Structural Effects of Substrate Utilization on the Adenosinetriphosphatase Chains of Sarcoplasmic Reticulum†

Takahide Watanabe and Giuseppe Inesi*

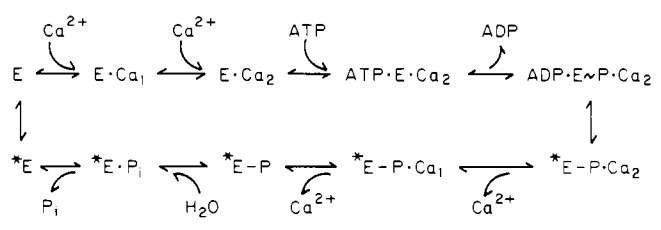
ABSTRACT: Addition of ATP to suspensions of fragmented sarcoplasmic reticulum (SR) containing low concentrations of a detergent that does not by itself produce major vesicular disruption is followed by a transient reduction in turbidity accompanied by solubilization of the vesicles. The effect of ATP is Ca^{2+} dependent and proceeds in parallel with utilization of the nucleotide as a substrate for the SR ATPase. Analogous effects are observed with other substrates producing enzyme phosphorylation at the catalytic site. The effect of ATP can also be detected in studies of fluorescence energy transfer between enzyme chains, by using the technique of Vanderkooi et al. [Vanderkooi, J., Ierokomas, A., Nakamura, H., & Martonosi, A. (1977) *Biochemistry 16*, 1262]. For this purpose, ATPase chains are labeled separately with *N*-(iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)ethylenediamine (IAEDANS) and 6-(iodoacetamido)fluorescein (IAF). Samples of vesicles uniformly labeled with either IAEDANS or IAF, mixtures of two populations of vesicles uniformly labeled with either fluorophore, and vesicles containing randomized chains labeled with either fluorophore are used as experimental systems. In the last system, significant energy transfer from IAEDANS (donor) to IAF (acceptor) is revealed by fluorescence spectra and measurements of donor fluorescence intensity and lifetime. This is attributed to close interactions between ATPase chains within the membrane bilayer. It is then found that in the presence of low detergent concentrations, ATP changes the extent of energy transfer between labeled ATPase chains, consistent with destabilization of the interaction of chains. The observed effects are attributed to a reversible structural transition concomitant with enzyme phosphorylation and related to catalytic and transport function.

The sarcoplasmic reticulum (SR) calcium pump discovered by Hasselbach & Makinose (1941, 1963), and Ebashi & Lipman (1962), entails ATP utilization for vectorial transport of calcium ion against a concentration gradient. To this effect, a number of sequential reactions have been proposed for completion of each catalytic and transport cycle [for reviews, see deMeis & Vianna (1979), Yamamoto et al. (1979), and Inesi et al. (1980)], as shown in Scheme I which is derived from that originally given by Carvalho et al. (1976).

In addition to chemical events, structural transitions of the enzyme and transport units are likely to accompany a vectorial phenomenon such as active transport. In fact, a Ca^{2+}-induced conformational change of the ATPase protein was demonstrated by spectroscopic methods (Coan & Inesi, 1977; Champeil et al., 1978; Ikemoto et al., 1978; Murphy, 1978; Dupont & Leigh, 1978). This effect, as well as site–site interaction, is required to explain the cooperative behavior of Ca^{2+} binding and the consequent enzyme activation (Inesi et al., 1980).

It was also proposed that another structural transition (represented by the asterisk notation in Scheme I) must follow utilization of ATP in order to produce a change in orientation and a reduction in affinity of the calcium binding sites (deMeis & Vianna, 1979). We report here a series of experiments demonstrating that a reversible structural transition of the ATPase chains occurs when the enzyme is phosphorylated by a variety of substrates, including ATP in the forward direction and P_i in the reverse direction of the catalytic cycle.

Scheme I



Materials and Methods

SR vesicles were obtained from rabbit hind leg white muscle as previously described (Eletr & Inesi, 1972). Protein concentrations were determined by the Lowry method.

Reagents. *N*-(Iodoacetyl)-*N'*-(5-sulfo-1-naphthyl)-ethylenediamine (IAEDANS) was obtained from Sigma. 6-(Iodoacetamido)fluorescein was a gift of Dr. S. Highsmith. Dodecyloctaethylene glycol monoether ($C_{12}E_8$) (Nikko, Japan) was obtained through the intervention of Professor Y. Tonomura. 1,4-Bis(4-methyl-5-phenyloxazol-2-yl)benzene (Me_2POPOP) was purchased from Eastman.

