Biochimica et Biophysica Acta 896 (1987) 187–195
Elsevier

BBA 73169

Effect of chemical modification on the crystallization of Ca^{2+}-ATPase in sarcoplasmic reticulum

Sandor Varga *, Peter Csermely **, Nandor Mullner **, Laszlo Dux *** and Anthony Martonosi

Department of Biochemistry, State University of New York, Health Sciences Center, Syracuse, NY (U.S.A.)

(Received 24 July 1986)

Key words: ATPase, Ca^{2+}; Sarcoplasmic reticulum; Membrane cystal

The influence of chemical modification on the morphology of crystalline ATPase aggregates was analyzed in sarcoplasmic reticulum (SR) vesicles. The Ca^{2+}-ATPase forms monomer-type (P1) type crystals in the E_1 and dimer-type (P2) crystals in the E_2 conformation. The P1 type crystals are induced by Ca^{2+} or lanthanides; P2 type crystals are observed in Ca^{2+}-free media in the presence of vanadate or inorganic phosphate. P1- and P2-type Ca^{2+}-ATPase crystals do not coexist in significant amounts in native sarcoplasmic reticulum membrane. The crystallization of Ca^{2+}-ATPase in the E_2 conformation is inhibited by guanidino-group reagents (2,3-butanedione and phenylglyoxal), SH-group reagents, phospholipases C or A_2, and detergents, together with inhibition of ATPase activity. Amino-group reagents (fluorescein 5′-isothiocyanate, pyridoxal phosphate and fluorescamine) inhibit ATPase activity but do not interfere with the crystallization of Ca^{2+}-ATPase induced by vanadate. In fluorescamine-treated sarcoplasmic reticulum the vanadate-induced crystals contain significant P1-type regions in addition to the dominant P2 form.

Introduction

The Ca^{2+}-ATPase of sarcoplasmic reticulum forms two distinct types of crystal [1–4] that are assumed to represent the E_1 and E_2 conformations of the enzyme defined by kinetic and fluorescence studies [5–8]. The E_1 type crystals form in the presence of Ca^{2+} or lanthanides and consist of chains of ATPase monomers evenly spaced in a right-handed helix on the surface of the vesicles. The space group of the E_1 crystals is P1 and their structural unit is the Ca^{2+}-ATPase monomer [4]. The E_2-type crystals are induced by vanadate or inorganic phosphate in a Ca^{2+}-free solution [1,2]. The characteristic features of the E_2 crystals are the helical chains of ATPase dimers separated from neighboring dimer chains by wider bands of negative stain. The space group of E_2 crystals is P2, with Ca^{2+}-ATPase dimers as structural units [3]. The two distinct crystal forms appear to be specifically related to the two major conformations of the Ca^{2+}-ATPase; mixtures of the two types of crystal were not observed in native

* On leave from the Central Research Laboratory, University Medical School, Debrecen, H-4012 Hungary.
** On leave from the 1st Institute of Biochemistry, Semmelweis University Medical School, Budapest, H-1088 Hungary.
*** On leave from the Institute of Biochemistry, University Medical School, Szeged, H-6701 Hungary.

Abbreviations: EGTA, ethylene glycol bis[β-aminoethyl ether]-*N,N,N′,N′*-tetraacetic acid; PLP, pyridoxal 5′-phosphate; Mops, morpholinepropanesulfonic acid; FITC, fluorescein 5′-isothiocyanate; Salyrgan, *O*-{[3-(hydroxymercury-2-methoxypropyl)]carbamoyl}phenoxyacetic acid sodium salt.

Correspondence: A. Martonosi, Department of Biochemistry, State University of New York, Health Sciences Center, Syracuse, NY 13210, U.S.A.

sarcoplasmic reticulum vesicles. In this report we analyze the effects of chemical modification of the Ca^{2+}-ATPase aggregates in sarcoplasmic reticulum.

Methods and Materials

Sarcoplasmic reticulum vesicles were isolated from predominantly white skeletal muscles of rabbits according to Nakamura et al. [9].

The labeling with pyridoxal 5′-phosphate (Sigma Chemical Co.) was a modification of the procedure described by Murphy [10]. The sarcoplasmic reticulum vesicles (2 mg protein/ml) were suspended in a buffer of 0.3 M sucrose/0.1 M Tris-maleate/0.1 mM EGTA/and 5 mM $MgCl_2$ (pH 7.0). Pyridoxal 5′-phosphate (PLP) was added from a 0.1 M stock solution (in 50 mM Tris-maleate adjusted to pH 7.0) to a final concentration of 10 mM and the mixture was incubated at 25°C for 60 min. The samples were cooled in ice for 5 min, and 10 mM $NaBH_4$ (Fisher Scientific Co.) was added. After 10 min at 2°C, the vesicles were collected by centrifugation at $80\,000 \times g$ for 20 min and washed twice with sucrose-Tris buffer. The samples were divided into two portions. For crystallization and ATPase activity measurements the final pellet was suspended in 0.1 M KCl/10 mM imidazole/5 mM $MgCl_2$/0.5 mM EGTA (pH 7.4) (standard buffer) to a final protein concentration of 1 mg/ml. For the determination of the bound PLP, the final pellet was suspended in 50 mM Tris-maleate (pH 6.8) ad solubilized with 1% sodium dodecyl sulfate (pH 6.8). The amount of PLP bound to the membrane was determined from the difference between the optical absorbance measured at 325 and 390 nm (the latter used to correct for light scattering), using the molar extinction coefficient of 10 000 [10].

