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Effect of calcium on the interactions between Ca^{2+}-ATPase molecules in sarcoplasmic reticulum

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The interaction between Ca^{2+}-ATPase molecules in the native sarcoplasmic reticulum membrane and in detergent solutions was analyzed by chemical crosslinking, high performance liquid chromatography (HPLC), and by the polarization of fluorescence of fluorescein 5′-isothiocyanate (FITC) covalently attached to the Ca^{2+}-ATPase. Reaction of sarcoplasmic reticulum vesicles with glutaraldehyde causes the crosslinking of Ca^{2+}-ATPase molecules with the formation of dimers, tetramers and higher oligomers. At moderate concentrations of glutaraldehyde solubilization of sarcoplasmic reticulum by $C_{12}E_8$ or Brij 36T (≅ 4 mg/mg protein) decreased the formation of higher oligomers without significant interference with the appearance of crosslinked ATPase dimers. These observations are consistent with the existence of Ca^{2+}-ATPase dimers in detergent-solubilized sarcoplasmic reticulum. Ca^{2+} (2–20 mM) and glycerol (10–20%) increased the degree of crosslinking at pH 6.0 both in vesicular and in solubilized sarcoplasmic reticulum, presumably by promoting interactions between ATPase molecules; at pH 7.5 the effect of Ca^{2+} was less pronounced. In agreement with these observations, high performance liquid chromatography of sarcoplasmic reticulum proteins solubilized by Brij 36T or $C_{12}E_{10}$ revealed the presence of components with the expected elution characteristics of Ca^{2+}-ATPase oligomers. The polarization of fluorescence of FITC covalently attached to the Ca^{2+}-ATPase is low in the native sarcoplasmic reticulum due to energy transfer, consistent with the existence of ATPase oligomers (Highsmith, S. and Cohen, J.A. (1987) Biochemistry 26, 154–161); upon solubilization of the sarcoplasmic reticulum by detergents, the polarization of fluorescence increased due to dissociation of ATPase oligomers. Based on its effects on the fluorescence of FITC-ATPase, Ca^{2+} promoted the interaction between ATPase molecules, both in the native membrane and in detergent solutions.

Introduction

The self-association of Ca^{2+}-ATPase into oligomers in native and in detergent-solubilized sarcoplasmic reticulum continues to be one of the major problems of the field. In spite of intense effort at its solution [1–5], there is still disagreement about the size of the putative ATPase oligomers, their association constants, and functional significance.

The tendency of the Ca^{2+}-ATPase to undergo self-association in the native membrane with the formation of ATPase oligomers of various sizes is supported by electron microscopy [6–20], by target inactivation data [21–24] and by fluorescence studies [25–33]. There are, however, considerable uncertainties about the precise implications of many of these results.

The interpretation of fluorescence energy transfer data, both in intact sarcoplasmic reticulum and in reconstituted Ca^{2+}-ATPase vesicles [25–33] is complicated by the possibility of energy transfer between non-associated ATPase molecules due to the high density of Ca^{2+}-ATPase in the membrane [34,35]. So, while these experiments provide strong indication for the existence of ATPase oligomers in both the native and

Abbreviations: Brij-36T, polyoxyethylene 10 lauryl ether; $C_{12}E_8$, octaethyleneglycol dodecyl ether; $C_{12}E_9$, polyoxyethylene 9 lauryl ether; $C_{12}E_{10}$, polyoxyethylene 10 lauryl ether; DOC, deoxycholate; DTT, dithiothreitol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; FITC, fluorescein-5′-isothiocyanate; HPLC, high performance liquid chromatography; Mops, 4-morpholinepropanesulfonic acid; SDS, sodium dodecyl sulfate; SR, sarcoplasmic reticulum; Tes, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid.

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reconstituted membranes, they leave the size of these oligomers uncertain.

Target inactivation data on skeletal [21,23,24] and cardiac sarcoplasmic reticulum [22], and on reconstituted vesicles of skeletal muscle Ca^{2+}-ATPase at varying lipid: protein mole ratios [23] suggest that the catalytic unit of the Ca^{2+}-ATPase is a dimer. However, Grover et al. [36] obtained a target size closer to a tetramer for the oxalate-stimulated Ca^{2+} transport in cardiac sarcoplasmic reticulum and a target size approaching one million for smooth muscle preparations. The target sizes of 400–1000 kDa are closer to the average size of ATPase oligomers suggested by the relationship between surface and intramembrane particles seen by electron microscopy [8,19] than to the values obtained by Chamberlain et al. [22] and Hymel et al. [23,24]. These discrepancies may be due to the uncontrolled effects of free radicals, that could extend the effects of radiation to ATPase molecules not involved directly in collision [37].

Covalent crosslinking of the Ca^{2+}-ATPase by a variety of crosslinking reagents, both in native sarcoplasmic reticulum and in detergent-solubilized preparations, generally leads to the formation of Ca^{2+}-ATPase dimers; however, in most studies the dimers form part of a series of crosslinked products of increasing size, up to very large polymers that do not enter the polyacrylamide gels. Such results were obtained with dimethyl suberimidate [38–40], glutaraldehyde [39,41,42], Cu-phenanthroline [39–41,43–45], I_2 [40], dithio-bis(succinimidyl propionate) [41,43,46,47], dimethyl 3,3′-dithiobis(propionimidate) [43], di-*N*-(2-nitro-4-azido-phenyl)-cystamine-*S*,*S*-dioxide [43], and 1,5-difluoro-2,4-dinitrobenzene [48,49]. The report by Murphy [50] on the selective, nonserial production of Ca^{2+}-ATPase tetramers by reaction of sarcoplasmic reticulum with Cu-phenanthroline was not confirmed [39,40,43–45]. Preliminary reports by Ikemoto et al. [51] on the selective formation of hexamers in the presence of *N*,*N*′-*p*-phenylenedimaleimide and by Giotta [52] of the formation of dimers have not been followed up by detailed reports. The interpretation of the crosslinking data is complicated by the serial nature of the crosslinking; most of the reagents produced a spectrum of ATPase polymers, with no clear indication of a preferred size of oligomers.

In the presence of detergents such as Triton X-100 [42,45], $C_{12}E_8$ [41], or deoxycholate [46], the extent of crosslinking was generally reduced; at low detergent concentration dimers were usually still observed [42,45], but at higher detergent concentration essentially complete conversion of the Ca^{2+}-ATPase into monomeric form was suggested to occur, even at relatively high protein concentration [4,41,42,45–46].

Considerable effort was expended to obtain information on the size of the ATPase oligomers, their association constants, and functional significance in detergent-solubilized preparations of the Ca^{2+}-ATPase, using ultracentrifugation and gel exclusion chromatography in conjunction with rapid and steady-state kinetic measurements of the elementary steps of ATP hydrolysis [1–5,53–60]. Silva and Verjovski-Almeida [57,58] concluded from gel filtration data on Sephacryl S-300 columns that at protein concentrations higher than $\cong 50$ μg/ml most of the soluble Ca^{2+}-ATPase remained in associated form even at $C_{12}E_8$: protein ratios as high as 5000: the dimeric enzyme showed half of the sites reactivity with respect to Ca^{2+} binding, and the association constant for the monomer-dimer equilibrium was estimated at $9.37 \cdot 10^7$ M^{-1}. They suggested that at a $C_{12}E_8$ concentration of 3–7 mM, monomeric solution of Ca^{2+}-ATPase is obtained only at protein concentrations of 10 μg/ml or less. In contrast to these observations, Andersen et al. [4,59,60] reported a pronounced tendency for reversible formation of ATPase oligomers only at detergent: protein ratios less than 2; according to their estimates the association constant for dimer formation was only 10^5–10^6 M^{-1}, depending on detergent concentration, i.e., much lower than the values obtained by Silva and Verjovski-Almeida [58]. Andersen et al. [4] suggested that irreversible aggregation of Ca^{2+}-ATPase into inactive dimers and higher oligomers in the experiments of Silva and Verjovski-Almeida [57,58] may have contributed to the differences between the conclusions of the two groups. According to Martin [54], monomers are the dominant species at protein concentrations ranging from 25 to 150 μg/ml; by active enzyme sedimentation only the monomers were found to be enzymatically active. Thus while these observations confirm the strong propensity of Ca^{2+}-ATPase for self-association, the size and association constant of the dominant oligomeric species of Ca^{2+}-ATPase is left undefined.