Fluorescent Labeling of SR. SR (5 mg/mL), suspended in 20 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, 80 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, and 20% glycerol, was labeled with 1.0 mM IAF or IAEDANS separately at 25 °C in the dark. Solutions of IAF and IAEDANS in dimethylformamide were made fresh each time. The reaction was terminated by the addition of 60 mM β-mercaptoethanol. The two labeled SR samples were then mixed in different ratios (still maintaining the same amount of total protein) and applied to a Sephadex G-50 column (1.3 × 28.0 cm) equilibrated with 20 mM 4-morpholinepropanesulfonic acid (Mops), pH 6.9, 80 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, and 20% glycerol, to remove the unreacted chromophores. In some cases, deoxycholate (DOC) was added at the concentration of 1.0 mg of DOC/mg of protein to solubilize the two labeled SR samples and reconstitute vesicles with a

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mixed population of ATPase chains labeled with IAF and IAEDANS.

Determination of Bound Chromophore. The concentrations of bound IAEDANS and IAF were determined by absorption after solubilizing the SR with 1.7% DOC by using the following molar extinction coefficients: 6.1×10^3 at 336 nm for IAEDANS and 8.5×10^4 at 492 nm for IAF.

Turbidity and Fluorescence Measurements. Steady-state fluorescence spectra and fluorescence intensity were obtained by using an Aminco-Bowman spectrofluorometer. Changes in turbidity were followed by measuring 90° light scattering at 350-nm wavelength.

Fluorescence lifetime measurements were performed with an SLM Instruments phase-modulation spectrofluorometer (Spencer & Weber, 1969) that was assembled and kindly made available to us by Dr. J. Lakowicz of our department. A modulation frequency of 10 MHz, with a cross-correlation signal of 10 Hz, was used. Excitation and emission wavelengths (340 and 470 nm) were selected by monochromators. At these wavelengths, interference by nonspecific chromophores or light scattering was minimal (<3%) as revealed by spectra of samples labeled with either donor or acceptor alone, or both donor and acceptor.

As the lifetime measurements were related to a reference solution (Lakowicz & Cherek, 1980; Lakowicz et al., 1981), sample and reference (Me_2POPOP) of approximately equal emission intensities were placed in a double cuvette holder, and several alternative measurements of phase shift and modulation were obtained. The lifetime of the reference was assumed to be 1.45 ns. The results were acquired and analyzed by an interfaced calculator.

The reaction mixtures for optical measurements contained 20 mM Tris-HCl, pH 8.0, 80 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, and 20% glycerol. SR samples were used within 1 day following labeling. ATPase activity was followed by determining either P_i with the molybdovanadate reaction or $^{32}P_i$ from [γ-^{32}P]ATP.

Results

Detergent and ATP Effects on Membrane Solubilization. In preliminary experiments, we found that an effect of ATP on the membranous association of ATPase chains is most clearly demonstrated when SR vesicles are exposed to low concentrations of a nonionic detergent such as $C_{12}E_8$. Therefore, we first determined the pattern of membrane solubilization upon stepwise addition of detergent. Solubilization is accompanied by a reduction in turbidity which we followed by measuring 90° light scattering.

The pattern of SR solubilization as a function of $C_{12}E_8$ concentration is diphasic. It is shown in Figure 1 that an early reduction in turbidity is obtained at low detergent concentrations, followed by a plateau and then by a further reduction in turbidity as more detergent is added. It is clear that not only the detergent concentration but also the detergent:protein ratio is important.

Electron microscopic inspection of negatively stained samples reveals that at low detergent concentrations (e.g., the plateau region in Figure 1) the vesicular structure of SR is mostly retained, although the appearance of the vesicles is somewhat abnormal due to loss in resolution of the outer granular layer (Inesi & Scales, 1974). With regard to function, the remaining vesicles are totally leaky to Ca^{2+} but retain high ATPase activity.