The reaction of microsomes with fluorescein 5′-isothiocyanate was carried out as described earlier [6]; the localization of covalently bound fluorescein among the tryptic peptides of Ca^{2+}-ATPase was similar to that described by Dux et al. [7].

The labeling with fluorescamine (Aldrich Chemical Co.) was performed according to Hidalgo et al. [11]. The sarcoplasmic reticulum vesicles (2 mg protein/ml) were suspended in a solution of 0.1 M KCl/20 mM Tris/0.1 mM EGTA/5 mM $MgCl_2$ (pH 7.0). Fluorescamine was added from a 50 mM stock solution in dimethylformamide to a final concentration of 0.5 mM. To the control sample the same amount of dimethylformamide was added. The samples were incubated at 25°C for 10 min and processed as described above for pyridoxal 5′-phosphate. The bound fluorescamine was determined by absorption measurements at 362 nm and 500 nm, using the molar extinction coefficient of 5 800. The absorption spectra of the free and bound fluorescamine have an isosbestic point around 360 nm; the absorption values at 500 nm were used for light-scattering corrections.

The chemical modifications with 2,3-butanedione or phenylglyoxal (Aldrich Chemical Co.) were carried out according to Murphy [12]. The vesicles (2 mg protein/ml) were suspended in 50 mM sodium borate/0.1 M Na-Mops/0.1 mM EGTA/5 mM $MgCl_2$ (pH 7.4). Freshly made 0.1 M stock solution of redistilled 2,3-butanedione or recrystallized phenylglyoxal in 50 mM sodium borate (pH 7.4) were added to final concentrations of 10 mM. After incubation for 60 min at 25°C, the samples were centrifuged at $80\,000 \times g$ for 20 min and the sediments were suspended in the same borate/Mops buffer to a final protein concentration of 1 mg/ml.

The Ca^{2+}-activated ATPase activity was measured as described earlier [9] in the presence of 1 μM ionophore A23187 (Calbiochem). The method of Lowry et al. [13] was used for the assay of protein with bovine serum albumin as standard.

Decavanadate stock solutions were prepared by adjusting the pH of a 50 mM monovanadate (Fisher Scientific Co.) solution to pH 2.0 and after several hours to 7.4. Due to the temperature- and pH-dependent decay of decavanadate [14], special care was taken to use only freshly diluted, ice-cold decavanadate solutions in each experiment.

For electron microscopy the vesicle suspensions were negatively stained with 1% uranyl acetate (Fisher Scientific Co.) and viewed in a Siemens Elmiskop I microscope at an accelerating voltage of 60 kV. The extent of crystallization of the Ca^{2+}-ATPase, denoted as the crystallization index, was determined by counting the vesicles with crystalline regions on their surface and expressing

their number as percent of the total number of vesicles [15].

Materials

Pyridoxal 5′-phosphate, phospholipase C (*C. welchii*), fluorescein 5′-isothiocyanate and *N*-ethylmaleimide were obtained from Sigma Chemical Co., St. Louis, MO. $NaBH_4$, Na-vanadate, and uranyl acetate were supplied by Fisher Scientific Co., Fairlawn, NJ. Fluorescamine, 2,3-butanedione, phenylglyoxal and *p*-chloromercuribenzoate were the products of Aldrich Chemical Co., Milwaukee, WI. A23187 came from Calbiochem. San Diego, CA; phospholipase A_2 (*Crotalus durissimus*) from Boehringer-Mannheim, Indianapolis, IN and Salyrgan from Mann Research Laboratories, NY.

Results and Discussion

The effect of amino and guanidino group reagents on the crystallization of Ca^{2+}-ATPase by vanadate

The crystallization of the Ca^{2+}-ATPase in the E_2 form is induced by vanadate (V) anions [1,2]. Oligovanadates such as decavanadate are particularly effective, promoting rapid crystallization at V concentrations as low as 10 μM [14]. Sedimentation equilibrium and ^{51}V-NMR studies suggest the binding of two decavanadate molecules per mol Ca^{2+}-ATPase [16,17]. To obtain further information about the role of positively charged side-chain groups in the effects of vanadate on the enzyme, we investigated the effects of amino-group reagents (fluorescein 5′-isothiocyanate, pyridoxal 5′-phosphate and fluorescamine) and two guanidino-group reagents (butanedione and phenylglyoxal) on the vanadate-induced crystallization of the Ca^{2+}-ATPase.