In our previous studies on the crystallization of Ca^{2+}-ATPase in detergent solutions [61–63] we observed that preservation of ATPase activity and the formation of three-dimensional arrays of Ca^{2+}-ATPase molecules was optimal at pH 6.0 and required the presence of 20% glycerol and 20 mM $CaCl_2$ in the incubation medium. These data suggested a relationship between the formation of ATPase aggregates and the long-term stability of the enzyme. In the studies reported here the effect of Ca^{2+} on the ATPase–ATPase interactions was further analyzed with the use of high pressure liquid chromatography, chemical crosslinking and fluorescence polarization spectroscopy. The observations suggest that the stability of Ca^{2+}-ATPase in detergent solutions, and by inference in native membranes, requires interactions between ATPase molecules.

A preliminary report was presented at the International Symposium on Molecular Basis of Biomembrane Transport, Bari, Italy, May, 1988.

Experimental procedures

Materials

Acrylamide, bis-acrylamide (*N,N'*-methylene-bis-acrylamide), ammonium persulfate, Coomassie brilliant blue R-250, and β-mercaptoethanol were purchased from Bio-Rad Laboratories, Richmond, CA. The detergents Brij 36T (polyoxyethylene 10-lauryl ether), and $C_{12}E_8$ (octaethyleneglycol dodecyl ether), as well as diethylamine, Mops, and Tris were obtained from Sigma Chemical Co., St. Louis, MO. Glutaraldehyde and sodium dodecyl sulfate were supplied by Polysciences, Inc., Warrington, Pennsylvania. Glycine was obtained from Aldrich Chemical Co., Milwaukee, WI, and TEMED (*N,N,N',N'*-tetramethylethylenediamine) from Eastman-Kodak Company, Rochester, NY. Glycerine and Bromophenol blue were supplied by Fisher Scientific Co., Fairlawn, NJ, and the molecular weight calibration kits by Pharmacia Fine Chemicals AB, Uppsala, Sweden.

Methods

Sarcoplasmic reticulum vesicles were isolated as described by Pikula et al. [62]. The measurement of ATPase activity was performed according to Varga et al. [64], and the assay of protein according to Lowry et al. [65].

For analysis of protein composition by SDS-polyacrylamide gel electrophoresis the samples were dispersed in a solution of 5% sodium dodecyl sulfate, 10 mM Tris-HCl buffer, pH 8.0, 1% β-mercaptoethanol and 10% glycerol, at a protein concentration of 1–2 mg/ml. Aliquots of 50 μl were applied for electrophoresis on 6–18% gradient gels, essentially according to Laemmli [66]. The Coomassie-blue stained gels were analyzed with an LKB 2202 Ultra-Scan laser densitometer, coupled with a Hewlett-Packard integrator plotter (3390A). The conditions for crosslinking of sarcoplasmic reticulum proteins with glutaraldehyde, either in intact vesicles or in detergent solubilized preparations, are described in the legends.

For high performance liquid chromatography of sarcoplasmic reticulum proteins, a Perkin-Elmer Series 10 liquid chromatography apparatus equipped with an LC-75 detector, an SW TSK guard column and a G 4000 SW TSK gel column was used, as described in the legends.

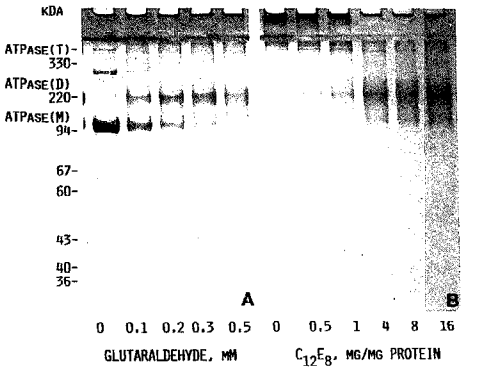
The polarization of fluorescence of FITC covalently attached to the Ca^{2+}-ATPase [25] was measured in an SLM 4800 fluorescence spectrophotometer.

Results and Discussion

In sarcoplasmic reticulum vesicles isolated from rabbit skeletal muscle, about 60–80 percent of the protein content is the Ca^{2+}-transport ATPase; the Ca^{2+}-ATPase migrates on SDS-polyacrylamide gel electrophoresis as a monomer of ≅ 110000 kDa (Fig. 1). Among the minor protein components of sarcoplasmic reticulum are the calsequestrin, with an apparent molecular mass of about 63 kDa, and the high-affinity Ca^{2+}-binding protein of 55 kDa [67]. A sharp band of ≅ 300 kDa may represent a component of the spanning protein complex, that links the T-tubules to the junctional sarcoplasmic reticulum [68,69]. Components of ≅ 400 kDa or higher are present only in trace amounts.

Reaction of sarcoplasmic reticulum vesicles with 0.1 mM glutaraldehyde at 25 °C for 1 h causes a significant decrease in the amount of Ca^{2+}-ATPase monomers, with a corresponding increase in a band at 220 kDa, that is assumed to represent crosslinked Ca^{2+}-ATPase dimers (Fig. 1). The conversion of Ca^{2+}-ATPase into dimers and higher oligomers becomes nearly complete at a glutaraldehyde concentration of ≅ 0.5 mM. After solubilization of sarcoplasmic reticulum with $C_{12}E_8$ (≅ 4 mg/mg protein) the formation of Ca^{2+}-ATPase oligomers larger than dimers is diminished and Ca^{2+}-ATPase dimers accumulate as the principal product of the glutaraldehyde reaction. The formation of ATPase dimers is observed even at $C_{12}E_8$ concentrations as high as 16 mg/mg protein. These observations are consistent with the view that ATPase molecules exist in an oligomeric state in the native sarcoplasmic reticulum membrane, and that ATPase dimers may represent the dominant oligomeric species in sarcoplasmic reticulum solubilized with $C_{12}E_8$.

Similar observations were made at pH 6.0 in a medium of 0.1 M KCl, 10 mM imidazole, pH 6.0, 1 mM EGTA, 3 mM $MgCl_2$, 5 mM NaN_3, 20% glycerol, 25 IU/ml Trasylol, 2 μg/ml 1,6-di-*tert*-butyl-*p*-cresol (Fig. 2A), except that the glutaraldehyde concentration required to achieve comparable crosslinking was slightly higher at pH 6.0 than at pH 7.5. Solubilization of sarcoplasmic reticulum with Brij 36T (4 mg/mg protein), as with $C_{12}E_8$, reduced the extent of crosslinking; this is particularly evident with respect to the higher polymers of Ca^{2+}-ATPase (Fig. 2B). The relatively large concentration of crosslinked ATPase tetramers, even in the presence of detergents, suggests, in agreement with the HPLC and fluorescence data (see below), that in addition to ATPase monomers and dimers, ATPase tetramers may also be present in significant amounts in detergent-solubilized sarcoplasmic reticulum. There was no indication of the formation of crosslinked ATPase trimers in any of the experiments, although on statistical grounds trimers should be present in larger amounts than tetramers. The protein band of ≅ 300 kDa apparent molecular weight, that is seen in untreated sarcoplasmic reticulum, is exceptionally sensitive to glutaraldehyde and is no longer observed at 0.4 mM glutaraldehyde concentration. This unique sensitivity of the ≅ 300 kDa protein to crosslinking supports the suggestion of Caswell and his colleagues [68,69] that this