When saturating concentrations of detergent are reached, the turbidity of the SR suspension is reduced to approximately

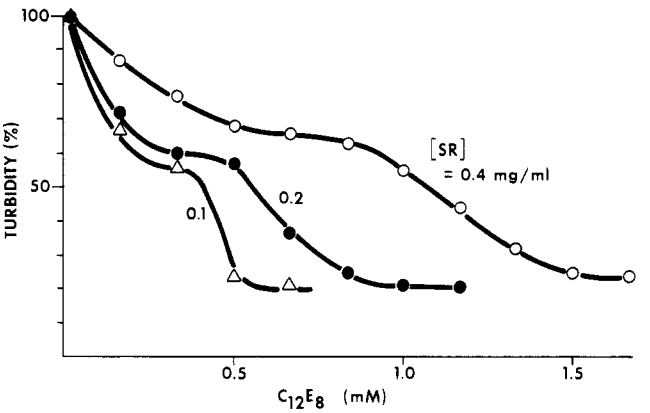

FIGURE 1: Turbidity changes induced by stepwise addition of $C_{12}E_8$ to samples containing different concentrations of SR vesicles. The reaction mixture contained 0.1 (△), 0.2 (●), or 0.4 mg (○) of SR protein/mL in 20.0 mM Tris-HCl, pH 8.0, 80 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, and 20% glycerol. Turbidity was measured by light scattering at a 350-nm wavelength.

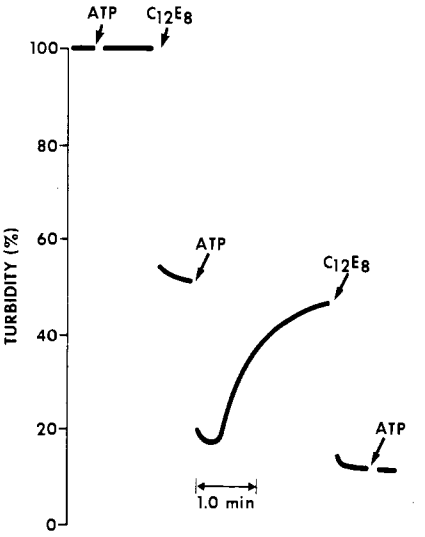

FIGURE 2: Turbidity change induced by $C_{12}E_8$ and ATP. 0.2 mg of SR/mL was suspended in a medium as described in Figure 1. The concentration of ATP and $C_{12}E_8$ added each time was 16.7 μM and 0.67 mM, respectively.

20% of the original and total loss of vesicular structure is noted. This is in accordance with the observations of Dean & Tanford (1978), who obtained dissociation of ATPase chains in similar conditions. ATPase activity is retained by the solubilized protein.

It is apparent that stepwise exposure of SR to increasing concentrations of $C_{12}E_8$ produces two consecutive and distinct effects: an initial alteration of membrane structure and then a complete solubilization of the membrane.

Addition of ATP to SR in the absence of detergent or in the presence of saturating detergent concentrations does not produce any significant turbidity changes (Figure 2). However, if ATP is added in the presence of low $C_{12}E_8$ concentrations (e.g., the plateau in Figure 1), a further and pronounced reduction in turbidity is obtained (Figure 2). A parallel loss of vesicular structure, and appearance of amorphous aggregates, is observed by electron microscopy.

It is of great interest that such an effect of ATP is reversible, as shown by an increase in turbidity (Figure 2), as well as by the reappearance of numerous vesicular structures after a few minutes. The duration of the ATP effect is directly propor-

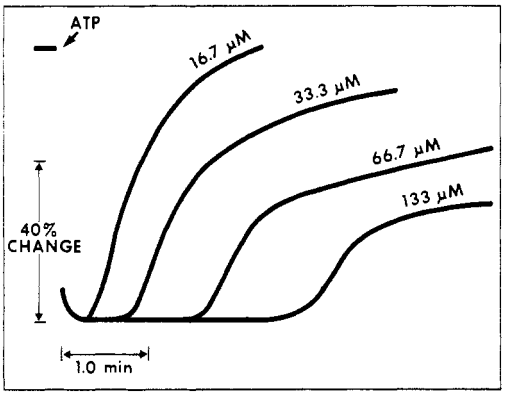

FIGURE 3: ATP dependence of the turbidity decrease in the presence of 0.67 mM $C_{12}E_8$. 0.2 mg of SR/mL was suspended in the same medium as described in Figure 1. The concentrations of ATP are shown in the figure. Percent turbidity change is calculated from its level after $C_{12}E_8$ addition.