Effect of fluorescein 5′-isothiocyanate. Coupling of the enzyme with fluorescein 5′-isothiocyanate inhibits the ATPase activity [18,19]. The binding of decavanadate to one of the two vanadate-binding sites is also blocked [16] without interference with the crystallization of the Ca^{2+}-ATPase (Fig. 1). The binding of monovanadate to the Ca^{2+}-ATPase [16] and its effect on the crystallization of the enzyme are not influenced by FITC (Fig. 1).

Effect of pyridoxal 5′-phosphate. Reaction of sarcoplasmic reticulum vesicles with pyridoxal 5′-phosphate inhibits the ATPase activity and ATP protects against this inhibition [10]. Murphy suggested [10] that the reaction of a single lysine residue, located in the 30 kDa tryptic cleavage fragment of the Ca^{2+}-ATPase may be associated with the inhibition of ATPase activity. In our experience, the Ca^{2+}-ATPase activity of sarcoplasmic reticulum is only partially lost even after reaction of 17 nmol of PLP per mg sarcoplasmic reticulum protein, representing 2–3 mol of PLP per mol ATPase (Table I). ATP (5 mM) fully protected the ATPase activity against inhibition by PLP, although the amount of bound PLP was not altered significantly (Table I). There is no difference between control and pyridoxal phosphate-labeled sarcoplasmic reticulum vesicles in the extent of crystallization of Ca^{2+}-ATPase in the presence of EGTA and vanadate (Table I).

Effect of fluorescamine. Inhibition of ATPase activity and Ca^{2+} transport is also observed upon limited labeling of sarcoplasmic reticulum vesicles with fluorescamine [11]. Fluorescamine reacts with the amino groups of phosphatidylethanolamine and in the presence of serum albumin this is the main target of chemical modification. ATP (5 mM) protects the ATPase activity from inhibition by fluorescamine, but the Ca^{2+} transport activity is lost [11]. Hidalgo et al. [11] attribtued this effect to uncoupling of ATP hydrolysis from Ca^{2+} transport. The ATPase activities and the amount of bound fluorescamine shown in Table I are in reasonable agreement with the results of Hidalgo et al. [11]. The change in the lipid environment and the possible modification of the Ca^{2+}-ATPase caused by fluorescamine do not diminish its ability to form two-dimensional crystals in the presence of decavanadate (Table I). In native sarcoplasmic reticulum vesicles, the Ca^{2+}-ATPase crystals induced by mono- or decavanadate are nearly exclusively of the P2 type (Fig. 1); P1-type crystals without clearly recognizable dimer chains [4] constitute 3% or less of the vesicle population with decavanadate (Table II) and 0.03% with monovanadate (not shown). After fluorescamine treatment, the occurrence of vesicles with P1-type crystals (Fig. 2) in the presence of either mono- or decavanadate, increases to 10–12% of the vesicle population (Table II). This slight increase in the