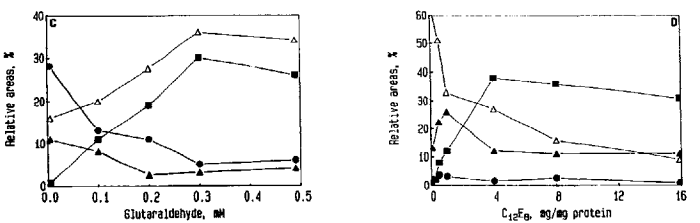


Fig. 1. Effect of glutaraldehyde and $C_{12}E_8$ concentration on the crosslinking of sarcoplasmic reticulum proteins by glutaraldehyde. (A) Sarcoplasmic reticulum vesicles (1 mg protein/ml) were suspended in 80 mM KCl, 50 mM potassium phosphate buffer, pH 7.5, 10 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 10.7 mg $C_{12}E_8$ per mg protein; the reaction was started at 25°C by the addition of glutaraldehyde to final concentrations indicated on the abscissa and was stopped 1 h later with diethylamine (10 mM). (B) The reaction was performed under the conditions described for A, except that the final concentration of glutaraldehyde was 0.5 mM and the concentration of $C_{12}E_8$ was varied between 0 and 16 mg/mg protein, as shown on the abscissa. The bands at 110 kDa, 220 kDa and 440 kDa are tentatively identified as ATPase monomer (M), dimer (D) and tetramer (T), respectively. The bands near 65 kDa and 56 kDa are the calsequestrin and the high-affinity Ca^{2+}-binding protein. For molecular weight determination, Pharmacia high and low molecular weight calibration kits were used in all experiments. (C) Densitometry of the gel patterns shown in Fig. 1A. The densitometry was performed as described under Experimental procedures. Symbols: ●, 110 kDa band (ATPase monomer); ■, 220 kDa band (ATPase dimer); ▲, 400–800 kDa band (putative ATPase tetra- to octamer); △, protein aggregates that did not enter the gel. (D) Densitometry of the gel patterns shown in Fig. 1B. Details as under (C).

group of proteins forms part of the large spanning protein complex that links T-tubules to the sarcoplasmic reticulum.

Solubilization of sarcoplasmic reticulum by Brij 36T (4 mg/mg protein) nearly completely prevented the crosslinking of calsequestrin (Fig. 2B) that occurred in intact sarcoplasmic reticulum (Fig. 2A).

The effect of Ca^{2+} on the crosslinking of Ca^{2+}-ATPase by glutaraldehyde

The Ca^{2+}-ATPase exists in two principal conformations as defined by the ion composition, temperature, and pH of the incubation medium [13,70,71]. The E_1 conformation can be stabilized by saturation of the high-affinity Ca^{2+}-binding sites of the Ca^{2+}-ATPase by

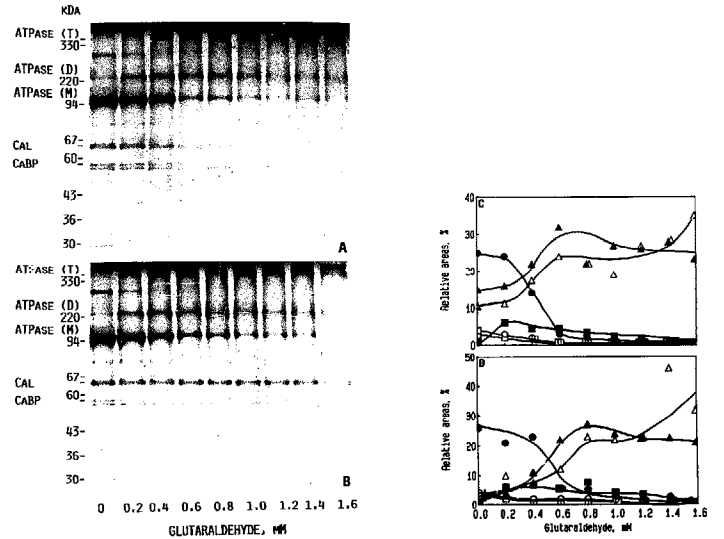


Fig. 2. The effect of glutaraldehyde concentration on the crosslinkig of sarcoplasmic reticulum proteins. (A,B) Sarcoplasmic reticulum vesicles (1 mg protein/ml) were suspended in a medium of 0.1 M KCl, 10 mM imidazole, pH 6.0, 1 mM EGTA, 3 mM $MgCl_2$, 5 mM NaN_3, 20% glycerol, 25 IU/ml Trasylol, 2 μg/ml 1,6-di-*tert*-butyl-*p*-cresol without (A), or with 4 mg/ml Brij 36T (B), and incubated for 1 h at 2°C. The reaction was started at 25°C by the addition of glutaraldehyde to final concentrations of 0–1.6 mM, as indicated on the abscissa; 1 h later the reaction was stopped with diethylamine (15 mM) and the samples were processed for electrophoresis. The following bands are marked: ATPase tetramer (T), dimer (D), monomer (M), calsequestrin (Cal) and the high-affinity Ca^{2+}-binding protein (CaBP). (C) Densitometry of the electrophoretograms of Fig. 2A. Symbols: ●, ATPase monomer; ■, ATPase dimer; ▲, ATPase tetra- to octamer; △, ATPase polymer; ○, calsequestrin; □, 300 kDa protein complex. (D) Densitometry data of the electropherograms of Fig. 2B. Details as in (C).

0.1 mM Ca^{2+}, or ≅ 10 μM lanthanides [72] accompanied by the formation of P1 type membrane crystals of Ca^{2+}-ATPase [15]. Vanadate (V) ions in the absence of calcium convert the enzyme into a stable E_2-V form [70,71] associated with the formation of P2 type crystals containing Ca^{2+}-ATPase dimers as structural units [11–14,16–18]. These observations indicate that the conformation of Ca^{2+}-ATPase exerts an influence on the interactions between ATPase molecules.

The effect of EGTA, EGTA + vanadate, and Ca^{2+} on the crosslinking of the Ca^{2+}-ATPase by 0.4 mM glutaraldehyde was analyzed at pH 6.0, in the absence of detergents and at Brij 36T concentrations of 4–12 mg/mg protein (Fig. 3). Solubilization of sarcoplasmic reticulum by Brij 36T had relatively little effect on the formation of Ca^{2+} ATPase dimers in solutions containing 1 mM EGTA (Fig. 3A) or 1 mM EGTA + 5 mM monovanadate (Fig. 3B). In both cases significant amount of Ca^{2+}-ATPase monomer remained in the system after reaction with glutaraldehyde (0.4 mM) for 1 h at 25°C. The crosslinking reaction was promoted by 0.2 mM Ca^{2+} (Fig. 3C), particularly at low detergent concentration (0–4 mg Brij 36T/mg); this effect became more pronounced with increasing Ca^{2+} concentration (Fig. 3D,E), and in the presence of 20 mM Ca^{2+} (Fig. 3F) extensive conversion of Ca^{2+}-ATPase into higher oligomers occurred, even at a Brij 36T concentration as high as 12 mg/mg protein.

