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Isolation and Characterization of Proteolipids from Sarcoplasmic Reticulum

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Summary. (1) A proteolipid fraction was isolated from the membranes of fragmented sarcoplasmic reticulum from rabbit skeletal muscle by extraction with acidified chloroform/methanol 2:1. This material was further resolved on Sephadex LH-20 in acetone/methanol 2:1. Four fractions were collected and converted into partly delipidated water-soluble form. Although the separation of the four fractions was not sharp, it was clear from SDS-urea polyacrylamide gel electrophoresis and amino acid composition that there are at least two different species of proteolipids in sarcoplasmic reticulum.

(2) Some of these fractions had ionophoric activity, catalyzing the translocation of Ca^{2+}, Mn^{2+}, Na^{+} into egg phosphatidylcholine vesicles after they were incorporated into the membrane by sonication. Under these conditions cardiolipin had no ionophoric activity.

(3) A Ca^{2+}-ATPase was isolated from sarcoplasmic reticulum by the method developed for delipidation of the enzyme [Green, N.M. (1975), *In:* Calcium Transport in Contraction and Secretion, E. Carafoli et al., editor. pp. 339–348 North Holland, Amsterdam.] This preparation which was depleted in proteolipids, catalyzed an ATP-dependent Ca^{2+} transport after incorporation into phospholipid vesicles demonstrating that a single component, Ca^{2+}-ATPase, can function in reconstituted vesicles. However, the efficiency of pumping in these vesicles was low (Ca^{2+}/ATP < 1). We propose that the proteolipid may increase the efficiency of pumping by contributing to the formation of the transmembranous channel either by aiding in its assembly or by participating as a functional subunit.

Proteolipids are found in a variety of membranes in animals, plants, and eucaryotic and procaryotic microorganisms [9]. Although these proteins are defined mainly operationally by their solubility in chloroform/methanol mixtures [8], proteolipids from different sources have additional common characteristics, namely a low molecular weight, formation of tight complexes with phospholipids, and, in some cases, covalently bound fatty acid residues. In spite of their ubiquity, only little is known about the structure and properties of proteolipids, and even less about possible functions. Some of the harsh isolation techniques which have been used, involving strong detergents (SDS) or prolonged exposure to organic solvents, can yield material suitable for physical and chemical studies, but result in loss of biological activity. There is abundant evidence for the function of a proteolipid in mitochondria, chloroplasts, and bacteria as a proton channel mainly based on experiments with dicyclohexylcarbodiimide [3,7] and reconstitutions [5,19].

The membrane of sarcoplasmic reticulum vesicles contains a proteolipid, which was isolated and characterized by MacLennan and coworkers [18]. The proteolipid is, in variable amounts, copurified with the Ca^{2+}-ATPase [17,22]. Its function is unknown; a structural role [18] and a direct involvement in the Ca^{2+} uptake process [22] have been proposed.

In the present communication, we describe a new method of isolation and fractionation of the sarcoplasmic reticulum proteolipids. We show further that several distinct proteolipid species can be extracted from the membrane and that some of them have ionophoric activity. Possible functions of the protolipids in sarcoplasmic reticulum are discussed.

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Materials and Methods

Materials. Phosphatidylcholine from egg yolk (type VII-E), glucuronic acid and Dowex-50W, 8% cross-linked, 50–100 mesh, were purchased from Sigma Chemical Co. Deoxycholic acid was obtained from Schwarz/Mann and recrystallized from ethanol. $^{45}CaCl_2$ (16 Ci/g calcium), $^{22}NaCl$ (carrier-free), $^{86}RbCl$ (5 Ci/g rubidium), and $^{54}MnCl_2$ (carrier-free), were from ICN. Sephedex LH-20 was purchased from Pharmacia. DEAE-Cellulose (DE-52) was from Whatman and Bio-beads SM-2 from Bio-Rad. Triton X100 (Sigma) was purified as described below. A-23187 was a gift of Dr. R. Hosley of Eli Lilly. Solvents and other chemicals were of analytical grade.

Preparation of sarcoplasmic reticulum. Fragmented sarcoplasmic reticulum was prepared according to MacLennan [16] (washed R_1 of Preparation *A*) except that the extraction buffer contained 0.5 mM of the protease inhibitor phenylmethanesulfonyl fluoride.