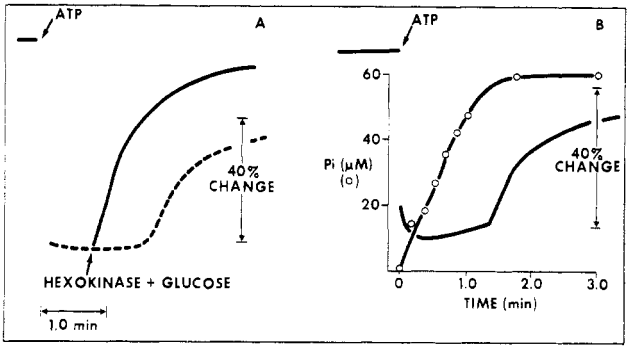

FIGURE 4: (A) Interference of hexokinase and glucose with the ATP effect on turbidity. Hexokinase (11 μg/mL) and glucose (0.8 mM) were added as indicated (—). A control shows the course of turbidity change in the absence of hexokinase and glucose (- - -). The concentration of ATP was 66.7 μM. (B) Relation between the time courses of turbidity change and $^{32}P_i$ liberation from [γ-^{32}P]ATP. Both measurements were done on the same sample. 60.7 μM [γ-^{32}P]ATP was added at time zero to a 3.0-mL reaction mixture, and a 100-μL aliquot was taken out at serial intervals to measure $^{32}P_i$ release. Other conditions are the same as in Figure 2. 0.67 mM $C_{12}E_8$ was present in (A) and (B).

tional to the concentrations of ATP used (Figure 3). However, if glucose and hexokinase are added to drain off ATP, a rapid reversal of the clearing effect is observed (Figure 4A). In fact, it can be demonstrated that reversal occurs soon after utilization of available ATP, as indicated by the release of a corresponding amount of P_i through the hydrolytic ATPase reaction (Figure 4B).

The clearing effect of ATP is not a result of calcium accumulation by SR since it is observed even in the presence of the ionophore A-23187. On the other hand, utilization of ATP by the ATPase is necessary, as AMP-P(NH)P and ADP are not effective. If an ATP regenerating system is added following ADP, the clearing effect is observed (Figure 5).

Another important feature of the ATP clearing effect is that Ca^{2+} is an absolute requirement (Figure 6). We also found that this requirement is identical with that exhibited by ATPase activity in the same experimental conditions. In the presence of Ca^{2+}, ITP and acetyl phosphate can substitute for ATP in producing the clearing effect, although at lower rates and to a lesser extent than ATP, in analogy to their slow utilization as substrates for the enzyme.

The clearing effect is obtained when the enzyme is phosphorylated with P_i instead of ATP (Figure 6C,D). In this case,

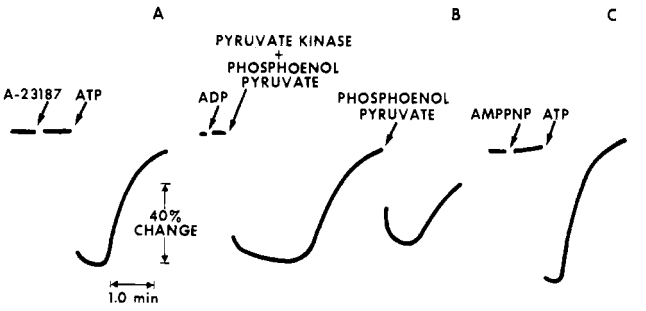

FIGURE 5: (A) Presence of the calcium ionophore A-23187 (3.3 μM) does not prevent the effect of ATP (66.7 μM) on turbidity. (B) ADP (33.3 μM) is not effective; however, the effect is observed repeatedly upon addition of an ATP-generating system: pyruvate kinase (4 μg/mL) and phosphoenolpyruvate (83 μM the first time and 17 μM the second time). (C) AMP-P(NH)P (31 μM) is not effective, but ATP (33 μM) is. Reaction media as described for Figure 2.

