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# Effect of Solubilization on Adenosine 5′-Triphosphate Induced Calcium Release from Purified Sarcoplasmic Reticulum Calcium Adenosinetriphosphatase†

William L. Dean* and Robert D. Gray

ABSTRACT: ATP-induced $Ca^{2+}$ release from the purified sarcoplasmic reticulum $Ca^{2+}$-ATPase has been monitored in several different ATPase environments. Arsenazo III was used as a $Ca^{2+}$ indicator in stopped-flow experiments and was shown to detect the early burst in $Ca^{2+}$ transport, slower steady-state transport, and release of $Ca^{2+}$ from fragmented sarcoplasmic reticulum. ATP-induced rapid release of $Ca^{2+}$ followed by a slower rebinding step could be demonstrated for purified $Ca^{2+}$-ATPase in leaky vesicles if the reaction was slowed by lowering the pH to 6.1 and by including dimethyl sulfoxide in the reaction medium. At a dodecyl octaoxyethylene glycol monoether ($C_{12}E_8$) to protein weight ratio of 0.2, a detergent concentration too low for solubilization to occur, ATP-induced $Ca^{2+}$ release occurred more rapidly than for native leaky membranes, whereas the rebinding step was slower. In contrast, no $Ca^{2+}$ release was observed for any soluble preparation. The kinetics of $Ca^{2+}$ release was studied under conditions where the ATPase was monomeric or aggregated, and also in the presence of added phospholipid. The ATPase was shown to be monomeric by sedimentation equilibrium measurements in the presence of $Ca^{2+}$, ADP, and $\beta,\gamma$-methylene-ATP at a $C_{12}E_8$ to protein weight ratio of 2.0. It is concluded that solubilization of the $Ca^{2+}$-ATPase may result in uncoupling of ATP hydrolysis from ATP-induced $Ca^{2+}$ release.

The $Ca^{2+}$-ATPase[1] from sarcoplasmic reticulum has recently been shown to exhibit cooperative binding of two calcium ions per phosphorylation site (Inesi et al., 1980; Verjovski-Almeida & Silva, 1981) although earlier studies did not reveal cooperativity (Meissner, 1973; Ikemoto, 1975). Since this $Ca^{2+}$ binding level represents only one $Ca^{2+}$ per ATPase polypeptide in native membranes, the observed pumping stoichiometry of two $Ca^{2+}$ per ATP hydrolyzed suggests that only half of the total ATPase molecules transport $Ca^{2+}$ at any given time and that the functional unit of the ATPase is at least a dimer (Inesi et al., 1980; Verjovski-Almeida & Silva, 1981; Ikemoto et al., 1981; Froehlich & Taylor, 1976). This conclusion thus supports the proposal that the $Ca^{2+}$-ATPase is aggregated in its native membrane (Ikemoto et al., 1981). In contrast, there are data showing that monomeric ATPase in detergent solution retains many properties of the native enzyme (Dean & Tanford, 1978; Jorgensen et al., 1978; Dean & Gray, 1980). Furthermore, structural studies indicate that a single $Ca^{2+}$-ATPase polypeptide possesses all of the necessary structures for $Ca^{2+}$ transport (MacLennan et al., 1980; Green et al., 1980). However, the solubilized, monomeric enzyme lacks negative cooperativity of ATP hydrolysis (Inesi et al., 1980; Dean & Tanford, 1978), cooperativity of $Ca^{2+}$ binding (Verjovski-Almeida & Silva, 1981), and the ability to form a covalent phosphoenzyme intermediate from inorganic phosphate (Nestruck-Goyke & Hasselbach, 1981).

Solubilization of the $Ca^{2+}$-ATPase results in several experimental advantages, although it eliminates the possibility of assaying $Ca^{2+}$ transport since there is no aqueous compartment in detergent solution for $Ca^{2+}$ accumulation. However, a closely related activity which should be measurable in detergent solution is ATP-induced $Ca^{2+}$ release from the ATPase. Ikemoto (1976) used the $Ca^{2+}$-sensitive dye arsenazo III to observe rapid, ATP-induced release of $Ca^{2+}$ from purified ATPase in leaky vesicles. More recently Watanabe et

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[1] Abbreviations: Bis-Tris, [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane; $C_{12}E_8$, dodecyl octaoxyethylene glycol monoether; Tes, 2-[[tris(hydroxymethyl)methyl]amino]ethanesulfonic acid; ATPase, adenosinetriphosphatase; EGTA, ethylene glycol bis($\beta$-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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al. (1981) reported that addition of dimethyl sulfoxide was necessary for ATP-induced $Ca^{2+}$ release. Neither of the groups presented data on solubilized preparations.