Isolation of proteolipids from sarcoplasmic reticulum by organic solvent extraction. Sarcoplasmic reticulum (600–800 mg protein) suspended in 0.25 M sucrose – 10 mM Tris-HCl, pH 7.5, was lyophilized. The lyophilized preparation was extracted with $CHCl_3$/MeOH/concHCl at a ratio of 2:1:0.01 ml for each 10 mg protein. The suspension was centrifuged in 12 ml thick-walled Sorvall glass centrifuge tubes at 10,000 rpm (12,000 × *g*) in SS34 rotors for 10 min in a Sorvall centrifuge. To the supernatant was added 1/4 volume of 1 N HCl. After mixing, a large spongy precipitate was removed manually and separation of phases was achieved by centrifugation in 200 ml glass bottles at 250 × *g* in a swing-out rotor for 15 min. The upper (aqueous) phase was discarded. The pH of the lower (organic) phase was adjusted to 6 to 7 with NH_3 vapor. To the neutralized chloroform extract was added 1/2 volume of a solution with the composition $CHCl_3$/MeOH/H_2O (3:47:48) containing 0.02% $CuSO_4$. The pH of the mixture was then raised with NH_3 vapor until a bluish tinge appeared.

The bluish mixture was centrifuged at 250 × *g* in a swing-out rotor for 15 min. The upper phase was removed completely by aspiration. The lower phase was dried under N_2 and the residue was dissolved in 2 to 3 ml of $CHCl_3$/MeOH (2:1). This solution contains proteolipids and almost all the lipids of sarcoplasmic reticulum and is termed the "purified lipid extract."

For further resolution of proteolipids, the purified lipid extract was then applied to a Sephadex LH-20 column (1.5 × 70 cm) equilibrated with acetone/methanol (2:1). The elution pattern for both protein and lipid is shown in Fig. 1.

The fractions under each protein peak were combined and the proteolipids were made water-soluble by the following procedure. To the proteolipid in acetone-methanol was added an equal volume of 5 mM Tris-HCl (pH 8.0) and of ether. The suspension was thoroughly mixed and then centrifuged at 400 × *g* in a swing-out rotor for 15 min. The ether phase containing extracted lipid was discarded and ether extraction was repeated once more. Residual ether in the aqueous phase was removed by bubbling N_2 through. The solution was dialyzed against 30 volumes of 5 mM Tris-HCl, pH 8.0, overnight at 4 °C and concentrated by lyophilization. The lyophilized proteolipid was usually dissolved in water at 1/20 of the original volume. Delipidation of proteolipid by ether extraction by the procedure described above was not complete. The proteolipid solutions were sometimes turbid. However, this did not interfere with the assay of ionophore activity.

Assays for ionophoric activity. The ionophoric activity of proteolipids was determined by measuring translocation of radioactive ions into liposomes containing proteolipids [10]. Liposomes were prepared by sonication of a suspension of egg phosphatidylcholine at 20 mg/ml with and without proteolipids.

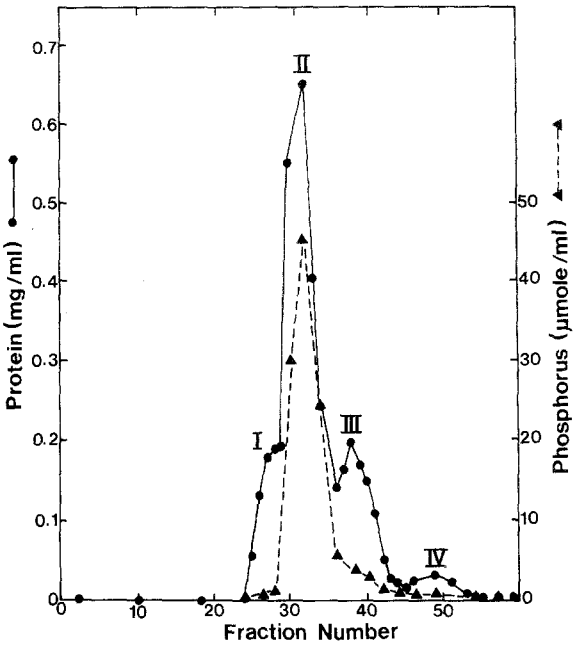

Fig. 1. Elution profile of proteolipids from a Sephadex LH-20 column. The conditions of the fractionation are described in "Materials and Methods." ●—●, protein; ▲--▲, total phosphorus. The numbers of the peaks refer to the designations used in the text.