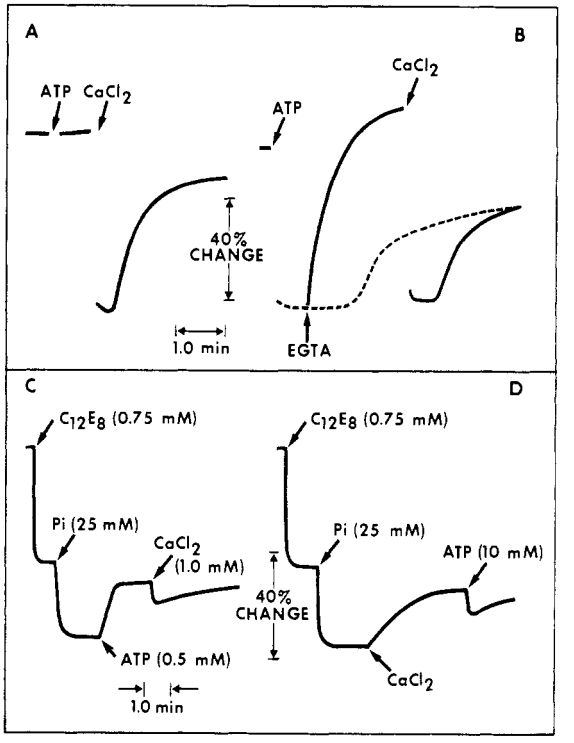

FIGURE 6: Ca^{2+} dependence of the ATP effect. (A) Reaction mixture as described for Figure 2, except for omission of $CaCl_2$ and addition of 1.0 mM EGTA. ATP and $CaCl_2$ were then added at 33.3 μM and 1.0 mM final concentrations, respectively. (B) Reaction mixture as described for Figure 2. ATP, EGTA, and $CaCl_2$ were added to yield 66.7 μM, 0.5 mM, and 0.5 mM final concentrations. The dashed line shows the time course of the ATP-induced turbidity change when no EGTA was added. (C and D) Clearing effect produced by phosphorylation of the enzyme with P_i in the absence of Ca^{2+}. The reaction mixture contained 20 mM Mops, pH 6.5, 5 mM $MgCl_2$, 1 mM EGTA, 20% glycerol, and 0.2 mg of SR protein/mL. $C_{12}E_8$, P_i, ATP, and $CaCl_2$ were added to reach the concentrations indicated.

Ca^{2+} must be removed as required for the P_i reaction (Masuda & deMeis, 1973). In the absence of Ca^{2+}, ATP inhibits the P_i effect.

ATP-Dependent Dissociation and Reassociation of ATPase Chains. We then explored further the clearing effect of ATP with respect to dissociation and reassociation of ATPase chains. The interaction of ATPase chains was the subject of an elegant study by Vanderkooi et al. (1977), who detected fluorescence energy transfer among chains that were labeled with a different fluorophore in each of two separate populations of vesicles,

and were then randomized by solubilization with deoxycholate and reconstitution of mixed vesicles upon removal of the detergent. The authors measured fluorescence intensities of donor and acceptor and checked by determination of nanosecond decay of donor fluorescence that the observed changes were related to energy transfer. We utilized their techniques to find out whether changes in the relationship among ATPase chains could be demonstrated with our conditions by measurements of fluorescence energy transfer.

In preliminary studies of fluorescence labeling kinetics, we incubated two separate populations of SR vesicles with IAEDANS (donor) or IAF (acceptor), quenched the reaction at serial times by addition of mercaptoethanol, and removed the unreacted label by column chromatography. In these experiments, we obtained seemingly asymptotic levels for incorporation of either label and demonstrated by sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis that the labels were associated exclusively with the ATPase fraction. We also excluded the association of significant amounts of label with chloroform–methanol (2:1) extracts of SR lipids. In most of the following experiments, labeling was obtained by a 2-h incubation, producing incorporation of approximately 1 mol of IAF or 2 mol of IAEDANS per mol of ATPase chains, based on a M_r of 100 000 for each chain, and assuming that ATPase accounts for 75% of the total SR protein. The enzyme activity of the labeled samples remained essentially the same as that of control vesicles exposed to the same incubation media in the absence of either label.

In accordance with the observations of Vanderkooi et al. (1977), we found that when two populations of vesicles are labeled separately with IAEDANS or IAF, and mixed in the absence of detergent, there is no significant exchange of labeled chains among different vesicles, and the two fluorophores remain at distances precluding energy transfer. However, when the two populations of vesicles are mixed and solubilized in the presence of DOC, and then reconstituted by removal of DOC, the emission spectra show a definite reduction of the IAEDANS (donor) fluorescence and an increase of the IAF (acceptor) fluorescence intensity (Figure 7A). This indicates that solubilization and reconstitution produce random assembly of chains reacted with different labels. Thereby, the average distance between different fluorophores becomes sufficiently short to allow energy transfer. We found that for our experiments an ideal ratio between the concentrations of protein labeled with IAEDNS (donor) and IAF (acceptor) was 3:7 (Figure 7B). Under these conditions, no significant overlap of the emission spectrum of IAF, or other nonspecific emission, was present at 470-nm wavelength. Therefore, we chose this wavelength to monitor changes in the fluorescence emission of the IAEDANS label, as an expression of energy transfer and interaction of ATPase chains.