The conditions of these experiments are essentially identical to those used for the long-term stabilization and crystallization of Ca^{2+}-ATPase in detergent solutions [62,63]. Therefore the Ca^{2+}-induced interaction

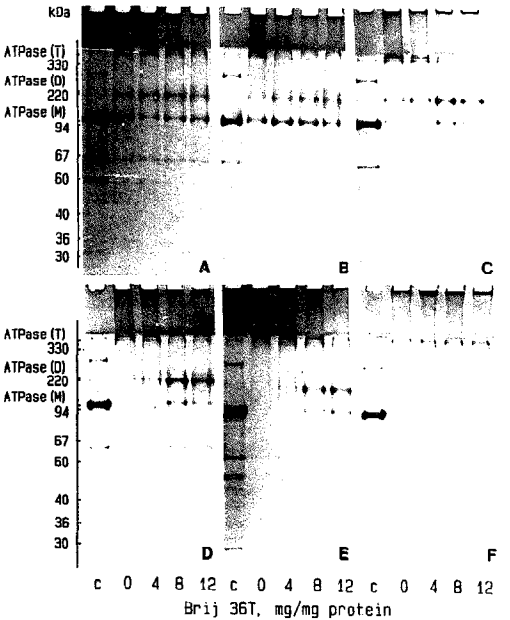


Fig. 3. Effect of detergent concentration on the crosslinking of sarcoplasmic reticulum proteins by glutaraldehyde. Sarcoplasmic reticulum vesicles (1 mg protein/ml) were suspended in a medium of 0.1 M KCl, 10 mM imidazole, pH 6.0, 3 mM $MgCl_2$, 3 mM NaN_3, 20% glycerol, 25 IU/ml Trasylol, 2 μg/ml 1,6-di-*tert*-butyl-*p*-cresol, and 1 mM EGTA (A), 1 mM EGTA + 5 mM monovanadate (B), 0.2 mM $CaCl_2$ (C), 2 mM $CaCl_2$ (D), 10 mM $CaCl_2$ (E), or 20 mM $CaCl_2$ (F), at detergent concentrations indicated on the abscissa. After incubation for 2 h at 2°C the reaction was started with the addition of glutaraldehyde (0.4 mM). The crosslinking was allowed to proceed for 1 h at 25°C. The reaction was stopped with diethylamine (15 mM) and the samples were processed for electrophoresis. Control samples (c) contained glutaraldehyde previously mixed with diethylamine without detergent. ATPase (M), (D), and (T), indicate Ca^{2+}-ATPase monomers, dimers and tetramers, respectively.

between ATPase molecules in detergent solutions at pH 6.0, reflected by the increased crosslinking with glutaraldehyde, and by the formation of three-dimensional crystals of Ca^{2+}-ATPase, probably contribute to the long-term preservation of the ATPase activity.

This conclusion is further supported by the experiments of Fig. 4, in which the extent of crosslinking was measured as the function of Ca^{2+} concentration in the absence of Brij 36T (Fig. 4A) and at Brij 36T concentrations of 4 mg/mg protein (Fig. 4B), 12 mg/mg protein (Fig. 4C), and 20 mg/mg protein (Fig. 4D). The accumulation of crosslinked ATPase dimers is particularly pronounced at Ca^{2+} concentrations of 0.2–5 mM in the presence of 12 mg Brij 36T per mg protein (Fig. 4C). At higher Ca^{2+} concentration (20 mM), large Ca^{2+}-ATPase aggregates formed that did not enter into the gel. The increase in the concentration of Ca^{2+}-ATPase dimers at 0.2 mM Ca^{2+}, particularly at high detergent concentration (12–20 mg/mg protein), occurred with a decrease in monomer concentration. These observations support the existence of stable Ca^{2+}-ATPase dimers in detergent solutions at pH 6.0 in the presence of Ca^{2+}. The effect of Ca^{2+} on the interactions between Ca^{2+}-ATPase molecules was less pronounced at pH 7.5; this may

explain the absence of three-dimensional Ca^{2+}-ATPase crystals [62], and the instability of the detergent-solubilized enzyme during long-term storage at pH 7.5.

The polarization of fluorescence of fluorescein-5′-isothiocyanate labeled Ca^{2+}-ATPase

Highsmith and Cohen [26] observed that the polarization of fluorescence of FITC covalently bound to Ca^{2+}-ATPase is relatively low in the native sarcoplasmic reticulum membrane but increases upon solubilization of Ca^{2+}-ATPase with $C_{12}E_9$. From the dependence of polarization on the extent of labeling of the Ca^{2+}-ATPase with FITC, they concluded that the dominant oligomeric form of the Ca^{2+}-ATPase in sarcoplasmic reticulum may be the tetramer.

We confirmed these observations under the experimental conditions used in our studies and utilized the method for the characterization of the effect of Ca^{2+}, Mg^{2+}, and K^+ on the state of association of the Ca^{2+}-ATPase in the native membrane and in solubilized preparations. As shown in Fig. 5, the polarization of fluorescence of FITC covalently bound to the Ca^{2+}-

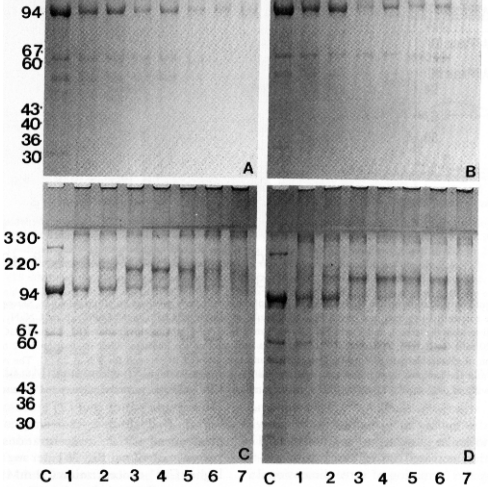


Fig. 4. Effect of [Ca^{2+}] concentration on the crosslinking of sarcoplasmic reticulum proteins by glutaraldehyde in the presence of detergents. (A–D) Sarcoplasmic reticulum vesicles (1 mg protein/ml) were suspended in a medium of 0.1 M KCl, 10 mM imidazole, pH 6.0, 3 mM $MgCl_2$, 3 mM NaN_3, 20% glycerol, 25 IU/ml Trasylol, 2 μg/ml 1,6-di-*tert*-butyl-*p*-cresol without detergent (A), or with Brij 36T at a concentration of 4 mg/mg protein (B), 12 mg/mg protein (C), or 20 mg/mg protein (D); the media of the samples in each series contained the following additions: lane 1, 1 mM EGTA; lane 2, 1 mM EGTA + 5 mM monovanadate; lane 3, 0.2 mM $CaCl_2$; lane 4, 1 mM $CaCl_2$; lane 5, 5 mM $CaCl_2$; lane 6, 10 mM $CaCl_2$; lane 7, 20 mM $CaCl_2$. After incubation for 2 h at 2°C, glutaraldehyde was added to a final concentration of 0.4 mM and incubation continued for 1 h at 25°C. The crosslinking reaction was stopped with diethylamine (15 mM) and the samples were prepared for SDS-polyacrylamide gel electrophoresis. Control samples (lane C) contained glutaraldehyde previously mixed with diethylamine. (E,F) Densitometry data on the electrophoretograms of Figs. 4 A–D. The relative areas under the 110 kDa ATPase monomer (E) and the 220 kDa ATPase dimer (F) bands was evaluated by densitometry. Symbols: ●, no detergent; ■, 4 mg Brij 36T/mg protein; ▲, 12 mg Brij 36T/mg protein; △, 20 mg Brij 36T/mg protein.

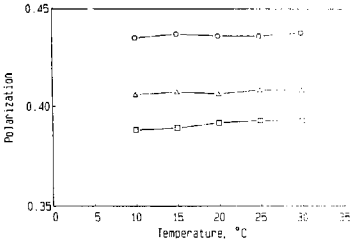


Fig. 5. The polarizaton of fluorescence of FITC-labeled Ca^{2+}-ATPase at different labeling ratios. Sarcoplasmic reticulum vesicles were labeled with FITC at a final concentration of 2.5 (○), 5.0 (△), and 7.5 (□) nmol/mg protein, as described earlier [25]. The polarization of the samples was measured at a final protein concentration of 0.1 mg/ml at 25 °C using 490 nm light beam for excitation, as described in Methods (λ_{em} = 520 nm).

ATPase decreased with increased saturation of the FITC binding sites and was nearly independent of temperature in the range of 10–30 °C (Fig. 5). At the highest labeling ratio used in this experiment (7.5 nmol FITC/mg sarcoplasmic reticulum protein) essentially all ATPase molecules are expected to be labeled by FITC; the low polarization of fluorescence arises from energy transfer between FITC-labeled Ca^{2+}-ATPase molecules and implies close proximity or interaction

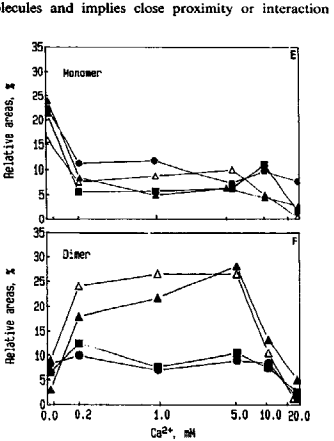


Fig. 4 (continued).