For measurement of Ca^{2+} translocation, egg phosphatidylcholine was sonicated in buffer containing 0.1 M K-glucuronate, pH 7.4, 50 mM sucrose and 20 mM Tris Cl, pH 7.4. The reaction was initiated by the addition of liposomes to 1.5 ml of a reaction mixture containing 50 mM sucrose, 20 mM Tris HCl (pH 7.4) and 1 mM $CaCl_2$ (2×10^7 cpm/μmol) at 2.5 mg lipid/ml. The reaction was carried out at 37 °C. At different time intervals, aliquots (0.25 ml) were taken and passed through a Dowex-Tris column prewashed with 0.25 M sucrose [10]. The vesicles were eluted with 3 ml of 0.25 M sucrose. Aliquots (1 ml) were counted in a liquid scintillation counter.

For measurement of $^{22}Na^+$ translocation, the vesicles were sonicated and then tested in 0.05 M ^{22}Na-phosphate, pH 7.4 (1300 cpm/nmol).

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was performed according to Swank and Munkres [25] using an acrylamide/N,N′-methylenebisacrylamide ratio of 15:1.

"Coupling factor." The preparation of the heat-stable factor from the Ca^{2+}-ATPase ("coupling factor") was as described [22]. Ca^{2+}-ATPase was reconstituted by the cholate dialysis procedure in the presence or absence of the "coupling factor" [20] and assayed for Ca^{2+} uptake and ATP hydrolysis [22].

Preparation of Ca^{2+}-ATPase from sarcoplasmic reticulum. The "coupling factor" was obtained from a Ca^{2+}-ATPase prepared according to MacLennan [16], as modified in [22]. Its effect on Ca^{2+} transport was tested in vesicles reconstituted with R_{3a} or a similar Ca^{2+}-ATPase fraction obtained by fractionation with ammonium sulfate of R_2 [16] after solubilization in cholate. Ca^{2+}-ATPase free of proteolipid was prepared according to Green [12]; the excess of Triton X100 was removed from the enzyme by treatment with Bio-beads SM-2 and the enzyme was relipidated with 30 μmol of egg phosphatidylcholine per μmol of ATPase. The Triton

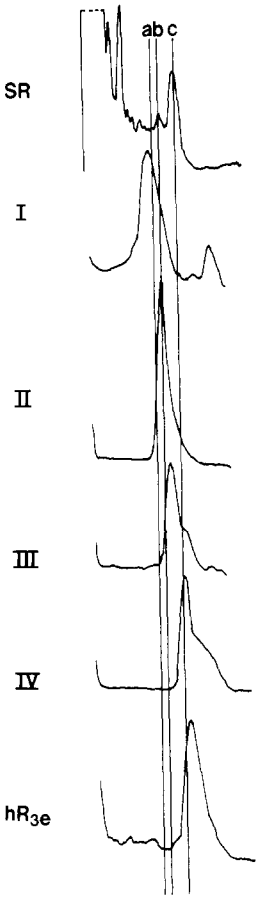

Fig. 2. Electrophoresis of sarcoplasmic reticulum and proteolipids on SDS-urea gels. Polyacrylamide gel electrophoresis was carried out according to [25]. The amount of protein was 100 μg for sarcoplasmic reticulum and 5 to 10 μg for the proteolipid fractions. The gels were stained with Coomassie blue and scanned at 550 nm.

X100 used in this preparation was first treated with stannous chloride in aqueous solution to remove peroxides: 100 ml of 10% Triton X100 in H_2O were flushed for 10 min with nitrogen and 280 mg solid $SnCl_2\ 2H_2O$ were added. The solution was stirred for 30 min under nitrogen, extracted twice with chloroform, and the chloroform removed from the combined extracts by rotary flash evaporation. The residue was diluted with 100 ml H_2O and reextracted twice with chloroform. The solvent was removed by rotary flash evaporation in an oil pump vacuum [28]. The purified detergent was stored in the dark and under nitrogen at room temperature.