In the present study we have assessed the ability of a variety of ATPase preparations to release $Ca^{2+}$ upon addition of ATP. Both vesicular and solubilized preparations were assayed for $Ca^{2+}$ release. In addition, soluble $Ca^{2+}$-ATPase preparations were analyzed by sedimentation equilibrium centrifugation to determine aggregation states. We conclude that interaction of the ATPase with a phospholipid bilayer is essential for the coupling of $Ca^{2+}$ release to ATP hydrolysis since no solubilized preparation exhibited ATP-induced $Ca^{2+}$ release regardless of the aggregation state or phospholipid concentration.

## Materials and Methods

Sarcoplasmic reticulum was isolated from rabbits by the method of Eletr & Inesi (1972) followed by sucrose density gradient centrifugation (Dean & Gray, 1980). Purified ATPase vesicles were prepared according to method 2 of Meissner et al. (1973). Delipidation of the purified ATPase was carried out as previously described (Dean & Tanford, 1978), and the phospholipid content was 4–6 mol of phospholipid/mol of enzyme based on a molecular weight of 119 000 (Rizzolo et al., 1976). Protein was determined by the method of Lowry et al. (1951) and was corrected as described previously (le Maire et al., 1976). All preparations of purified ATPase, both vesicular and solubilized, exhibited specific activities from 15 to 25 $\mu$mol $min^{-1}$ $mg^{-1}$ at 37 °C as determined by a coupled assay (Dean & Tanford, 1977).

Stopped-flow experiments were performed at 20 °C with a Durrum-Gibson stopped-flow spectrophotometer. Changes in transmittance were recorded under computer contol by using an OLIS Model 3820 data acquisition system (On Line Instrument Systems, Jefferson, GA), which samples the photomultiplier output at selected time intervals and stores the resulting data on magnetic diskettes (Gray, 1982). In all experiments the reaction was monitored initially at a rapid sampling rate for the first 100 or 120 data points followed by a slower sampling rate for the next 120 data points. A reference voltage from which changes in absorbance were calculated was recorded 20 s after the final data point.

Arsenazo III (Aldrich Chemical Co.) was purified by acid precipitation and Chelex chromatography (Kendrick et al., 1977). Changes in the absorbance of arsenazo III were initially monitored by taking the difference in absorbance between stopped-flow traces at 654 and 578 nm. However, it was later demonstrated that measuring the absorbance at 654 nm alone yielded the same $Ca^{2+}$ concentrations, and all results reported herein are derived from data at 654 nm only. Traces obtained at the specified concentrations of ATP are presented in this study after subtraction of the trace obtained from mixing of ATPase with buffer containing no ATP. All traces are the averages of three mixing experiments. $Ca^{2+}$ concentrations are based on the absorbance of arsenazo III at 654 nm which was standardized by using a $Ca^{2+}$ electrode (Radiometer) in the experimental buffers.

Sedimentation equilibrium measurements were made in a Beckman L5-75 centrifuge equipped with a Prep UV scanner (Beckman). A 12-mm double sector cell was used, and centrifugation was carried out at 22 000 rpm for 3 h and then at 15 000 rpm until equilibrium was reached (~30 h). Absorbance measurements were at 280 nm. The density of the buffer used in most experiments (0.01 M Tes buffer, pH 7.5, 0.1 M KCl, 2.74 M glycerol, 1 mM dithiothreitol, and 1.86 mM $C_{12}E_8$) was 1.065 g/mL at 19 °C (Dean & Tanford, 1978), and the density of the above buffer with 20% (v/v) dimethyl sulfoxide was calculated to be 1.075 g/mL. A partial specific volume of 0.740 $cm^3/g$ was used for the ATPase and 0.973 $cm^3/g$ for bound $C_{12}E_8$. Binding was assumed to be 1.3 g/g (Dean & Tanford, 1978). The molecular weight was corrected for bound detergent according to Tanford et al. (1974).