TABLE I

Effect of FITC labeling on the ATPase and acetylphosphatase activity and on the polarization of FITC fluorescence

Sarcoplasmic reticulum vesicles were labelled with FITC at a final concentration of 0, 2.5, 5.0, 7.5 and 15.0 nmol/mg protein, as described earlier [25]. After labeling, the vesicles were resuspended in 10 mM K-Mops, pH 7.5 at 2 mg/ml protein concentration and were used for assay of ATPase activity as described earlier [64]. Acetylphosphatase activity was measured by assaying the concentration of unhydrolyzed acetyl phosphate. Incubation was carried out in a medium of 0.1 M KCl, 20 mM imidazole, pH 7.4, 0.5 mM EGTA, 0.5 mM $CaCl_2$, 5 mM $MgCl_2 \pm 1$ μM A23187, 5 mM acetyl phosphate and 0.5 mg protein/μl at 25 °C for 20 min in a volume of 1.2 ml. Reaction was stopped by cooling 0.5 ml aliquots and the acetyl phosphate content was measured according to Lipmann and Tuttle [74]. For measuring Ca^{2+}-insensitive acetyl phosphatase activity, Ca^{2+} was omitted from the medium. The polarization of FITC fluorescence in the samples were measured at 25 °C (λ_{exc} = 490 nm; λ_{em} = 520 nm).

FITC labeling (nmol/mg protein	ATPase activity (μmol·mg^{-1}·min^{-1})		Acetylphosphatase activity (μmol·mg^{-1}·min^{-1})		Polarization of fluorescence
	Ca^{2+}-activated	Basal	Ca^{2+}-activated	Basal	
0.0	1.65	0.30	0.111	0.066	-
2.5	1.13	0.32	0.112	0.066	0.414
5.0	0.98	0.32	0.095	0.061	0.397
7.5	0.63	0.31	0.063	0.052	0.337
15.0	0.50	0.31	0.028	0.043	0.327

between them. At the lowest labeling ratio (2.5 nmol of FITC/mg protein) only one out of four ATPase molecules are labeled, and the high value of polarization is consistent with the absence of significant energy transfer. FITC does not interfere with ATPase–ATPase interactions [25] or with the crystallization of Ca^{2+}-ATPase in the presence of EGTA and vanadate [73]. Excitation of FITC-labeled sarcoplasmic reticulum at wavelengths ranging from 475 to 510 nm yielded fluorescence at 520 nm with a constant polarization of 0.432 ± 0.003 at a labeling ratio of 2.5 nmol/mg protein, and 0.367 ± 0.004 at a labeling ratio of 7.5 nmol FITC/mg protein. This is consistent with chemical evidence that FITC selectively labels lysine-515 in the Ca^{2+}-ATPase, without major contribution by labeling at secondary sites.

The Ca^{2+}-stimulated ATPase activity progressively decreased with increasing labeling by FITC, accompanied by the decrease in fluorescence polarization (Table I), while the Ca^{2+}-insensitive (basal) ATPase and acetylphosphatases activities were essentially unaffected by FITC. There was also a moderate inhibition of the Ca^{2+}-stimulated acetylphosphatase activity by FITC, in contrast to the observations of Pick and Bassilian [75]. This difference may be due to the different labeling conditions used in our experiments.

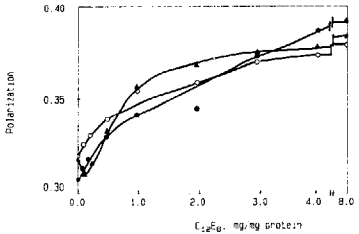


Fig. 6. Polarization of fluorescence of FITC-SR as the function of $C_{12}E_8$ concentration. Sarcoplasmic reticulum vesicles were labelled with FITC at 5 nmol/mg protein concentration, as described earlier. The vesicles were in 0.1 M KCl, 10 mM K-Mops, pH 6.0, 3 mM $MgCl_2$, 3 mM NaN_3, 5 mM DTT, 25 IU/ml Trasylol, 2 μg/ml 1,6-di-*tert*-butyl-*p*-cresol and 20% glycerol. After addition of 1.0 mM EGTA (○), 0.2 mM $CaCl_2$ (●) and 1.0 mM EGTA + 5 mM Na_3VO_4 (▲), the polarization of the vesicles was determined as described in Methods, in the presence of different concentrations of $C_{12}E_8$ at 25°C (λ_{exc} = 490 nm; λ_{em} = 520 nm).

The effect of $C_{12}E_8$ concentration on the polarization of fluorescence of FITC-labeled Ca^{2+}-ATPase

In sarcoplasmic reticulum vesicles labeled with 5 nmol of FITC per mg protein the polarization of FITC fluorescence measured at pH 6.0 was ≅ 0.31 ± 0.32 (Fig. 6). Addition of 0.2 mM Ca^{2+} or 1 mM EGTA, with or without 5 mM vanadate immediately prior to the polarization measurement had relatively little effect. Treatment of the membranes with nonsolubilizing concentrations of $C_{12}E_8$ (0.2–0.5 mg per mg protein) sharply increased the polarization; the polarization of fluorescence continued to rise with increasing $C_{12}E_8$ concentration up to (and probably beyond) 8 mg $C_{12}E_8$/mg protein.

The similar values of fluorescence polarization obtained with Ca^{2+}, that stabilizes the E_1 conformation, and with EGTA + vanadate that stabilize the E_2 conformation of the Ca^{2+}-ATPase imply either that the proximity between ATPase molecules is not affected by transitions between the E_1 and E_2 states, or that compensatory changes in the relative orientation of ATPase molecules within the oligomers cancel the effects of the changes in intermolecular distances.

The effect of Ca^{2+} on the polarization of fluorescence of FITC-labeled Ca^{2+}-ATPase

Ca^{2+}, at millimolar concentrations, decreased the polarization of fluorescence of FITC both in native sarcoplasmic reticulum (Fig. 7A) and in detergent-solubilized preparations (Fig. 7B), suggesting that Ca^{2+} promotes the formation of ATPase aggregates. This is consistent with the effect of Ca^{2+} on the crosslinking of Ca^{2+}-ATPase oligomers (Fig. 3) and on the crystallization of Ca^{2+}-ATPase in detergent-solubilized sarcoplasmic reticulum [62]. The decrease in fluorescence polarization with increasing concentration of the vesicles is not fully accounted for by the effect of light scattering on the polarization of fluorescence [76], and interaction between vesicles probably contributes. Vesicle aggregation that brings the ATPase molecules located in adjacent vesicles within range for efficient energy transfer may also explain the greater effect of Ca^{2+} on fluorescence polarization at higher protein concentration. Therefore, two distinct mechanisms are expected to play a role in the Ca^{2+}-induced decrease in the polarization of fluorescence of FITC-ATPase in intact sarcoplasmic reticulum vesicles. (1) Interaction between ATPase molecules located in the same membrane. (2) Interaction between headgroups of ATPase molecules located in distinct vesicles.