Reconstitution of the relipidated enzyme was performed with cholate and deoxycholate as described previously [15].

Results and Discussion

I. Evidence for More than One Proteolipid

The proteolipids of sarcoplasmic reticulum appeared as a single diffuse band after electrophoresis in 7.5 or 10% polyacrylamide gels according to Weber et al. [27]. They were resolved, however, into three bands

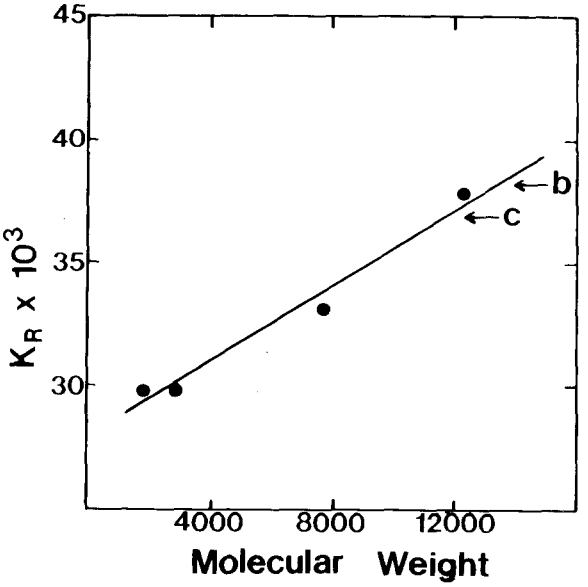

Fig. 3. Plot of molecular weight *vs.* retardation coefficient in SDS-urea polyacrylamide gels. Electrophoresis was performed according to [25] in 12, 14, 16, 18, and 20% gels. The ratio of monomer to crosslinker was 32 and the gels contained 10% vol/vol glycerol. Horse cytochrome *c* (mol wt = 12,300) and its cyanogen bromide fragments (mol wt = 1810, 2780, and 7760) were used as standards. The retardation coefficients K_R were calculated from the slope of Ferguson plots: $\log A = \log A_0 - K_R T$, where A is the mobility relative to bromophenol blue, A_0 the extrapolated relative mobility at gel concentration = 0, T the gel concentration in percent, and K_R the retardation coefficient. The arrows (*b* and *c*) refer to the proteolipid bands (*see* Fig. 2, top).

when electrophoresis was performed in gels containing SDS[1] and urea [25] (Fig. 2, top). Band *a* was small and not always visible. The free mobility of bands *b* and *c* was considerably higher than the free mobility of standard polypeptides, as determined from a Ferguson plot [cf. 2 and 4]. Therefore, molecular weights were estimated from a plot of M vs. the retardation coefficient K_R [2, 4] (Fig. 3). They were found to be 11,900 and 13,600 for *c* and *b*, respectively.

Fractionation of sarcoplasmic reticulum proteolipids on Sephadex LH-20, as described under "Materials and Methods," yielded four incompletely resolved peaks (Fig. 1). Their electrophoretic profile in SDS-urea gels is shown in Fig. 2. Peak *I*, and possibly *IV*, is a mixture of two components; peak *III* is contaminated with a small amount of *IV*. Purification of the proteolipids to homogeneity has proved difficult mainly because of association with phospholipids, but even at the present level of purity, the differences in electrophoretic mobility of the fractions are obvious. The position of the bands in the gel is consistent with the order of their elution from the

[1] *Abbreviation:* SDS – sodium dodecylsulfate.

Table 1. Amino acid analysis of sarcoplasmic reticulum proteolipids

Amino acid	I	II	III	IV
Lysine	0.57	3.27	4.18	5.21
Histidine	0.00	0.65	0.78	2.17
Arginine	15.77	9.73	5.67	4.99
Aspartic acid	4.67	6.95	8.65	7.81
Threonine	6.35	6.30	7.23	6.29
Serine	12.46	11.12	9.29	9.98
Glutamic acid	16.95	13.25	11.63	9.33
Proline	2.35	3.60	4.61	5.64
Glycine	1.35	6.87	10.35	10.41
Alanine	1.48	7.36	10.71	9.54
Valine	4.73	5.64	5.74	4.99
Methionine	3.58	1.47	1.77	1.30
Isolucine	1.63	3.03	3.12	2.82
Leucine	14.84	11.94	8.87	11.71
Tyrosine	10.75	5.48	4.04	1.74
Phenylalanine	2.53	3.35	3.33	6.07

Proteolipids were separated on Sephadex LH-20 as described in "Methods." The amino acid composition of the four peaks from the same experiment was determined on a Beckman 120 C Amino Acid Analyzer after hydroysis in 3 N mercaptoethane sulfonic acid. Amino acid content is given per 100 moles.