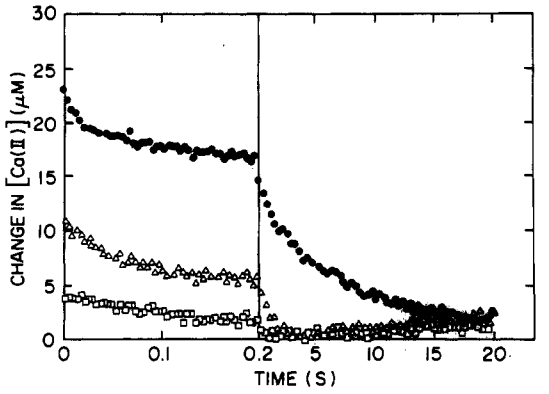


FIGURE 1: $Ca^{2+}$ transport by sarcoplasmic reticulum vesicles. One syringe contained sarcoplasmic reticulum vesicles (2 mg of protein/mL) in 0.01 M Tes buffer, pH 7.5, containing 0.1 M KCl, 2.74 M glycerol, 5 mM $MgCl_2$, 0.1 mM $Ca^{2+}$, and 30 $\mu$M arsenazo III. The other syringe contained MgATP in the same buffer. Assuming that 70% of the sarcoplasmic reticulum protein is the $Ca^{2+}$-ATPase (Meissner et al., 1973) and that the molecular weight of the $Ca^{2+}$-ATPase is 119 000 (Rizzolo et al., 1976) yields a final ATPase concentration of 5.9 $\mu$M. $Ca^{2+}$ concentrations were calculated as described under Materials and Methods. Filled circles represent data obtained at a final ATP concentration of 50 $\mu$M, triangles at 5 $\mu$M, and squares at 2.5 $\mu$M.

Highly purified phosphatidylcholine was purchased from Lipid Products. All other materials used in the present study and their preparation are the same as those reported earlier (Dean & Tanford, 1977).

## Results

Arsenazo III has been used by others to measure $Ca^{2+}$ transients in sarcoplasmic reticulum (Chiu & Haynes, 1977). For determination of the feasibility of using arsenazo III as a $Ca^{2+}$ indicator in our experiments, the kinetics of $Ca^{2+}$ transport into sarcoplasmic reticulum vesicles was analyzed as shown in Figure 1. The line at 0.2 s in Figure 1 emphasizes the change in time scale that occurs at this time point. For all three ATP concentrations employed, a rapid burst of $Ca^{2+}$ transport (decrease in extravesicular $Ca^{2+}$) occurred in the first 100 ms. This phenomenon has been observed by others using rapid quench techniques and radioactive $Ca^{2+}$ (Kurzmack et al., 1977; Verjovski-Almeida & Inesi, 1979) and has been found to correspond with a burst in phosphoenzyme formation (Yamada et al., 1971; Froehlich & Taylor, 1976). As also observed by Verjovski-Almeida & Inesi (1979), the amount of $Ca^{2+}$ transported in this initial burst (4–5 $\mu$M) in our experiments was approximately equal to a molar ratio of one $Ca^{2+}$ per ATPase polypeptide. The total amount of $Ca^{2+}$ transported was equal to twice the amount of ATP hydrolyzed at the two lower ATP levels, whereas at the highest ATP level, transport was not complete at 20 s. The total amount of $Ca^{2+}$ transported at this ATP concentration (100 $\mu$M) cannot be related to the amount of ATPase polypeptide since ATP was present at a 10-fold excess over the ATPase and several cycles of pumping could occur before ATP depletion. The release of $Ca^{2+}$ after complete hydrolysis of ATP, which occurred after about 1 s at 2.5 and 5 $\mu$M ATP, was also detected by arsenazo III and is quite similar to the results of Katz et al. (1977), who used direct radioactive measurement of $Ca^{2+}$. Thus it is ev-

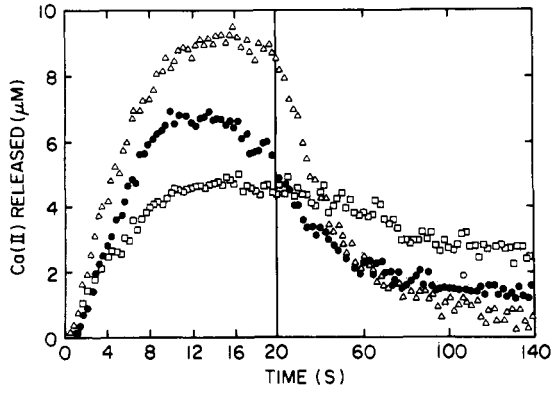


FIGURE 2: ATP-induced $Ca^{2+}$ release from purified $Ca^{2+}$-ATPase in leaky vesicles. Purified $Ca^{2+}$-ATPase and MgATP were mixed to give a final ATPase concentration of 4.2 μM. The buffer used was 20 mM Bis-Tris, pH 6.1, containing 20% (v/v) dimethyl sulfoxide, 80 mM KCl, 5 mM $MgCl_2$, 50 μM $Ca^{2+}$, and 50 μM arsenazo III. Triangles represent data obtained at a final ATP concentration of 10 μM, filled circles at 2.5 μM, and squares at 100 μM.

