would suggest that the rate constants of five thiols, \(n = 2, \ldots, 6\), vary in the range from a higher value to about \(k_{7-12}\).

The results described above suggest that there are several kinetically distinguishable classes of thiols characterized by \(k_1, k_2, k_3\) to \(k_9\), and \(k_{12}\). However one cannot exclude the possibility that blocking any one or two of the 12 reactive thiols might reduce the rate constant of the others (negative cooperativity).

**[Ca²⁺] Dependence of the Rates of Probe Incorporation**—The rate of incorporation into the most reactive thiol (\(k_1\)) is virtually independent of [Ca²⁺] (Fig. 6A), whereas \(k_2\) (Fig. 6B) and \(k_{7-12}\) (Fig. 6C) show appreciably large changes depending upon [Ca²⁺]. The value of \(k_2\) increases in the range of 10⁻⁶ to 10⁻⁴ M Ca²⁺ in which the activation of enzyme activity (cf. Refs. 6, 7, 14) and Ca²⁺ binding to the high affinity (14, 15) sites take place; \(k_3\) decreases in the range of [Ca²⁺] ≥ 10⁻⁴ M, again parallel with the inhibition of ATPase activity (6, 7, 12, 14) and with Ca²⁺ binding to the low affinity (14, 15) sites. The [Ca²⁺]-dependent changes of \(k_{7-12}\) show a reciprocal relation to those of \(k_3\), namely \(k_{7-12}\) decreases in the

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**Table I**

<table>
<thead>
<tr>
<th>Dns-Cys-Hg/enzyme</th>
<th>Number of blocked thiols/10⁵ daltons</th>
</tr>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>mol/mol</td>
<td>1.72</td>
</tr>
<tr>
<td>1 × 10⁻³ × df(t)/dt, M⁻¹ s⁻¹</td>
<td>1.12</td>
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</table>

**Fig. 5.** Second order expression of the process of thiol blockage with Dns-Cys-Hg. The data of the same experiment shown in Fig. 4 were calculated as described in the text. Values calculated by using the left side of Equation 1 were plotted as a function of reaction time.
activation range of \([\text{Ca}^{2+}]\) and increases in the inhibitory range of \([\text{Ca}^{2+}]\). These results suggest that changes of \(k_1\) and \(k_{2-12}\) would reflect \(\text{Ca}^{2+}\)-controlled conformational states of the enzyme involved in the mechanism by which the regulation of enzyme activity takes place. The fact that, in both activating and inhibitory ranges of \([\text{Ca}^{2+}]\), changes of \(k_5\) and \(k_{7-12}\) take place in a reciprocal fashion would indicate that the changes of \(k_5\) and \(k_{7-12}\) reflect changes of enzyme conformation in different regions of the enzyme molecule.

**DISCUSSION**

Various types of thiol-directed probes have been used in the studies of sarcoplastic reticulum (29–43). As shown here, the fluorescent probe, Dns-Cys-Hg (21), has several new advantages as follows. (a) The reaction of Dns-Cys-Hg with thiols of SR \(\text{Ca}^{2+}\)-ATPase is stoichiometric, and unlike the reaction with other reagents, in which the number of blocked thiols has to be determined at various times of reaction, the number of thiols to be blocked can be controlled by the amount of reagent mixed with the enzyme. (b) Because of the rapidity of the reaction, the enzyme would be less susceptible to the secondary effects, such as oxidation, compared with the conventional pseudo-first order analysis of thiol reaction with covalently reacting reagents, which normally requires an excess of reagent over the enzyme and rather lengthy times of reaction. (c) Because of the relatively large increase in fluorescence intensity upon incorporation of the probe into the protein, the time course of thiol blocking can be easily monitored. Furthermore, the fact that various thiols of the purified ATPase react at distinctly different rates with the probe, has permitted the blocking of different classes of thiol in a sequential fashion.

According to the work of Leavis and Lehrer (21), the spectral property of the protein-attached Dns-Cys-Hg varies depending upon the environment of the attached probe. Thus, Dns-Cys-Hg may serve as a probe for the studies of microenvironment of protein thiols (21). In the case of SR \(\text{Ca}^{2+}\)-ATPase, however, the protein-attached Dns-Cys-Hg shows the same emission characteristics regardless of the thiol class (cf. Fig. 1). This would indicate that the thiol-attached Dns-Cys-Hg is in a nonpolar domain regardless of the classes of thiol described here. A similar finding has been reported in the Dns-Cys-Hg-labeled (Na\(^+\) + K\(^+\))-ATPase (44).

Attempts to classify the thiols in terms of reactivity with various thiol reagents and of their involvement in the ATPase activity have already been made by other workers in studies of the purified ATPase and the sarcoplasmic reticulum membrane (Table II). Assignment of several kinetic classes shown in this study is in reasonable agreement with the previous reports. It appears that blocking the most reactive thiol, characterized by \(k_1\), probably identical with the class that reacts rapidly with N-ethylmaleimide and Nbs\(_5\) (Table II), has little or no effect on ATPase activity. Upon further blocking of five thiols, both the phosphoenzyme formation and ATP hydrolysis rate are almost completely inhibited; these thiols may be equivalent with the reported intermediate class (Table II). The 7th through 12th thiols characterized by \(k_{7-12}\) would correspond with the reported slow class.