The polarization of fluorescence of FITC-ATPase is greater in the presence of solubilizing concentrations of

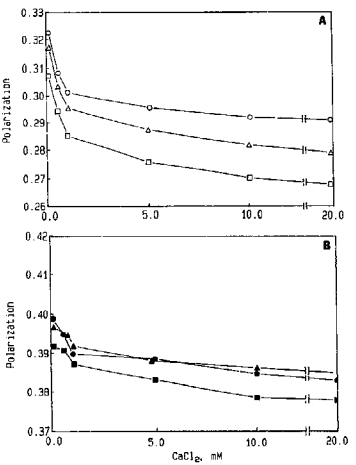


Fig. 7. The effect of [Ca^{2+}] on the polarization of FITC fluorescence in sarcoplasmic reticulum. Sarcoplasmic reticulum vesicles were labeled with FITC at final concentration of 7.5 nmol/mg protein, as described earlier [25]. After labeling, the vesicles were resuspended in 10 mM K-Mops, pH 7.5 at final protein concentrations of 0.05 mg/ml (○), 0.2 mg/ml (△), 0.4 mg/ml (□) and the polarization of the FITC fluorescence was measured after additions of $CaCl_2$ (0–20 mM final concentration) (A). A similar experiment was also performed (B) in the presence of 4 mg $C_{12}E_8$/mg protein. Protein concentrations: ●, 0.05 mg/ml; ▲, 0.2 mg/ml and ■, 0.4 mg/ml.

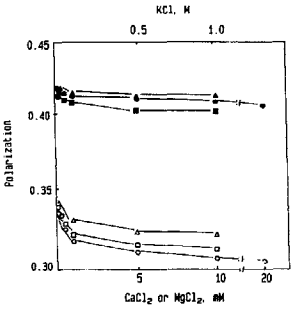


Fig. 8. Effect of Ca^{2+}, Mg^{2+} and K^+ on the polarization of FITC fluorescence in SR. Sarcoplasmic reticulum vesicles were labeled with FITC at final concentrations of 7.5 nmol/mg, as described earlier [25]. The polarization of FITC fluorescence was measured after additions of $CaCl_2$ (○,●), $MgCl_2$ (□,■) and KCl (△,▲) at 25°C, at a final protein concentration of 0.1 mg/ml. The measurements were carried out in 10 mM K-Mops, pH 7.5 buffer (open symbols) or in 10 mM K-Mops pH 7.5, that contained 4 mg/mg protein $C_{12}E_8$ (filled symbols). The polarization values were determined as described under Methods.

$C_{12}E_8$ (Fig. 7B), and the influence of Ca^{2+} and protein concentration on the polarization is somewhat less pronounced, consistent with an increase in the average distance between ATPase molecules. While the effect of $C_{12}E_8$ on the polarization of FITC fluorescence develops rapidly, the decrease in the polarization induced by Ca^{2+} had a slower time course and required 2–4 h to develop fully.

The effect of Ca^{2+} was not specific, since qualitatively similar change in polarization was observed at high concentration of Mg^{2+}, either in native membranes or in membrane preparations solubilized with 4 mg $C_{12}E_8$/mg protein (Fig. 8). The concentration of K^+ required to produce the decrease in polarization in the native membrane was about 100-times greater than that of the divalent cations, and K^+ had no significant effect on solubilized sarcoplasmic reticulum.

High performance liquid chromatography of detergent-solubilized sarcoplasmic reticulum

Resolution of Ca^{2+}-ATPase monomers from the various oligomeric forms can be achieved by high performance liquid chromatography on TSK G3000 or G4000 SW molecular sieve columns [4,77]. The speed of separation is important to prevent the denaturation and aggregation of Ca^{2+}-ATPase that is known to occur in detergent solutions [4]. We utilized this technique for the analysis of the effect of Ca^{2+} and glycerol on the monomer-oligomer equilibrium of Ca^{2+}-ATPase under conditions that promote the formation of Ca^{2+}-ATPase crystals.

Typical elution profiles of sarcoplasmic reticulum proteins solubilized by Brij 36T (5 mg/mg protein) in a medium of 0.1 M KCl, 10 mM Tes, pH 7.0, 3 mM $MgCl_2$, 3 mM NaN_3, 25 IU/ml Trasylol and 2 μg/ml 1,6-di-*tert*-butyl-*p*-cresol are given in Fig. 9. The observed band pattern is influenced by the treatment of the solubilized protein sample before application to the column, by the protein concentration and protein/detergent ratio and by the pH, ion composition and glycerol content of the incubation medium.

It is customary to subject the samples to centrifugation, usually in a Beckman airfuge centrifuge at 130000 × g for 30 min, to remove 'unsolublized material' before application for column chromatography [4,58,77, 78]. A comparison of samples centrifuged in a Beckman microfuge at ≅ 8000 × g for 5 min (Fig. 9A,C,E), and in a Beckman Airfuge at 130000 × g for 5 min (Fig. 9B,D,F) shows that the high speed centrifugation decreases the contribution of ATPase oligomers and shifts

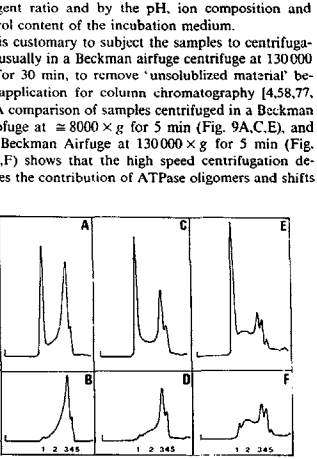


Fig. 9. High performance liquid chromatography of sarcoplasmic reticulum proteins. Sarcoplasmic reticulum vesicles were washed with a solution containing 0.1 M KCl, 10 mM Tes, pH 7.0, 3 mM $MgCl_2$, 3 mM NaN_3, 25 IU/ml Trasylol and 2 μg/ml 1,6-di-*tert*-butyl-*p*-cresol to remove sucrose. After centrifugation the sediment was resuspended in the same solution to a protein concentration of 5 mg/ml. Brij 36T was added to a final concentration of 5 mg/mg protein followed by homogenization in a Potter homogenizer. After incubation for 60 min at 2°C the samples were centrifuged in microfuge (8000 × g for 5 min) (upper panel), or in airfuge (130000 × g for 5 min) (lower panel); 20 μl samples were applied to HPLC in a TSK Gel 4000 SW column (7.5 mm ID × 30 cm) protected with TSK guard column SW (7.5 mm i.d. × 7.5 cm) and eluted with the above buffer containing 5 mg of Brij 36T/ml at a flow rate of 0.5 ml/min. (A,B) No further additions; in samples (C,D) 20 mM $CaCl_2$, and in samples (E,F) 20 mM $CaCl_2$ and 20% glycerol were also present both in the sample and in the eluant. Elution positions 1, 2, and 3 represent the high molecular weight oligomers (void volume), dimers, and monomers of Ca^{2+}-ATPase, respectively; calsequestrin, Ca^{2+}-binding protein and other low molecular weight components are eluted in bands 4 and 5, respectively.

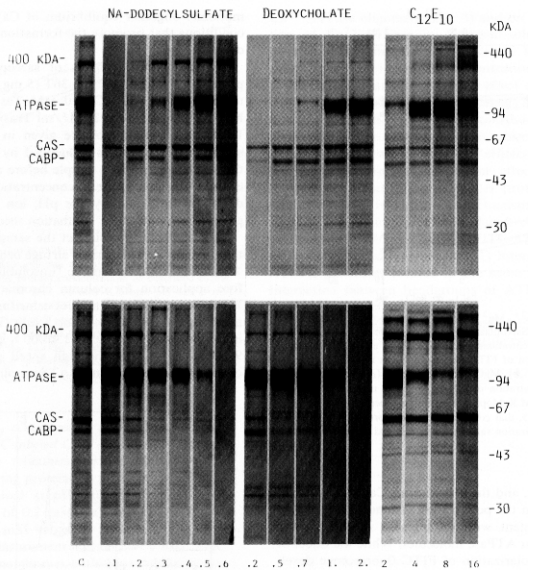


Fig. 10. Solubilization of sarcoplasmic reticulum proteins with sodium dodecyl sulfate (SDS), sodium deoxycholate (DOC) and $C_{12}E_{10}$. Sarcoplasmic reticulum vesicles (2 mg/ml) were solubilized with SDS, deoxycholate and $C_{12}E_{10}$ at the detergent: protein ratios indicated, in a medium containing 0.1 M NaCl, 10 mM Na-Mops, pH 8.0 (SDS and DOC) or 0.1 mM KCl, 10 mM K-Mops, pH 8.0 ($C_{12}E_{10}$), together with 3 mM $MgCl_2$, 5 mM DTT, 3 mM NaN_3, 25 IU/ml Trasylol, and 2 μg/ml 1,6-di-*tert*-butyl-*p*-cresol. After 30 min incubation at 2°C the unsolubilized material was removed by brief centrifugation (10 min at room temperature), using Beckman airfuge (130000× *g*) and the protein composition of the supernatants (upper panels) and pellets (lower panels) were analyzed by SDS-polyacrylamide gel electrophoresis [63]. C, control samples before centrifugation.

the apparent equilibrium in favor of the ATPase monomers. The amount of proteins removed by the preliminary high speed centrifugation was 20–30% in the experiments of Andersen and Vilsen [77], and as high as 55% in our studies and in the experiments of Silva and Verjovski-Almeida [58]. The net effect of this is that the contribution of oligomers to the system is minimized, with an apparent increase in the dissociation constant of monomer-oligomer equilibrium.