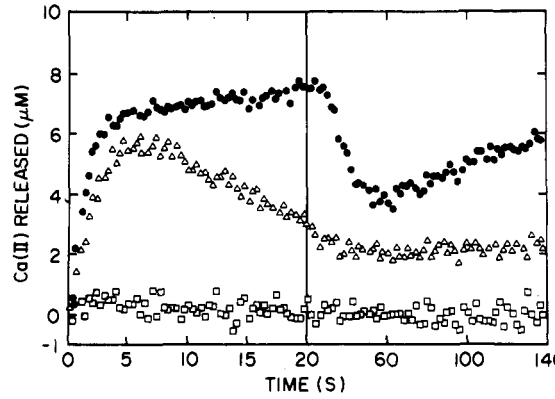


FIGURE 3: ATP-induced $Ca^{2+}$ release from purified $Ca^{2+}$-ATPase in the presence of $C_{12}E_8$. In the two traces exhibiting $Ca^{2+}$ transients, purified $Ca^{2+}$-ATPase in leaky vesicles was mixed with MgATP to give a final ATPase concentration of 4 μM and a final ATP concentration of 10 μM for data represented by filled circles and 2.5 μM for triangles. The buffer used was 20 mM Bis-Tris, pH 6.1, containing 20% (v/v) dimethyl sulfoxide, 80 mM KCl, 5 mM $MgCl_2$, 50 μM $Ca^{2+}$, 50 μM arsenazo III, and 0.1 g/L $C_{12}E_8$. No release was observed with three solubilized preparations (squares) at the same final ATPase and MgATP concentrations as stated above and with 2.74 M glycerol included in the buffer: (1) delipidated ATPase in 1.0 g/L $C_{12}E_8$; (2) delipidated ATPase in 0.1 g/L $C_{12}E_8$; (3) delipidated ATPase in 1.0 g/L $C_{12}E_8$ with a final concentration of 2 g/L purified egg phosphatidylcholine.

ident that arsenazo III is an excellent reagent for analysis of $Ca^{2+}$ transients under the conditions reported here.

Having confirmed the utility of arsenazo III for $Ca^{2+}$ transient detection, we attempted to repeat the experiments described by Ikemoto (1976) using purified ATPase in leaky vesicles. However, when the conditions specified were used, no release of $Ca^{2+}$ was detected although we used an ATPase preparation purified by a different procedure (Meissner et al., 1973). For this reason the modifications described by Watanabe et al. (1981) were utilized. Lowering the pH to 6.1 caused a reversible inhibition in ATPase activity of 70%, and inclusion of 20% (v/v) dimethyl sulfoxide resulted in a final reversible inhibition of 93% for both vesicular and solubilized preparations. As shown in Figure 2, the results of mixing ATP with purified ATPase under these conditions in leaky vesicles yielded the same results observed by Watanabe et al. (1981): a rapid release of $Ca^{2+}$ followed by a slow rebinding. The time course of $Ca^{2+}$ rebinding was dependent on ATP concentration as observed by the other group. A maximum of three $Ca^{2+}$ per ATP molecule appeared to be released. This result points out a problem inherent to the methodology employed. At 10 μM ATP where the greatest amount of $Ca^{2+}$ was released, the maximum absorbance change was 0.04 at 654 nm. Since this was obtained after subtraction of the results of an identical experiment with ATP omitted, it is likely that the method is not precise enough to differentiate between two and three $Ca^{2+}$ per ATP molecule. The decrease in the amount of $Ca^{2+}$ released at the highest ATP concentration (100 μM) is puzzling but may be a result of steady-state enzyme turnover at this 10-fold molar excess of ATP.