Similar conclusions were reached by measurements of donor (IAEDANS) fluorescence lifetime by phase fluorometry, for which we obtained values ranging between 15.0 and 15.4 ns (Table I) in the absence of acceptor. These values are in agreement with those obtained by Vanderkooi et al. (1977), who differentiated time-resolved decay measurements in two components corresponding to 2.7 and 18.8 ns. Our phase measurements include the averaged contribution of the two components.

Nearly identical values were obtained in samples of vesicles labeled separately with IAEDANS or IAF, and then mixed without randomization of chains (e.g., no DOC solubilization and reconstitution). On the other hand, occurrence of energy transfer was definitely demonstrated by a reduction of the donor fluorescence lifetime to 11.4–11.8 ns following chain randomization (Table I).

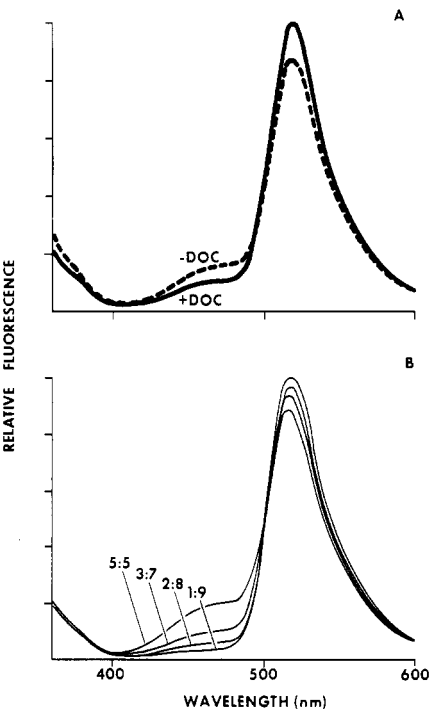

FIGURE 7: (A) Fluorescence spectra of a 3:7 mixture of SR vesicles labeled separately with IAEDANS and IAF. The two populations of vesicles were simply mixed or treated with DOC for randomization of chains before chromatography for removal of nonreacted (and quenched) labels. (B) Fluorescence spectra of different ratios of SR vesicles labeled separately with IAEDANS and IAF. The two populations of vesicles *were* mixed and treated with DOC for randomization of chains before chromatography. Protein concentration was 0.163 mg/mL in both (A) and (B). Excitation wavelength was 340 nm. Fluorescence units are relative to maximal emission.

Table I: Phase Lifetimes (ns) of Donor (IAEDANS) Fluorescence in Vesicles Containing ATPase Chains Labeled As Specified[a]

labeling	control	$C_{12}E_8$, 0.5 mM	ATP, 66.7 μM
IAEDANS only	15.0–15.4	15.8–17.6	14.9–15.6
IAEDANS and IAF, in separate vesicles	14.0–15.2	13.2–15.0	11.4–13.0
IAEDANS and IAF, randomized	11.4–11.8	12.0–12.3	13.0–13.4

[a] See Materials and Methods for conditions of labeling and lifetime measurements. The figures given for each condition are the lowest and highest values obtained by four to five determinations in each of two separate preparations. Reaction medium was as described for Figure 10.

In samples containing two populations of vesicles reacted with the two labels separately and then mixed without DOC treatment (thereby preventing randomization of the labeled chain and energy transfer), additions of nonsaturating concentrations of $C_{12}E_8$, and then of ATP (Figure 8), produced a slow reduction in the fluorescence intensity of IAEDANS (donor). A parallel reduction in the donor fluorescence lifetime was also observed (Table I). This effect is consistent with structural destabilization of the vesicles by the two reagents, favoring exchange of chains between the two populations of vesicles and randomization of chains labeled with the two fluorophores. Thereby, energy transfer is permitted. In this case, addition of excess $C_{12}E_8$ produced chain dissociation and