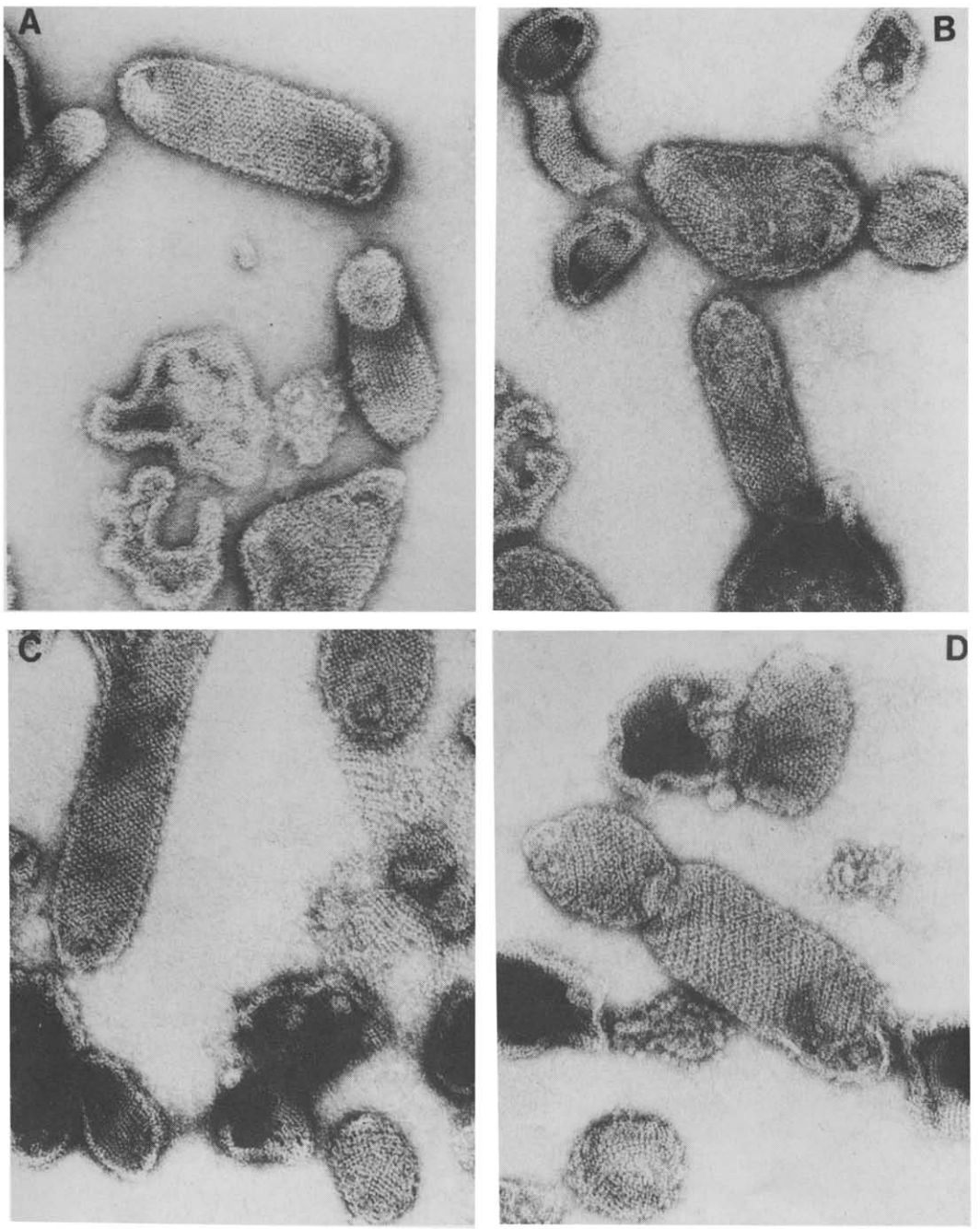

Fig. 1. Vanadate-induced crystals of Ca^{2+}-ATPase in control and FITC-treated microsomes after 24 h of incubation. Control (panel A, C) and FITC-labeled (panels B, D) sarcoplasmic reticulum vesicles were incubated with mono- (panels A, B) or decavanadate (panels C, D) at 1 mM total V concentration in 0.1 M KCl/10 mM imidazole/5 mM $MgCl_2$/0.5 mM EGTA (pH 7.4) for 24 h at 4°C. Samples were negatively stained with 1% uranyl acetate. (A) Monovanadate-induced crystals in control vesicles. ×150000. (B) Monovanadate-induced crystals in FITC-treated vesicles. ×150000. (C) Decavanadate-induced crystals in control vesicles. ×150000. (D) Decavanadate-induced crystals in FITC-treated vesicles. ×150000.

TABLE I

THE EFFECT OF CHEMICAL MODIFICATION ON THE EXTENT OF DECAVANADATE-INDUCED CRYSTALLIZATION OF THE SARCOPLASMIC RETICULUM Ca^{2+}-ATPase

The chemical modification and the various assays were carried out as described under Methods and Materials. The crystallization of the Ca^{2+}-ATPase was determined afte 24 h incubation with 2 mM decavanadate in a medium of 0.1 M KCl/10 mM imidazole (pH 7.4)/5 mM $MgCl_2$/0.5 mM EGTA. The crystallization index is a measure of the extent of crystallization under optimum conditions, given by expressing the number of vesicles with crystalline regions on their surface as percent of the total number of vesicles [15]. n.d., not determined.

Addition	Bound reagent (nmol/mg protein)	ATPase activity (μmol P_i/mg protein per min)	Crystallization index (±S.E.) (%)
Control, no addition	–	1.38	85.8±2.3
Pyridoxal phosphate (10 mM)	17	0.96	82.2±3.0
Pyridixal phosphate (10 mM) and 5 mM Ca^{2+}	17	0.87	82.6±4.0
Pyridoxal phosphate (10 mM) + 5 mM Ca^{2+} and 4 mM ATP	19	1.26	81.6±3.2
Control, no addition	–	0.93	91.0±1.1
Fluorescamine (0.5 mM)	51	0.52	85.5±2.8
Fluorescamine (0.5 mM) and 5 mM ATP	47	0.93	88.8±3.9
Fluorescamine (0.5 mM) + 5 mM ATP and 5 mg/ml bovine serum albumin	41	0.85	89.5±0.7
Control, no addition	–	1.05	90.2±2.3
Butanedione (10 mM)	n.d.	0.07	0.8±1.7
Butanedione (10 mM) and 5 mM ATP	n.d.	1.06	9.3±0.6
Control, no addition	–	1.29	90.8±1.4
Phenylglyoxal (10 mM)	n.d.	0.09	5.2±3.6
Phenylglyoxal (10 mM) and 5 mM ATP	n.d.	0.40	14.0±5.2

occurrence of the P1 crystal form may result from modification of the protein or phospholipids, or both, and further studies are required to identify the precise molecular basis of the effect. Modifica-

TABLE II

THE EFFECT OF FLUORESCAMINE ON THE CRYSTAL FORM

Microsomes were labeled with 0.5 mM fluorescamine in the absence of ATP or bovine serum albumin, as described under Methods and Materials and in Table I. Crystallization was induced by incubation in the presence of 2 mM decavanadate at 2°C in 0.1 M KCl/10 mM imidazole/5 mM $MgCl_2$/0.5 mM EGTA (pH 7.4). After negative staining with uranyl acetate, the crystallization index and the proportion of P1- and P2-type crystals were determined.