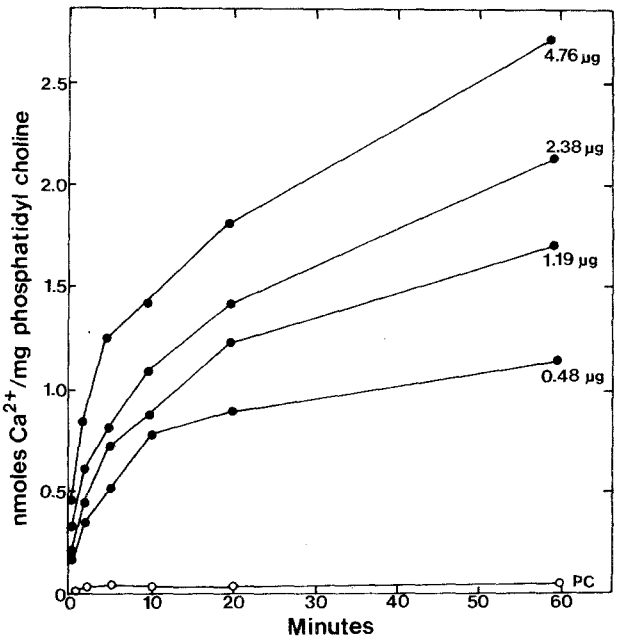

Fig. 4. Proteolipid-mediated Ca^{2+} uptake into liposomes. The uptake was measured at 37 °C as described in "Materials and Methods." The amounts of proteolipid (fraction *III*) indicated in the figure are in µg per mg phosphatidylcholine.

Sephadex column (peak *I* has the lowest, peak *IV* the highest mobility).

Because of the incomplete separation of the fractions on the Sephadex LH-20 column, amino acid analysis of the individual proteolipids has yielded variable results with different batches. However, within each experiment the four fractions differed markedly from each other in the amino acid composition, thus corroborating the assumption of at least two proteolipids. An example of amino acid analysis of the four fractions is shown in Table 1.

II. Ionophoric activity of proteolipids from sarcoplasmic reticulum

When incorporated into the membrane of liposomes by sonication, the proteolipids from sarcoplasmic reticulum caused an uptake of Ca^{2+} into the vesicles. Since fraction *III* had the highest activity, subsequent experiments were carried out with this fraction.

Figure 4 shows the time course of Ca^{2+} influx mediated by the proteolipid. The rate of the process was proportional to the amount of protein used and increased with increasing assay temperature (Fig. 5); the calculated activation energy is 9.8 kcal/mole. This value is similar to the activation energy of gramicidin-mediated transport of Na^+ or K^+ in comparable lipids [1, 11]. It may be of interest to note that in the absence of proteolipids, but in the presence of A-23187 (18 ng) and nigericin (450 ng), 6.4 nmol of Ca^{2+} per mg phosphatidylcholine were taken up after 5 min and 7.6 nmol after 10 min. Almost no Ca^{2+} flux was observed with liposomes without protein (Fig. 4) or with lipids extracted with ether from the proteolipid fraction used in the assay (not shown). This is especially important because certain phospholipids can act as ionophores in the Pressman cell [26]. If acidic phospholipids (e.g., cardiolipin) were added externally to vesicles instead of being sonicated together with the bulk of lipids, they caused Ca^{2+} influx, in agreement with [26]. Because of that, the proteolipid preparations, which contain residual phospholipid, were always incorporated into liposomes by sonication. Under such conditions, no Ca^{2+} influx was observed in control experiments with cardiolipin.