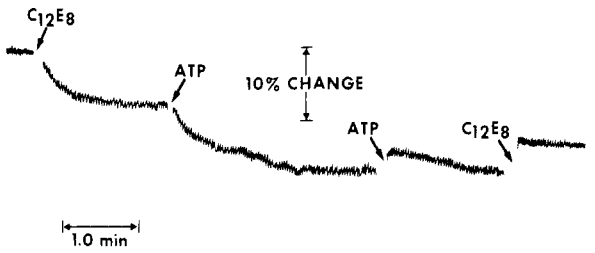

FIGURE 8: Effects of $C_{12}E_8$ and ATP on donor (IAEDANS) fluorescence. The sample contains a 3:7 mixture of IAEDANS- and IAF-labeled SR vesicles which *were not* treated with DOC for randomization of chains. The reaction medium contained 0.15 mg of protein/mL, 20 mM Tris-HCl, pH 8.0, 80 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, and 20% glycerol. $C_{12}E_8$ was added to yield a 0.5 mM concentration each time and ATP to yield 66.7 μM the first time and 33.3 μM the second time. Excitation, 340 nm; emission, 470 nm.

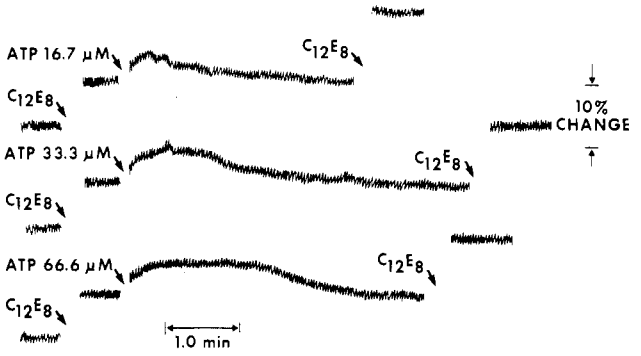

FIGURE 9: Effects of $C_{12}E_8$ and ATP on donor (IAEDANS) fluorescence. The sample contained a 3:7 mixture of IAEDANS- and IAF-labeled SR vesicles which *were* treated with DOC for randomization of chains. Reaction mixture was as described for Figure 8. The concentrations of added $C_{12}E_8$ were 0.50 and 0.67 mM the first and second time, respectively, for each run.

loss of energy transfer, manifested by an increase in IAEDANS fluorescence intensity (Figure 8).

Another series of observations was made with vesicles that were reconstituted following labeling with the two fluorophores separately and solubilization with DOC. As described above, these vesicles contain randomized chains, permitting significant energy transfer between the two fluorophores. In this case, addition of low $C_{12}E_8$ concentrations produced a rise of the donor fluorescence intensity (Figure 9), consistent with an increase in the average distance among chains and a reduction in energy transfer. When ATP was added in the presence of such low detergent concentrations, a further increase in fluorescence intensity (Figure 9) and lifetime (Table I) was noted. The effect of ATP on fluorescence intensity was transient, and its duration was proportional to the concentration of ATP used. If more $C_{12}E_8$ was added, a further and final increase in fluorescence was observed, corresponding to total dissociation of the labeled chains.

The effect of ATP was not observed if the vesicles were not pretreated with a low concentration of $C_{12}E_8$ (Figure 10); however, the vesicles were readily sensitized by the addition of this detergent. Furthermore, in reaction mixtures in which the free Ca^{2+} concentration was reduced to less than 10^{-8} M with [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA), $C_{12}E_8$ produced its effect, but ATP did not (Figure 10). The effect was readily produced upon addition of Ca^{2+} following ATP.

As expected, corresponding effects in the opposite direction (e.g., reduction in intensity) were obtained when the fluorescence emission of IAF (acceptor) was measured at 515-nm wavelength. In this case, however, interference by the

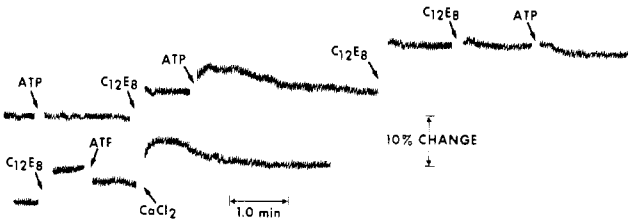

FIGURE 10: $C_{12}E_8$ and Ca^{2+} requirements for the effect of ATP on donor (IAEDANS) fluorescence. Sample and reaction mixture as described for Figure 9. In the lower run, the mixture contained 0.5 mM EGTA instead of 50 μM $CaCl_2$. The $C_{12}E_8$ concentrations were 0.5 mM for the first addition on both runs and 0.67 mM for the second and third additions on the upper run. In the lower run, $CaCl_2$ was added to yield a 0.5 mM concentration.

overlapping IAEDANS spectrum and other effects of ATP not related to energy transfer precluded accurate quantitation.