The precise assignment of molecular weights to the various protein bands is complicated by differences in the behavior of different calibration standards [77]. We tentatively assign band 1 to high molecular weight components, band 2 to ATPase dimers, band 3 to ATPase monomers and bands 4–5 to low molecular weight proteins.

Addition of 20 mM Ca^{2+} (Fig. 9C,D) or 20 mM Ca^{2+} and 20% glycerol (Fig. 9E,F) to the solubilizing and elution media shifted the equilibrium from monomers in favor of ATPase oligomers, as indicated by the accumulation of material in bands eluted near the exclusion volume of the columns. This effect was particularly clear in samples (Fig. 9C and E) that were not subjected to high speed centrifugation prior to application to the HPLC columns. Much of the high molecular weight ATPase oligomers are actually removed during

high speed centrifugation (Fig. 9B,D,F). Therefore under these conditions the increased association of Ca^{2+}-ATPase caused by 20 mM Ca^{2+} (Fig. 9D) or 20 mM Ca^{2+} 20% glycerol (Fig. 9F) resulted in a decreased yield of protein mass throughout the elution diagram, except in the region of band 2 that was tentatively assigned to Ca^{2+}-ATPase dimers.

The observations made by high performance liquid chromatography support the earlier conclusions from glutaraldehyde crosslinking, and fluorescence polarization studies that Ca^{2+} promotes the formation of ATPase oligomers. The Ca^{2+}-induced formation of ATPase oligomers probably contributes to the long-term stability of solubilized Ca^{2+}-ATPase in the presence of 20 mM $CaCl_2$ [62], and may represent the structural basis of the Ca^{2+}-induced crystallization of the enzyme in detergent solutions [63].

Differential solubilization of sarcoplasmic reticulum proteins by detergents

Interesting differences were observed between anionic and nonionic detergents in their ability to solubilize different proteins from sarcoplasmic reticulum vesicles.

Sodium dodecyl sulfate caused substantial solubilization of calsequestrin and of the high-affinity Ca^{2+}-binding protein already at concentrations as low as 0.1–0.2 mg SDS/mg protein; this was followed by solubilization of the 300 kDa proteins and of the Ca^{2+}-ATPase as the SDS concentration was increased to 0.5–0.6 mg/mg protein (Fig. 10). The extraction of the Ca^{2+}-ATPase by sodium dodecyl sulfate was inhibited by 2–10 mM $CaCl_2$ in the extraction medium, presumably due to the formation of Ca^{2+}-dodecyl sulfate aggregates.

The sequence of the solubilization of various proteins by increasing concentrations of deoxycholate was similar to that observed with sodium dodecyl sulfate, but complete solubilization of the Ca^{2+}-ATPase required deoxycholate concentrations as high as 1–2 mg/mg protein (Fig. 10).

The pattern of solubilization by the nonionic detergent $C_{12}E_{10}$ was quite different from that observed with either of the two anionic detergents (Fig. 10). $C_{12}E_{10}$ preferentially solubilized the high-affinity Ca^{2+}-binding protein at a detergent/protein ratio of 2, followed by solubilization of the Ca^{2+}-ATPase as the $C_{12}E_{10}$ concentration was raised to 4 mg/mg protein or above (Fig. 10). Much of the calsequestrin, the 300 kDa proteins, and other accessory proteins remained in the unsolubilized sediment fraction even at $C_{12}E_{10}$ concentrations as high as 16 mg/mg protein (Fig. 10). Similar observations were made with Brij 36T and other nonionic detergents (not shown). In view of the known association of calsequestrin with the junctional complex [68,69], these observations imply that the elements of the triad are susceptible to disruption by anionic detergents, such as sodium dodecyl sulfate or deoxycholate, but relatively resistant to nonionic detergents, such as $C_{12}E_{10}$ or Brij 36T.

The different patterns of solubilization of sarcoplasmic reticulum proteins by anionic and nonionic detergents suggest the existence of protein–protein and protein–lipid interactions in the sarcoplasmic reticulum membrane that are selectively affected by different detergents.

Conclusions

(1) Preferential crosslinking of Ca^{2+}-ATPase dimers by glutaraldehyde was observed at pH 6.0 in intact sarcoplasmic reticulum vesicles and after solubilization by nonionic detergents, suggesting that the dominant oligomeric form of the Ca^{2+}-ATPase under both conditions is the dimer.

(2) Ca^{2+}, at millimolar concentration, promoted the formation of Ca^{2+}-ATPase oligomers, both in the native membrane and in detergent solutions, as shown by chemical crosslinking, polarization of fluorescence and high performance liquid chromatography. The shift in equilibrium in favor of ATPase oligomers probably contributes to the increased stability and crystallization of the Ca^{2+}-ATPase in detergent solutions containing 20 mM Ca^{2+}.

(3) The existence of Ca^{2+}-ATPase oligomers in detergent solutions that were previously thought to contain primarily or only monomers of the Ca^{2+}-ATPase, reopens the problem of the functional role of the ATPase oligomers.

Acknowledgements

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References

1 Tanford, C. (1984) Crit. Rev. Biochem. 17, 123–151.
2 Møller, J.V., Andersen, J.P. and Le Maire, M. (1982) Mol. Cell. Biochem. 42, 83–107.
3 Møller, J.V., Le Maire, M. and Andersen, J.P. (1986) in Progress in Protein-Lipid Interactions, Vol. 2 (Watts, A. and De Pont, J.J.H.H.M., eds.), pp. 147–196, Elsevier, Amsterdam.
4 Andersen, J.P., Vilsen, B. Nielsen, H. and Møller, J.V. (1986) Biochemistry 25, 6439–6447.
5 Andersen, J.P. (1989) Biochim. Biophys. Acta 988, 47–72.
6 Martonosi, A., Taylor, K.A., Varga, S., Ting-Beall, H.P. and Dux, L. (1987) in Electron Microscopy of Proteins, Vol. 6, Membrane Structures (Harris, J.R. and Horne, R.W., eds.), pp. 255–276, Academic Press, London.