The ionophoric activity of the proteolipid was not affected by treatment with up to 25 mM dithiothreitol or β-mercaptoethanol, 0.5 mM N-ethylmaleimide, 0.5 mM cyclohexylmaleimide, 0.5 mM mersalyl, 0.5 mM *p*-chloromercuriphenylsulfonic acid. Activity was lost upon storage of a dilute aqueous preparation at room temperature (but not at 4 °C) overnight.

The proteolipid-mediated cation transport across the membrane is not specific for Ca^{2+}. Mn^{2+}, Na^+ (Table 2) and Rb^+ were transported at similar rates.

It may be noted that several other proteolipids and oligopeptides tested had no ionophore activity

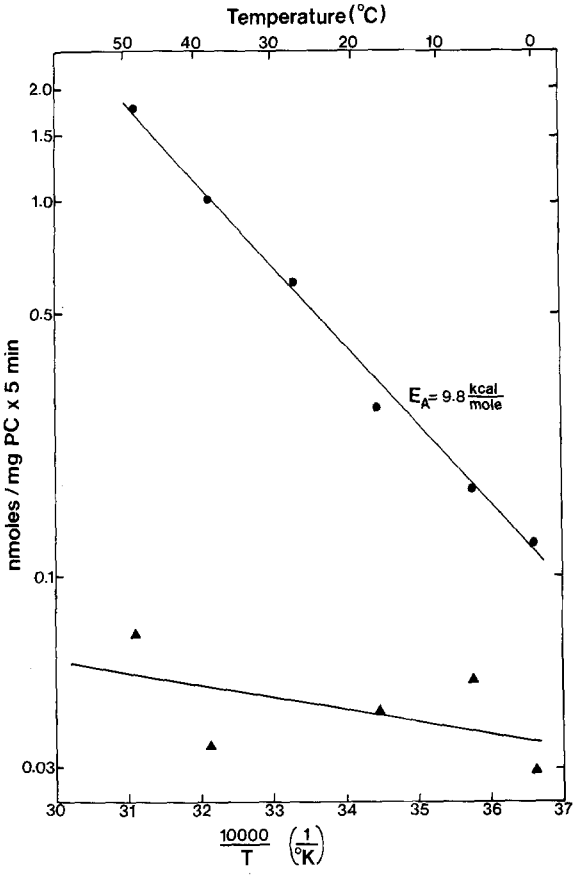

Fig. 5. Dependence of proteolipid-mediated Ca^{2+} transport on the temperature. The uptake of Ca^{2+} was measured as described under "Materials and Methods" at different temperatures. ▲—▲, control vesicles without proteolipid; ●—●, 1.2 μg proteolipid (fraction *III*) per mg phosphatidylcholine. The lines were fitted by least square analysis.

Table 2. Proteolipid-mediated ion transport

Expt.	μg proteolipid/mg phosphatidylcholine	Ion transported	Liposomes	
			Phosphatidylcholine (nmol/mg	Proteolipid + phosphatidylcholine
1	4.76	Ca	0.05	1.35
		Mn	0.81	1.82
2	1.19	Ca	0.07	0.57
		Na	0.21	2.38

Vesicles used for measuring Ca^{2+} and Mn^{2+} transport were prepared in 0.1 M K-glucuronate, pH 7.4, 50 mM sucrose, 20 mM Tris-HCl, pH 7.4. The reaction mixture contained 50 mM sucrose, 20 mM Tris-HCl, pH 7.6, and 1 mM $^{45}CaCl_2$ or 1 mM $^{54}MnCl_2$. Vesicles used for measuring Na^+ exchange were prepared in 50 mM Na-phosphate, pH 7.4. Ion fluxes in the Table were values obtained after 5 min reaction at 37 °C.

for Ca^{2+} under these experimental conditions. Among those tested were the mitochondrial proteolipid and a synthetic oligopeptide, discussed below. In fact, the proteolipid from sarcoplasmic reticulum prepared according to MacLennan et al. [18] was inactive.