In order to determine the requirements for $Ca^{2+}$ release from the ATPase, we carried out experiments in the presence of $C_{12}E_8$. At a weight ratio of 0.2 $C_{12}E_8$ to vesicular ATPase, the results shown in Figure 3 were obtained. Comparison with Figure 2 shows that the stoichiometry of release was unaffected by $C_{12}E_8$. However, the rate of release was increased while the rebinding rate and extent were diminished. The failure of the ATPase to rebind all of the initially bound $Ca^{2+}$ may be a result of enzyme inactivation caused by $Ca^{2+}$ depletion in the presence of $C_{12}E_8$ (Dean & Gray, 1981). At this ratio of detergent to protein little solubilization occurs (le Maire et al., 1976). Thus $C_{12}E_8$ per se does not inhibit $Ca^{2+}$ release if a bilayer is present.

In contrast to the above results, solubilized preparations of the ATPase did not exhibit ATP-dependent $Ca^{2+}$ release as shown in Figure 3. At a $C_{12}E_8$ to protein weight ratio of 2.0, the delipidated ATPase is a fully active monomer (Dean & Tanford, 1978), but no $Ca^{2+}$ release was observed from this preparation. At a lower detergent concentration (0.2 g $C_{12}E_8$/g protein) where the ATPase is aggregated (Dean & Tanford, 1978, Figure 4), again the release reaction was not observed. Since this is the same concentration of detergent used with vesicular ATPase where $Ca^{2+}$ release was observed (Figure 3), the loss of $Ca^{2+}$ release activity cannot be attributed to the presence of $C_{12}E_8$. Control experiments showed that glycerol had no effect on $Ca^{2+}$ release. Furthermore, more than half of the $Ca^{2+}$-ATPase activity was retained during the time required for the experiment. These results indicate that the state of aggregation of ATPase is not a determinant for $Ca^{2+}$ release when the ATPase is solubilized and that the loss of the $Ca^{2+}$ release activity is correlated with loss of membrane structure. This conclusion is further strengthened by the observation that addition of phosphatidylcholine at a weight ratio of 2, in the presence of sufficient $C_{12}E_8$ to solubilize the lipid and protein (2:1 $C_{12}E_8$ to protein weight ratio), yielded no reconstitution of $Ca^{2+}$ release activity (Figure 3). Freshly solubilized ATPase was also tested in order to rule out the possibility that the delipidation procedure caused the loss of $Ca^{2+}$ release activity. ATPase vesicles were solubilized at a $C_{12}E_8$ to protein weight ratio of 2 in the buffer used for $Ca^{2+}$ release experiments, and unsolubilized ATPase was removed by centrifugation. The soluble ATPase was fully active with respect to ATP hydrolysis but did not exhibit $Ca^{2+}$ release under the same conditions employed in the experiments reported in Figure 3. Thus solubilization and not lipid removal obliterates the $Ca^{2+}$ release reaction.

Recently Verjovski-Almeida & Silva (1981) reported that solubilized, monomeric $Ca^{2+}$-ATPase did not exhibit cooperativity of $Ca^{2+}$ binding displayed by membrane-bound ATPase. However, if solubilization was carried out in the presence of 1 mM $Ca^{2+}$, cooperativity was retained, and the authors suggested that $Ca^{2+}$ affected the aggregation state of the ATPase in $C_{12}E_8$. Consequently, we performed sedimentation equilibrium measurements on delipidated $Ca^{2+}$-ATPase in the presence of substrates for the enzyme. As shown in Table I,

Table I: Effect of Substrates on the State of Aggregation of Delipidated $Ca^{2+}$-ATPase in $C_{12}E_8$

| substrated added[a] | state of aggregation[b] |
|---|---|
| $Ca^{2+}$, $1 \times 10^{-2}$ M | monomer |
| $Ca^{2+}$, $1 \times 10^{-5}$ M | monomer |
| $Ca^{2+}$, $1 \times 10^{-11}$ M | dimer |
| MgADP, $5 \times 10^{-3}$ M; $Ca^{2+}$, $1 \times 10^{-5}$ M | monomer |
| Mg-$\beta,\gamma$-$CH_2$ATP, $5 \times 10^{-3}$ M; $Ca^{2+}$, $1 \times 10^{-5}$ M | monomer |
| 20% dimethyl sulfoxide; $Ca^{2+}$, $1 \times 10^{-5}$ M | monomer |