	Crystal form (% abundance)		Crystallization index (%)
	P1	P2	
Control microsomes	3.03	96.97	91 ±2.8
Fluorescamine-labeled microsomes	13.50	86.50	85.5±2.8

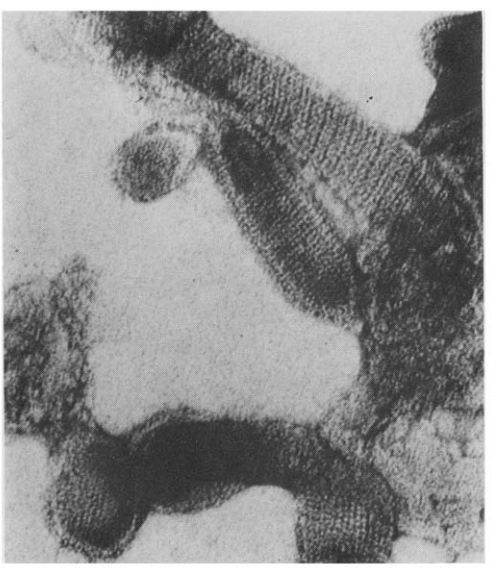

Fig. 2. Ca^{2+}-ATPase crystals in microsomes treated with fluorescamine. After treatment with fluorescamine as described in the legend to Table I and under Methods and Materials, the microsomes were negatively stained with 1% uranyl acetate. Magnification: ×150000.

tion of a fraction of ATPase molecules (about 13%) in a homogeneous population would be expected to affect all vesicles containing Ca^{2+}-ATPase to a moderate extent. Instead, P1-type crystals occur in about 13% of the vesicles, covering much of their surface, and we never observed a mixture of the two crystal forms (P1 and P2) within the same vesicle. It is possible that fluorescamine selectively modifies a subpopulation of sarcoplasmic reticulum vesicles that possess a distinctive structure; vesicles derived from slow-twitch fibers in the predominantly white muscles may yield such a subpopulation.

Effect of guanidino-group reagents. The inhibition of the Ca^{2+}-ATPase of sarcoplasmic reticulum by the guanidino-group reagent, butanedione, was ascribed by Murphy [12] to the modification of 'essential' arginine group(s) at the active site. The complete loss of ATPase activity after chemical modification with butanedione (Table I) is accompanied by inability of the Ca^{2+}-ATPase to form two-dimensional crystalline arrays in the presence of decavanadate (Table I). Phenylglyoxal, another guanidino-group reagent, produced similar effects (Table I). The presence of ATP (5 mM) during the reaction significantly protected the ATPase activity from inhibition by either butanedione or phenylglyoxal (Table I), and increased the crystallization index from near zero to 10–14% (Table I).

Decavanadate is a known inhibitor of phosphotransferase enzymes such as phosphofructokinase [20], adenylate kinase [21], phosphorylase [22] and hexokinase [23]. Paj et al. [21] located six arginine residues in the vicinity of the decavanadate-binding site of the adenylate kinase. The involvement of guanidino groups in the binding of decavanadate to phosphorylase was demonstrated by Soman et al. [22]. The findings described in this report suggest that arginine residue(s) may also play an important role in the binding of decavanadate to sarcoplasmic reticulum Ca^{2+}-ATPase.

The effect of Ca^{2+} on the Ca^{2+}-ATPase crystals induced by mono- or decavanadate

Ca^{2+} in low concentration prevents the binding of monovanadate to the Ca^{2+}-ATPase [24,25], inhibits formation of two-dimensional Ca^{2+}-ATPase crystals [1,2] and disrupts the crystals formed previously [2].

The observed differences between mono- and decavanadate in binding affinity and stoichiometry [14], in their effectiveness in promoting the crystallization of Ca^{2+}-ATPase [14], and in their capability to compete with FITC [16], prompted us to compare the effects of Ca^{2+} on the mono- and decavanadate-induced crystallization of the Ca^{2+}-ATPase.

In agreement with the earlier results [2], Ca^{2+} at submicromolar concentrations readily dispersed the monovanadate-induced Ca^{2+}-ATPase crystals (Fig. 3). Surprisingly, the disruption of the Ca^{2+}-ATPase crystals induced by decavanadate required millimolar free Ca^{2+} concentrations (Fig. 3). The marked difference in the sensitivity of the mono- and decavanadate-induced Ca^{2+}-ATPase crystals to Ca^{2+} is a further evidence for differences in the mode of action of mono- and oligovanadate anions on the Ca^{2+}-ATPase.