III. Proteolipid obtained by heating of the Ca^{2+}-ATPase ("coupling factor")

A proteolipid can be obtained from sarcoplasmic reticulum without extraction with organic solvents. This is accomplished by a short heating of a Ca^{2+}-ATPase preparation obtained by ammonium acetate fractionation in the presence of deoxycholate [22]. After removal of denatured protein by centrifugation, the supernatant contains phospholipids, deoxycholate, buffer components (sucrose, ammonium acetate, histidine, Tris), and a low-molecular weight protein which, by the criteria of its behavior on SDS-urea gels (Fig. 1, bottom) and extractability with chloroform/methanol 2:1, appears to be a proteolipid. This preparation, when reconstituted together with certain fractions of Ca^{2+}-ATPase, increased their pumping efficiency (Ca^{2+}/ATP ratio) [22]. It should be noted that proteolipids isolated by chloroform/methanol extraction and Sepedex LH-20 fractionation were not active in this assay. Only Ca^{2+}-ATPase preparations with relatively high ATPase activity but low Ca^{2+} transport activity after reconstitution, responded to the "coupling factor" (such preparations contain low levels of endogenous proteolipid, as judged by gel electrophoresis [22]). Moreover, the effect could be observed in the cholate dialysis reconstitution [20], but not in the sonication reconstitution [21] or freeze-thaw-sonication reconstitution [13, 29]. In the cholate dialysis reconstitution, the increase of pumping efficiency was not specific for the proteolipid; various agents, including some non-ionic detergents, phospholipids (e.g., phosphatidylcholine), and polypeptides (e.g., cytochrome *c*) stimulated Ca^{2+} uptake under proper experimental conditions, though the extent of stimulation was usually greater with the proteolipid preparation. Especially interesting was a water-soluble synthetic oligopeptide[2] which strongly stimulated Ca^{2+} uptake in a narrow range of concentrations (6–8 μg per 150 μg Ca^{2+}-ATPase). In order to exclude the possibility that all these agents act via the residual endogenous proteolipid which is always present in the Ca^{2+}-ATPase preparation of MacLennan [16], an enzyme preparation free of proteolipid was sought.

[2] The peptide (Phe-Glu-Glu-Ala-Tyr-Ile-Pro-Lys-Glu-Gln-Lys-Tyr-Ser-Phe) was donated by the Armour Pharmaceutical Company, Kankakee, Ill.

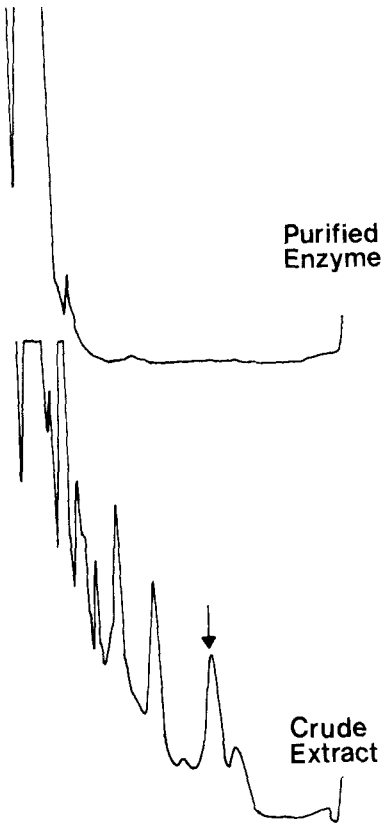

Fig. 6. SDS-polyacrylamide gel electrophoresis of a Triton X100 extract of sarcoplasmic reticulum and of purified Ca^{2+}-ATPase according to Green [12]. Electrophoresis was carried out in 10% gels according to [27]. Forty μg protein were applied on each gel. The gels were stained with Coomassie blue, destained with 7% acetic acid (no methanol), and scanned at 550 nm. The proteolipid band marked with an arrow showed red fluorescence in incandescent light.

The delipidation method of Green [12] was found to remove both phospholipids and proteolipids from the Ca^{2+}-ATPase, as judged from SDS-urea gel electrophoresis (Fig. 6). However, in some preparations in which the protein was eluted from DEAE-cellulose stepwise rather than by linear gradient, residual proteolipids were detected after extraction with acidic chloroform-methanol. Other delipidation methods [6, 14] remove only phospholipids. After relipidation with egg phosphatidylcholine, the enzyme prepared according to Green [12] had a specific ATPase activity of 10 to 12 μmol/mg × min at 37 °C and was reconstitutively active. The reconstituted Ca^{2+} pump was not stimulated by the proteolipid (Table 3). In other words, the experiment shows that only a single protein – the Ca^{2+}-ATPase – can function in the reconstitution of an active Ca^{2+} pump. The Ca^{2+} transport was dependent on ATP and sensitive to 1 mM mersalyl and 2 μg A-23187/ml (data not shown).