Discussion

With the experimentation described above, we have first monitored the turbidity of suspensions of SR vesicles upon stepwise addition of the detergent $C_{12}E_8$. In the presence of low $C_{12}E_8$ concentrations which do not produce major disruption of the vesicles, we have then obtained a transient reduction in turbidity upon addition of ATP (Figure 2). This effect suggests that ATPase chains undergo reversible dissociation when ATP is used as a substrate by the Ca^{2+}-activated enzyme.

Further evidence for the dissociating effect of ATP was obtained by following fluorescence energy transfer between chains labeled with two different fluorophores. This technique was first used in vesicular preparation of SR ATPase by Vanderkooi et al. (1977), who demonstrated energy transfer by measurements of fluorescence intensities and by determination of nanosecond decay of donor fluorescence.

We found that under conditions identical with those used to observe the clearing effect of ATP, utilization of this substrate by the Ca^{2+} ATPase affects energy transfer between neighboring chains of the enzyme. In fact, when we used vesicles reconstituted by randomization of two populations of chains that were labeled separately with donor and acceptor fluorophores, energy transfer was transiently reduced in parallel with utilization of ATP (Figure 9). On the other hand, if the two populations of vesicles were simply mixed without randomization of the chains (e.g., without "DOC treatment"), ATP produced a gradual *increase* in energy transfer (Figure 8) evidently related to exchange of chains among vesicles of the two populations, occurring during the ATP-induced dissociation. These diverging effects, which were obtained depending on whether randomized or nonrandomized ATPase preparations were used, demonstrate that the observed fluorescence effects are in fact due to energy transfer, and are not caused primarily by turbidity changes which are also associated with enzyme phosphorylation in both native and reconstituted systems. This was further demonstrated by the lack of fluorescence changes in vesicles labeled with only one fluorophore. Measurements of fluorescence spectra and donor fluorescence intensity and lifetime were all supportive of these conclusions.

It is by now well established that the ATPase protein accounts for more than half the mass of the SR membrane and that the amphiphilic ATPase chains are tightly packed with a polar portion protruding from the cytoplasmic membrane surface and a nonpolar portion inserted into the membrane bilayer [for a review, see Inesi (1979)]. This arrangement evidently permits energy transfer between fluorophores located

on different chains. Our observation indicates that enzyme phosphorylation produces a structural change which, in the presence of minimal detergent concentrations, causes dissociation of chains and changes in energy transfer.

It should be noted that low concentrations of $C_{12}E_8$ are required in order to observe the substrates's effect on light scattering and fluorescence energy transfer. It is likely that small amounts of detergent which do not produce major disruption still reduce the structural stability of the vesicles, thereby permitting fusion and/or protein exchange as revealed by a slow increase in energy transfer (Figure 10). Under these conditions, enzyme phosphorylation associated with substrate utilization evidently facilitates transition from the lamellar to the mixed micelle structure and dissociation of the ATPase chains. We found that the substrate-induced dissociation occurs in preference with nonionic (i.e., $C_{12}E_8$ or Triton X-100) rather than with ionic (i.e., DOC or $NaDodSO_4$) detergents.

An important feature of the effect of ATP is that it requires Ca^{2+}, and ATP can be substituted only by analogues which are utilized as substrates by the ATPase. Therefore, the structural effect is intimately related to the catalytic and transport cycle. No structural evidence for a structural change associated with substrate utilization by the ATPase has been reported as yet, even though a change in orientation of the calcium sites ($ADP{\cdot}E{\sim}P{\cdot}Ca_2 \rightleftharpoons ADP + {}^*E{-}P{\cdot}Ca_2$; Scheme I) must occur to satisfy the vectorial requirements of active transport (deMeis & Vianna, 1979). Although low detergent concentrations are required to observe total dissociation of chains upon substrate utilization, our observations indicate that a structural perturbation is produced by enzyme phosphorylation at the active site. We suggest that this perturbation plays a role in the mechanism of catalysis associated with vectorial transport.

Acknowledgments

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