[a] Substrates were added to 0.01 M Tes buffer, pH 7.5, containing 2.74 M glycerol, 0.1 M KCl, 0.001 M dithiothreitol, and 1.86 mM $C_{12}E_8$. [b] Sedimentation equilibrium centrifugation and molecular weight calculation were carried out as described under Materials and Methods. For all experiments, at least 80% of the ATPase in the analytical cell was within ±10% of the reported aggregation state where monomer is 119 000 daltons and dimer is 238 000 daltons (Rizzolo et al., 1976). Initial $Ca^{2+}$-ATPase concentration was ~4 μM in all experiments, and centrifugation time was 24 h.

at a $C_{12}E_8$ to protein ratio of 2 which is similar to that used by Verjovski-Almeida & Silva (1981), no aggregation of the ATPase occurred at a $Ca^{2+}$ concentration as high as 10 mM. However, when $Ca^{2+}$ was removed with EGTA, the ATPase was inactivated and aggregated to a dimer in 24 h. This aggregation was time dependent, and higher aggregation states were observed after longer times. This phenomenon has also been reported by others (le Maire et al., 1976; Jorgensen et al., 1978). Inclusion of MgADP or the ATP analogue $\beta,\gamma$-methylene-ATP did not result in aggregation of the ATPase. The presence of 20% dimethyl sulfoxide also had no effect on the aggregation state. All preparations except that with $Ca^{2+}$ removed by EGTA retained at least 80% of their initial activity during the 24 h required for sedimentation equilibrium measurements. Thus at high detergent to protein ratios (>2) the active $Ca^{2+}$-ATPase remains monomeric in the presence of substrates.

## Discussion

It can be inferred from the work of several groups that the loss of functional characteristics of the $Ca^{2+}$-ATPase (cooperative $Ca^{2+}$ binding, negative cooperativity of ATP hydrolysis, and phosphoenzyme formation from inorganic phosphate) caused by solubilization is a result of formation of monomeric ATPase from an initially aggregated form of the enzyme (Inesi et al., 1980; Verjovski-Alemida & Silva, 1981; Nestruck-Goyke & Hasselbach, 1981). This concept is consonant with experimental findings showing that approximately half of the ATPase chains are phosphorylated by ATP in native sarcoplasmic reticulum membranes (Froehlich & Taylor, 1976; Verjovski-Almeida & Inesi, 1979; Ikemoto et al., 1981). In the present study we report another function of the $Ca^{2+}$-ATPase that appears to be lost upon solubilization: ATP-induced $Ca^{2+}$ release. It is possible that failure to detect release and rebinding of the $Ca^{2+}$ was caused by a greatly increased rate of both phenomena upon solubilization. An increase by at least a factor of 20 would be necessary to render this reaction undetectable in the stopped-flow apparatus, which seems unlikely.

ATP-induced $Ca^{2+}$ release is an indicator of the coupling of ATP hydrolysis to a protein conformational change resulting in release of tightly bound $Ca^{2+}$ and is presumably directly related to $Ca^{2+}$ transport (Ikemoto, 1976). We demonstrated that the apparent loss of coupling for the solubilized enzyme is not a direct result of monomer formation or delipidation. This follows from experiments carried out at a low detergent to protein ratio where the ATPase is known to be aggregated (Dean & Tanford, 1978) and also from the data obtained with added phospholipid. Thus, we conclude that loss of coupling between ATP hydrolysis and $Ca^{2+}$ release is a result of membrane bilayer disruption.

Uncoupling of $Ca^{2+}$ transport from ATP hydrolysis has been reported after modification of fragmented sarcoplasmic reticulum by the following procedures: incubation at pH 5.5 and at 37 °C (Berman et al., 1977), modification of phosphatidylethanolamine with fluorescamine (Hidalgo et al., 1982), and limited proteolysis with trypsin (Scott & Shamoo, 1982). In all three cases it was shown that the sarcoplasmic reticulum retained $Ca^{2+}$-stimulated ATPase activity but that most of the $Ca^{2+}$ transport activity was lost. This was shown not to be a result of increased $Ca^{2+}$ efflux in all three systems. The results of the present report indicate that solubilization also may uncouple $Ca^{2+}$-stimulated ATPase activity from $Ca^{2+}$ release. The mechanism for the uncoupling is unclear, but our results and those of Hidalgo et al. (1982) suggest that protein–lipid interactions are involved in the coupling process.