The question of the function of the proteolipids in sarcoplasmic reticulum thus remains largely open.

Table 3. Influence of proteolipid on the pumping efficiency of Ca^{2+}-ATPase

Enzyme preparation	Additions	Ca^{2+} uptake (nmol / mg × min/in	ATPase (nmol / mg × min/in	Ca^{2+}/ ATP
Ca^{2+}-ATPase according to Green [12]	– –	332	608	0.55
	2.5 μg hR_3e	316	552	0.57
	300 μg Triton X100	348	420	0.83
R_3a according to MacLennan [16]	–	72	108	0.67
	2.5 μghR_3e	173	139	1.24
	300 μg Triton X100	148	107	1.38
Sarcoplasmic reticulum	–	583	532	1.10

Reconstitution with asolectin by cholate dialysis and assays were as described in "Materials and Methods." For each reconstitution, 150 μg Ca^{2+}-ATPase were used. hR_3e: "coupling factor" obtained by heating of Ca^{2+}-ATPase.

At present, three possibilities are being considered. The first is that it is involved in the Ca^{2+} uptake process. The fact that ATP-driven Ca^{2+} transport can take place in a reconstituted system in the absence of proteolipids, does not rule out a possible role in the native system which is more efficient. Indeed, the most striking effect of the proteolipid observed was the increase in the efficiency of pumping [22]. Moreover, a drawback of the experiments with the enzyme preparation of Green [12] is the presence of residual Triton X100. This detergent can act as an ionophore for monovalent cations in planar lipid bilayers [24]; under the same conditions[3], Triton X100 introduced a permeability for Ca^{2+} which was about twenty times lower than that for K^+. Therefore, the detergent could substitute for the proteolipid in the reconstituted system.

A second possible function for proteolipids in sarcoplasmic reticulum is as a structural aid during assembly. In reconstitution methods involving sonication, the membrane is ruptured and its hydrophobic core transiently exposed, thus allowing the incorporation of lipophilic proteins. During cholate dialysis reconstitution, however, the insertion of proteins into the forming (or already formed) membrane might require additional factors. Especially substances with amphiphilic properties would be good candidates for such "pilot molecules." This would explain the wide range of amphiphiles able to increase the Ca^{2+}/ATP ratio, perhaps by improving the proper orientation of the Ca^{2+}-ATPase in the membrane and thus reducing "idle" ATP hydrolysis. It seems likely that cho-

[3] We thank Dr. Christopher Miller, Brandeis University, for permission to quote his unpublished observation.

late dialysis is closer than sonication to the *in vivo* situation where a natural detergent such as lysolecithin may facilitate incorporation. The proteolipid may participate in the proper insertion of the Ca^{2+}-ATPase into the sarcoplasmic reticulum membrane. During development of the fetus and the new-born rabbit, the proteolipid is formed before the Ca^{2+}-ATPase [23].

The third possibility of a function of proteolipids in the sarcoplasmic reticulum membrane is an involvement in the rapid Ca^{2+} release wich causes muscle contraction. Additional data, especially concerning the electrical properties of the proteolipids in a membrane, will be necessary to test this assumption. It should be noted that the ionophoric activity of the isolated proteolipid is consistent with the first and third possibility presented above. The lack of specificity for Ca^{2+} does not weaken the argumentation: in the case of Ca^{2+} uptake, the specificity is more likely to reside in the Ca^{2+}-ATPase; for the Ca^{2+} release, no specificity is required, since Ca^{2+} is the only ion forming a steep gradient across the sarcoplasmic reticulum membrane. Moreover, the lack of specificity could allow for K^+ influx and equilibration of electrical charges. The fact that there are at least two and perhaps more species of proteolipids allows for the possibility that they participate in more than one of the above mentioned functions.

We thank Margaret Matheson for technical assistance during parts of the work and Tinh Nguyen for preparation of sarcoplasmic reticulum and help in isolation of the proteolipids. We wish to acknowledge the suggestion by Dr. N. Nelson to detect proteolipids in acrylamide gels by fluorescence in incandescent light.

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