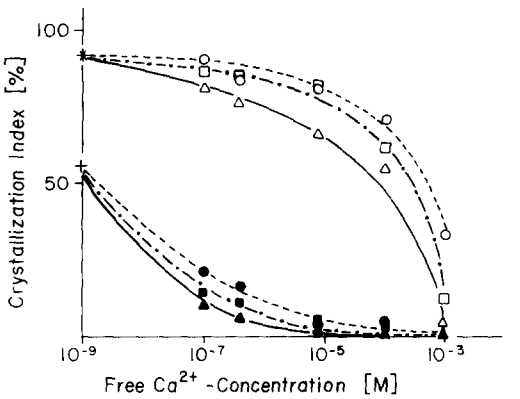

Fig. 3. Effect of Ca^{2+} on the crystallization of the Ca^{2+}-ATPase induced by mono- or decavanadate. Sarcoplasmic reticulum vesicles (1 mg protein/ml) were incubated in 0.1 M KCl/10 mM imidazole (pH 7.4)/5 mM $MgCl_2$/0.5 mM EGTA (standard buffer) and 1 mM monovanadate (closed symbols) or 1 mM decavanadate (open symbols) for 24 h at 2°C. After the preincubation $CaCl_2$ was added in a total concentration ranging from 0 to 1.5 mM to adjust the free Ca^{2+} concentration as indicated. Samples were taken for negative staining 4 (○, ●), 8 (□, ■), and 24 (△, ▲) h later. The electron micrographs were evaluated as described under Methods and Materials. * and + denote the control crystallization indices with deca- and monovanadate, respectively. The free calcium concentration of the solutions was checked by calcium-sensitive electrode (Orion). Mono- and decavanadate at the concentrations used did not cause any significant change in the free calcium concentration in the standard buffer.

Effect of phospholipase treatment on the crystallization of the Ca^{2+}-ATPase

Phospholipase C treatment of sarcoplasmic reticulum vesicles inhibits the ATPase activity and Ca^{2+} transport parallel with the hydrolysis of a major part of the phosphatidylcholine content of the membrane [26–28]. The products of phospholipase C action – diacylglycerols and phosphorylcholine – are both released from the membrane [29]. Therefore, the net effect of phospholipase C action is a decrease in the surface area of the membrane, with a corresponding increase in the density of Ca^{2+}-ATPase molecules, and drastic change in lipid composition.

The crystallization of Ca^{2+}-ATPase is markedly inhibited in phospholipase-C-treated vesicles (Table III). Comparable cleavage of membrane phospholipids by phospholipase A_2 into lysophospholipids and fatty acids causes only slight change in either ATPase activity or in the ability of the Ca^{2+}-ATPase to crystallize (Table III). However, the removal of the cleavage products of phospholipase A_2 action from the membrane by adsorption on fatty-acid-free serum albumin decreases the membrane lipid content and surface area to an extent similar to that obtained by phospholipase C treatment, and under these conditions the ATPase activity [28] and crystal formation are both drastically inhibited (Table III). These observations indicate that the conditions for crystallization (or interaction) of Ca^{2+}-ATPase molecules include the full complement of membrane lipids.

Effect of SH-group reagents

The Ca^{2+} transport ATPase is asymmetrically distributed in the sarcoplasmic reticulum membrane with a major portion of its mass located on the cytoplasmic surface and in the cytoplasmic leaflet of the bilayer. This is reflected in the electron density profiles of stacked sarcoplasmic reticulum membranes obtained by X-ray and neutron diffraction [30–32]. On treatment of the sarcoplasmic reticulum with 2-chloromercury-4-nitrophenol or *N*-ethylmaleimide, the electron density distribution becomes more symmetrical, indicating deeper penetration of the Ca^{2+}-ATPase into the bilayer [31]. This structural change is accompanied by increased permeability of the sarcoplasmic reticulum to calcium and other solutes [33].

Salyrgan, *p*-hydroxymercurybenzoate, and *N*-ethylmaleimide cause significant inhibition of the crystallization of Ca^{2+}-ATPase at concentrations as low as 10 nmol per mg protein, when added before vanadate (Table IV), and an essentially complete inhibition was obtained with 0.1–1.0 μmol reagent per mg protein. Preformed Ca^{2+}-ATPase crystals are more resistant to SH-group reagents, but even under these conditions signifi-

TABLE III

THE EFFECTS OF PHOSPHOLIPASE C AND A_2 ON THE CRYSTALLIZATION OF Ca^{2+}-ATPase IN SARCOPLASMIC RETICULUM

Microsomes (1 mg protein/ml) were treated with 10 μg/ml phospholipase C (*C. welchii*), or 5 μg/ml phospholipase A_2 (*Crotalus durissus*), in a medium of 0.1 M KCl/10 mM imidazole (pH 7.3)/1 mM $CaCl_2$ for 30–60 min at 25°C. To some samples 1% fatty-acid-free serum albumin was also added to adsorb the products of phospholipase A_2 action. After washing by centrifugation at 100000 × *g* for 30 min the microsomes were suspended in 0.1 M KCl/10 mM imidazole (pH 7.3) solution to a protein concentration of 1 mg/ml and used for ATPase assay as described under methods. Crystallization was performed in 0.1 M KCl/10 mM imidazole (pH 7.4)/5 mM $MgCl_2$/0.5 mM EGTA with either 5 mM monovanadate or 5 mM decavanadate for 1 weeks at 2°C. After negative staining with 1% uranyl acetate, the crystallization indices were determined as described under Methods and Materials.