We have found that the rates of incorporation of Dns-Cys-Hg into each of these classes are changed as a function of \([\text{Ca}^{2+}]\) during the incorporation. The changes of \(k_5\) and \(k_{7-12}\) in the \([\text{Ca}^{2+}]\) range of 10\(^{-6}\) to 10\(^{-3}\) \(\text{M}\) at \([\text{Ca}^{2+}]\) higher than 10\(^{-4}\) \(\text{M}\) parallel the \(\text{Ca}^{2+}\) binding to the \(\gamma\) sites (\(K_\gamma = 3 \times 10^4 \text{M}^{-1}\)) and that to the \(\alpha\) sites (\(K_\alpha = 6 \times 10^5 \text{M}^{-1}\)), respectively. Thus, it appears that the \(\text{Ca}^{2+}\)-dependent changes of \(k_5\) and \(k_{7-12}\) reflect the changes in conformation of the enzyme molecule controlled by binding of \(\text{Ca}^{2+}\) to the two types of sites. The conformational states could be expressed as \((\alpha(+) \gamma(-)), (\alpha(+) \gamma(+)\), and \((\alpha(+) \gamma(+)\), which predominate at pH 9.0, 5.7, and 2.7, respectively. The symbols + and − indicate occupied and empty sites, respectively. To facilitate further discussion, values of \(k_1\), \(k_5\), and \(k_{7-12}\) in the three conformational states are tabulated in Table III. The \((\alpha(+) \gamma(-))\) conformation is clearly distinguishable from the \((\alpha(-) \gamma(-))\) and \((\alpha(+) \gamma(+)\) conformations, by its higher value of \(k_2\) and the lower value of \(k_{7-12}\). The \((\alpha(-) \gamma(-))\) and \((\alpha(+) \gamma(+)\) conformations are somewhat similar to each other but there is an appreciable difference, in that the former has a considerably higher value of \(k_2\).

The important point in this study is that the three conformational states described above have an intimate relation to the activation and inhibition of the enzyme activity. Upon the addition of ATP, the enzyme in the \((\alpha(+) \gamma(-))\) state carries out a whole cycle of the ATPase reaction, as seen from the activation of both the phosphoenzyme formation and ATP hydrolysis; whereas in the \((\alpha(+) \gamma(+)\) state the enzyme forms the phosphorylated intermediate but the reaction steps in which phosphoenzyme is decomposed are inhibited, and in
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The rates of Dns-Cys-Hg incorporation into thiol classes at three representative states of enzyme

<table>
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<th>pCa</th>
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<tr>
<td>Fast (h₁)</td>
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<tr>
<td>Intermediate (h₁)</td>
<td>5.7</td>
</tr>
<tr>
<td>Slow (h₁-12)</td>
<td>2.7</td>
</tr>
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</table>

The results of three experiments are given as mean ± S.D.

<table>
<thead>
<tr>
<th>Reactive thiols</th>
<th>Material</th>
<th>Reagent</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>Fast n</td>
<td>Intermediate n</td>
<td>Slow n</td>
<td>Purified ATPase</td>
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<tr>
<td>1-</td>
<td>5+</td>
<td>6-</td>
<td>4</td>
</tr>
<tr>
<td>2-</td>
<td>12+</td>
<td></td>
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</tr>
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</tr>
<tr>
<td>1-</td>
<td>4+</td>
<td>5-</td>
<td>FSR</td>
</tr>
</tbody>
</table>

TABLE II

Thiol classes of purified Ca²⁺-ATPase and of sarcoplasmic reticulum

n, number of thiols per 10⁶ daltons; FSR, fragmented sarcoplasmic reticulum; + and −, blocking of thiol has and does not have an inhibitory effect on ATPase activity, respectively.

TABLE III

The α ( α)/γ ( γ) state both the phosphoenzyme formation and decomposition are inhibited. This suggests that various conformational states controlled by Ca²⁺ binding are directly involved in the mechanism by which the regulation of enzyme activity takes place.

It should be noted that Ca²⁺-dependent changes of k₂ and k₁-12 always take place in opposite directions. This would suggest that Ca²⁺ binding to the α sites produces a conformation in the enzyme in which the local region represented by the second thiol would become more exposed, whereas the region represented by the least reactive class would become hindered; and upon Ca²⁺ binding to the γ sites the former becomes hindered and the latter becomes more exposed. However, the thiol reactivity would not necessarily be proportional to the degree of exposure of the group (cf. Ref. 45), but may be influenced by many factors, such as hydrophobicity and charge of the microenvironment around the thiol and of the reagent as well. Although at the moment we are unable to elucidate the details of the mechanism by which the reactivity of the thiol is altered, it appears that Ca²⁺ binding exercises differential effects on the enzyme conformation in different regions of the enzyme molecule.

All experiments shown in this paper have been carried out in the absence of ATP. It is conceivable that upon addition of ATP a variety of new conformational states would be produced, each of which presumably corresponds to some intermediate in the ATPase reaction cycle (for the proposed reaction sequence, see Refs. 4, 15, 46–50). In fact a number of reports have suggested that changes in enzyme conformation are associated with some steps of the enzyme reaction (17, 31, 38–42). Obviously there remains the important task of establishing the precise correspondence between the conformational states described here and the dynamic conformational changes occurring during the enzyme reaction cycle.

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