7 Martonosi, A. (1975) in Calcium Transport in Contraction and Secretion (Carafoli, E., Clementi, F., Drabikowski, W. and Margreth, A., eds.), pp. 313–327, North Holland, Amsterdam.
8 Jilka, R.L., Martonosi, A. and Tillack, T.W. (1975) J. Biol. Chem. 250, 7511–7524.
9 Scales, D. and Inesi, G. (1976) Biophys. J. 16, 735–751.
10 Martonosi, A., Nakamura, H., Jilka, R.L. and Vanderkooi, J.M. (1977) in Biochemistry of Membrane Transport (Semenza, G. and Carafoli, E. eds.), pp. 401–415, Springer-Verlag, Berlin.
11 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 2599–2603.
12 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 10111–10115.
13 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 11896–11902.
14 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 11903–11907.
15 Dux, L., Taylor, K.A., Ting-Beall, H.P. and Martonosi, A. (1985) J. Biol. Chem. 260, 11730–11743.
16 Taylor, K., Dux, L. and Martonosi, A. (1984) J. Mol. Biol. 174, 193–204.
17 Taylor, K.A., Dux, L. and Martonosi, A. (1986) J. Mol. Biol. 187, 417–427.
18 Taylor, K.A., Ho, M.H. and Martonosi, A. (1986) Ann. NY Acad. Sci. 483, 31–43.
19 Franzini-Armstrong, C. and Ferguson, D.G. (1985) Biophys. J. 48, 607–615.
20 Ferguson, D.G., Franzini-Armstrong, C., Castellani, L., Hardwicke, P.M.D. and Kenney, L.J. (1985) Biophys. J. 48, 597–605.
21 Vegh, K., Spiegler, P., Chamberlain, C. and Mommaerts, W.F.H.M. (1968) Biochim. Biophys. Acta 163, 266–268.
22 Chamberlain, B.K., Berenski, C.J., Jung, C.Y. and Fleischer, S. (1983) J. Biol. Chem. 258, 11997–12001.
23 Hymel, L., Maurer, A., Berenski, C.J., Jung, C.Y. and Fleischer, S. (1984) J. Biol. Chem. 259, 4890–4895.
24 Hymel, L., Maurer, A., Berenski, C.J., Jung, C.Y. and Fleischer, S. (1985) in Structure and Function of Sarcoplasmic Reticulum, Symp. on Structure and Function of Sarcoplasmic Reticulum, Kobe, Japan, Nov. 1–4, 1982 (Fleischer, S. and Tonomura, Y., eds.), pp. 155–162, Academic Press, Orlando, FL.
25 Papp, S., Pikula, S. and Martonosi, A. (1987) Biophys. J. 51, 205–220.
26 Highsmith, S. and Cohen, J.A. (1987) Biochemistry 26, 154–161.
27 Vanderkooi, J.M., Ierokomas, A., Nakamura, H. and Martonosi, A. (1977) Biochemistry 16, 1262–1267.
28 Gingold, M.P., Rigaud, J.L. and Champeil, P. (1981) Biochimie 63, 923–925.
29 Champeil, P., Rigaud, J.L. and Gingold, M.P. (1982) Z. Naturforsch. 37c, 513–516.
30 Watanabe, T. and Inesi, G. (1982) Biochemistry 21, 3254–3259.
31 Yantorno, R.E., Yamamoto, T. and Tonomura, Y. (1983) J. Biochem. (Tokyo) 94, 1137–1145.
32 Fagan, M.H. and Dewey, T.G., 1986, J. Biol. Chem. 261, 3654–3660.
33 Munkonge, F., Michelangeli, F., Rooney, E.K., East, J.M. and Lee, A.G. (1988) Biochemistry 27, 6800–6805.
34 Estep, T.N. and Thompson, T.E. (1979) Biophys. J. 26, 195–208.
35 Dewey, T.G. and Hammes, G.G. (1980) Biophys. J. 32, 1023–1036.
36 Grover, A.K., Samson, S.E., Berenski, C.J. and Jung, C.Y. (1985) Life Sci. 37, 2193–2198.
37 Andersen, J.P. and Vilsen, B. (1988) FEBS Lett. 234, 120–126.
38 Louis, C.F. and Shooter, E.M. (1972) Arch. Biochem. Biophys. 153, 641–655.
39 Chyn, T. and Martonosi, A. (1977) Biochim. Biophys. Acta 468, 114–126.
40 Hebdon, G.M., Cunningham, L.W. and Green, N.M. (1979) Biochem. J. 179, 135–139.
41 Kosk-Kosicka, D., Kurzmack, M. and Inesi, G. (1983) Biochemistry 22, 2559–2567.
42 McIntosh, D.B. and Ross, D.C. (1985) Biochemistry 24, 1244–1251.
43 Huang, C.-K. (1977) Lateral protein distribution in membranes of vesicles from sarcoplasmic reticulum, Ph.D. Thesis, Yale University.
44 Louis, C.F., Saunder, M.J. and Holroyd, J.A. (1977) Biochim. Biophys. Acta 493, 78–92.
45 Baskin, R.J. and Hanna, S. (1979) Biochim. Biophys. Acta 576, 61–70.
46 Louis, C.F. and Holroyd, J.A. (1978) Biochim. Biophys. Acta 535, 222–232.
47 Kurobe, Y., Nelson, R.W. and Ikemoto, N. (1983) J. Biol. Chem. 258, 4381–4389.
48 Bailin, G. (1980) Biochim. Biophys. Acta 624, 511–521.
49 Bailin, G. (1981) Physiol. Chem. Phys. 13, 121–127.
50 Murphy, A.J. (1976) Biochem. Biophys. Res. Commun. 70, 160–166.
51 Ikemoto, N., Garcia, A.M., O'Shea, P.A. and Gergely, J. (1975) J. Cell Biol. 67, 187a.
52 Giotta, G.J. (1976) J. Biol. Chem. 251, 1247–1252.
53 Tanford, C. (1977) in Structure and Function of Biological Membranes (Abrahamsson, S. and Pascher, I., eds.), pp. 497–508, Plenum, New York.
54 Martin, D.W. (1983) Biochemistry 22, 2276–2282.
55 Martin, D.W. and Tanford, C. (1984) FEBS Lett. 177, 146–150.
56 Martin, D.W., Tanford, C. and Reynolds, J.A. (1984) Proc. Natl. Acad. Sci. USA 81, 6623–6626.
57 Silva, J.L. and Verjovski-Almeida, S. (1983) Biochemistry 22, 707–716.
58 Silva, J.L. and Verjovski-Almeida, S. (1985) J. Biol. Chem. 260, 4764–4769.
59 Andersen, J.P., Jørgensen, P.L. and Møller, J.V. (1985) Proc. Natl. Acad. Sci. USA 82, 4573–4577.
60 Andersen, J.P., Lassen, K. and Møller, J.V. (1985) J. Biol. Chem. 260, 371–380.
61 Dux, L., Pikula, S., Mullner, N. and Martonosi, A. (1987) J. Biol. Chem. 262, 6439–6442.
62 Pikula, S., Mullner, N., Dux, L. and Martonosi, A. (1988) J. Biol. Chem. 263, 5277–5286.
63 Taylor, K.A., Mullner, N., Pikula, S., Dux, L., Peracchia, C., Varga, S. and Martonosi, A. (1988) J. Biol. Chem. 263, 5287–5294.
64 Varga, S., Mullner, N., Pikula, S., Papp, S., Varga, K. and Martonosi, A. (1986) J. Biol. Chem. 261, 13943–13956.
65 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
66 Laemmli, U.K. (1970) Nature 227, 680–685.
67 Michalak, M. (1985) in The Enzymes of Biological Membranes, 2nd Edn., Vol. 3 (Martonosi, A., ed.), pp. 115–155, Plenum Press, New York.
68 Cadwell, J.J.S. and Caswell, A.H. (1982) J. Cell Biol. 93, 543–550.
69 Caswell, A.H. and Brunschwig, J.-P. (1984) J. Cell Biol. 99, 929–939.
70 Pick, U. (1982) J. Biol. Chem. 257, 6111–6119.
71 Pick, U. and Karlish, S.J.D. (1982) J. Biol. Chem. 257, 6120–6126.
72 Jona, I. and Martonosi, A. (1986) Biochem. J. 234, 363–371.
73 Varga, S., Csermely, P., Mullner, N., Dux, L. and Martonosi, A. (1987) Biochim. Biophys. Acta 896, 187–195.
74 Lipmann, F. and Tuttle, L.C. (1945) J. Biol. Chem. 159, 21–28.
75 Pick, U. and Bassilian, S. (1981) FEBS Lett. 123, 127–130.
76 Teale, F.W.J. (1969) Photochem. Photobiol. 10, 363–374.
77 Andersen, J.P. and Vilsen, B. (1985) FEBS Lett. 189, 13–17.
78 Vilsen, B. and Andersen, J.P. (1987) Eur. J. Biochem. 170, 421–429.