Treatment	ATPase (μmol P_i/mg per min)	Crystallization index (%)	
		monovanadate (5 mM)	decavanadate (5 mM)
Control	2.1–2.4	54–73	87–92
Phospholipase C (10 μg/ml)	0.51	16.3 ± 2.5	11.3 ± 3.5
Phospholipase A_2 (5 μg/ml)	1.50	41.6 ± 4.9	79.0 ± 1.0
Phospholipase A_2 (5 μg/ml) + 1% fatty acid free serum albumin	0.41	17.3 ± 1.5	22.6 ± 1.5

TABLE IV

EFFECT OF SH-GROUP REAGENTS ON THE CRYSTALLIZATION OF Ca^{2+}-ATPase

Sarcoplasmic reticulum vesicles (1 mg protein/ml) were treated with the various SH reagents in a medium of 0.1 M KCl/10 mM imidazole (pH 7.4)/5 mM $MgCl_2$/0.5 mM EGTA/5 mM monovanadate for 24 h at 2°C. The crystallization indices were determined after negative staining with 1% uranyl acetate. The crystallization index of control samples was 81.9±3.3%.

Reagent concentration (nmol/mg protein)	Crystallization index (%)		
	salyrgan	PCMB	*N*-ethylmaleimide
1	81.8±3.8	67.3±0.9	68.5±1.4
5	62.8±2.6	64.0±1.2	56.9±1.5
10	58.7±2.8	58.2±1.5	46.7±1.2
100	12.2±3.8	13.1±2.3	31.3±4.4
1000	1.9±2.0	2.9±3.5	8.9±0.8

cant loss of crystallinity was observed within 2 h after the addition of SH reagents at final concentrations of 10 nmol per mg protein (Table V).

N-Ethylmaleimide inhibits the conversion of the ADP-sensitive into the ADP-insensitive intermediate of the Ca^{2+}-ATPase [34,35]. Since the vanadate-induced crystallization of the Ca^{2+}-ATPase requires the accumulation of the E_2-V form of the enzyme, *N*-ethylmaleimide and other SH-group reagents may inhibit the vanadate-induced crystallization, in part, by interference with the $E_1 \rightarrow E_2$ conversion of the Ca^{2+}-ATPase.

The effect of detergents

The E_2 crystals induced by vanadate are sensitive to detergents [2]. The relationship between detergent concentration, alkyl chain length and the crystallization index is shown in Fig. 4, for a series of zwitterionic detergents denoted as zwittergents. Zwittergent 3-08, with an eight-carbon alkyl chain, has no effect on the stability of Ca^{2+}-ATPase crystals at concentrations ranging between 0.01 and 5 mg/ml. Zwittergents of increasing alkyl chain length are progressively more effective in disrupting the crystals, as shown by the decrease in the value of crystallization index at progressively lower detergent concentration. The

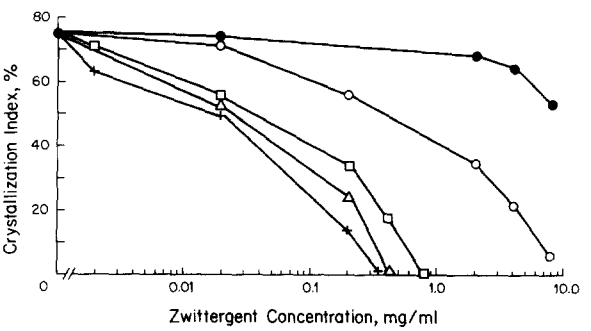

Fig. 4. The effect of zwitterionic detergents (zwittergents) on the Ca^{2+}-ATPase crystals. Sarcoplasmic reticulum vesicles (1 mg protein/ml) were preincubated with 1 mM monovanadate in 0.1 M KCl/10 mM imidazole/5 mM $MgCl_2$/0.5 mM EGTA (pH 7.4) for 24 h at 2°C. Detergents were added to the final concentrations indicated on the abscissa, and the crystallization indices of the preparations were determined as described under Methods and Materials. ●, 3-08; ○, 3-10; □, 3-12; △, 3-14; +, 3-16.

TABLE V

EFFECT OF SH-GROUP REAGENTS ON PREFORMED CRYSTALS OF Ca^{2+}-ATPase

Sarcoplasmic reticulum vesicles (1 mg protein/ml) were incubated in a medium of 0.1 M KCl/10 mM imidazole (pH 7.4)/5 mM $MgCl_2$/0.5 mM EGTA/5 mM monovanadate for 24 h at 2°C. SH-group reagents were then added to final concentrations indicated in the table and the crystallization indices were determined 10 min and 2 h after the addition of the reagents. The crystallization index of the control preparation was 78.1±2.6%.