In conclusion, we propose that loss of coupling between ATP hydrolysis and $Ca^{2+}$ release is not a consequence of monomer formation per se but results from disruption of the phospholipid bilayer. This conclusion is in agreement with earlier work which showed that loss of negative cooperativity of ATP hydrolysis was independent of the aggregation state of $C_{12}E_8$ solubilized ATPase (Dean & Tanford, 1978).

## Acknowledgments

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**Registry No.** Ca, 7440-70-2; ATP, 56-65-5; ATPase, 9000-83-3.

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# Characterization of the Aldehyde Binding Site of Bacterial Luciferase by Photoaffinity Labeling†

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ABSTRACT: A photoaffinity probe 1-diazo-2-oxoundecane has been synthesized and used to examine the aldehyde-binding site of the nonidentical dimeric luciferase ($\alpha\beta$) from *Vibrio harveyi* cells. In the dark, the probe competes against aldehyde in binding to luciferase. Irradiation of luciferase and the probe at 254 nm resulted in primarily specific labeling of both $\alpha$ and $\beta$ subunits with concomitant enzyme inactivation, but significant ($\simeq$40%) nonspecific labeling of mainly the $\beta$ subunit also occurred. The addition of decanal to protect the active center reduced the rate of inactivation. When 2-mercaptoethanol was included to quench the nonspecific labeling, the amounts of probe incorporated into $\alpha$ and $\beta$ correlated stoichiometrically with the quantities of enzyme photoinactivated. On the basis of these findings, we postulate that the aldehyde binding site is at or near the subunit interface of luciferase.

Bacterial luciferase catalyzes the monooxygenation reaction in which reduced FMN ($FMNH_2$)[1] and a long-chain aliphatic aldehyde react with $O_2$ to yield FMN, carboxylic acid, water, and light (Hastings & Nealson, 1977; Ziegler & Baldwin, 1981, and references therein). The luciferases from at least four strains of marine bacteria, including *Vibrio harveyi* (Baumann et al., 1980) previously designated as *Beneckea harveyi* (Reichelt & Baumann, 1973), have been purified, and each was shown to be a nonidentical dimer with the heavy and light subunits referred to as $\alpha$ and $\beta$, respectively (Hastings et al., 1969; Ruby & Hastings, 1980; Meighen & Bartlet, 1980). Earlier hybridization studies using chemically (Meighen et al., 1971) and mutationally (Cline & Hastings, 1972) modified *V. harveyi* luciferase subunits indicated that $\alpha$ ($M_r$ 42 000) participated directly in catalysis whereas the specific function of $\beta$ ($M_r$ 37 000) remained unclear. Selective modifications of the $\alpha$ subunit by sulfhydryl (Nicoli et al., 1974) and histidyl (Cousineau & Meighen, 1976) reagents or proteases (Baldwin et al., 1978; Holzman et al., 1980) were subsequently shown to completely inactivate luciferase.

Recently, a functional role in $FMNH_2$ binding has also been indicated for the luciferase $\beta$ subunit. It was proposed, based on properties of a hybrid consisting of the $\alpha$ from *V. harveyi* luciferase and $\beta$ from *Photobacterium phosphoreum* enzyme, that both $\alpha$ and $\beta$ were involved in the initial interaction with $FMNH_2$ whereas subsequent catalytic steps were dictated by the $\alpha$ subunit (Meighen & Bartlet, 1980). Chemical modification of a single amino group on either $\alpha$ or $\beta$ of the *V. harveyi* luciferase was found to result in the loss of $FMNH_2$-binding ability (Welches & Baldwin, 1981). A study using luciferase immobilized through amino groups on either $\alpha$ or $\beta$ also indicated that the binding of $FMNH_2$, but not the subsequent catalytic steps, was dependent upon some exposed amino groups on both subunits (Watanabe et al., 1982).

In the present study we have investigated the subunit function of *V. harveyi* luciferase with respect to aldehyde binding. A diazo ketone photoaffinity labeling probe, 1-diazo-2-oxoundecane (DOU), was synthesized and, in the dark, found to be an aldehyde-competitive inhibitor for luciferase. Upon irradiation, covalent attachment of the probe to luciferase occurs. The labeling of either $\alpha$ or $\beta$ causes inactivation

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[1] Abbreviations: FMN, flavin mononucleotide; $FMNH_2$, reduced FMN; DOU, 1-diazo-2-oxoundecane; [$^{14}$C]DOU, 1-diazo-2-oxo[1-$^{14}$C]undecane.

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