Reagent concentration (nmol/mg protein)	Crystallization index (%)					
	salyrgan		PCMB		*N*-ethylmaleimide	
	10 min	2 h	10 min	2 h	10 min	2 h
5	50.1±2	44.9±4	54.8±3	40.8±3	50.7±3	45.1±4
10	42.3±4	34.5±2	37.8±3	31.6±3	44.1±3	38.1±3
100	18.3±3	3.3±1	12.3±3	7.3±3	39.9±4	31.5±2

disruption of crystals occurs generally at lower detergent concentrations than those required for the solubilization of the vesicles. These observations, together with structural information obtained by reconstruction of the three-dimensional structure of Ca^{2+}-ATPase from electron microscope data [36,37], suggest that the interactions between ATPase molecules forming the crystalline arrays are stabilized by lipid–protein interactions in the bilayer, in the intramembranous contact regions between ATPase molecules.

Acknowledgement

This work was supported by research grants AM 26545 from the National Institutes of Health, PCM 84-03679 from the National Science Foundation, and by a grant-in aid from the Muscular Dystrophy Association to A.M.

References

1 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 2599–2603
2 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 11896–11902
3 Taylor, K.A., Dux, L. and Martonosi, A. (1984) J. Mol. Biol. 74, 193–204
4 Dux, L., Taylor, K.A., Ting-Beall, H.P. and Martonosi, A. (1985) J. Biol. Chem. 260, 11730–11743
5 Martonosi, A. and Beeler, T.J. (1983) in Handbook of Physiology: Skeletal Muscle (Peachey, L.D. and Adrian, R.H., eds.), Ch. 15, pp. 417–485, American Physiological Society, Bethesda
6 Jona, I. and Martonosi, A. (1986) Biochem. J. 234, 363–371
7 Dux, L., Papp, S. and Martonosi, A. (1985) J. Biol. Chem. 260, 13454–13458
8 Inesi, G. and de Meis, L. (1985) in The Enzymes of Biological Membranes, Vol. 3 (Martonosi, A., ed.), pp. 157–191, Plenum, New York
9 Nakamura, H., Jilka, R.L., Boland, R. and Martonosi, A. (1976) J. Biol. Chem. 251, 5414–5423
10 Murphy, A.J. (1977) Arch. Biochem. Biophys. 180, 114–120
11 Hidalgo, C., Petrucci, D.A. and Vergara, C. (1982) J. Biol. Chem. 257, 208–216
12 Murphy, A.J. (1976) Biochem. Biophys. Res. Commun. 79, 1048–1054
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
14 Varga, S., Csermely, P. and Martonosi, A. (1985) Eur. J. Biochem. 148, 119–126
15 Dux, L. and Martonosi, A. (1984) Eur. J. Biochem. 141, 43–49
16 Csermely, P., Varga, S. and Martonosi, A. (1985) Eur. J. Biochem. 150, 455–460
17 Csermely, P., Martonosi, A., Levy, G.C. and Ejchart, A.J. (1985) Biochem. J. 230, 807–815
18 Pick, U. and Karlish, S.J.D. (1982) J. Biol. Chem. 257, 6120–6126
19 Mitchinson, C., Wilderspin, A.F. Trinnaman, B.J. and Green, M.N. (1982) FEBS Lett. 146, 87–92
20 Choate, G. and Mansour, T.E. (1979) J. Biol. Chem. 254, 11457–11462
21 Pai, E.F., Sachsenheimer, W., Schirmer, R.H. and Schulz, G.E. (1977) J. Mol. Biol. 144, 37–45
22 Soman, G., Chang, Y.C. and Graves, D.J. (1983) Biochemistry 22, 4994–5000
23 Boyd, D.W., Kustin, K. and Niva, M. (1985) Biochim. Biophys. Acta 827, 472–475
24 Hasselbach, W., Medda, P., Migala, A. and Agostini, B. (1983) Z. Naturforsch. 38C, 1015–1022
25 Medda, P. and Hasselbach, W. (1983) Eur. J. Biochem. 137, 7–14
26 Martonosi, A. (1964) Fed. Proc. 23, 913–921
27 Martonosi, A., Donley, J. and Halpin, R.A. (1968) J. Biol. Chem. 243, 61–70
28 Martonosi, A., Donley, J.R., Pucell, A.G. and Halpin, R.A. (1971) Arch. Biochem. Biophys. 144, 529–540
29 Finean, J.B. and Martonosi, A. (1965) Biochim. Biphys. Acta 98, 547–553
30 Dupont, Y., Harrison, J.C. and Hasselbach, W. (1973) Nature 244, 555–558
31 Dupont, Y. and Hasselbach, W. (1973) Nature New Biol. 246, 41–44
32 Blasie, J.K., Herbette, L., Pierce, D., Pascolini, D., Scarpa, A. and Fleischer, S. (1982) Ann. N.Y. Acad. Sci. 402, 478–484
33 Martonosi, A. (1984) Physiol. Rev. 64, 1240–1320
34 Kawakita, M., Yasuoka, K. and Kaziro, Y. (1980) J. Biochem. (Tokyo) 87, 609–617
35 Yasuoka-Yabe, K. and Kawakita, M. (1983) J. Biochem. (Tokyo) 94, 665–675
36 Taylor, K.A., Dux, L. and Martonosi, A. (1986) J. Mol. Biol. 187, 417–427
37 Ho, M.-H., Taylor, K.A. and Martonosi, A. (1986) Biophys. J. 49, 570a