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MEMBRANE-SURFACTANT INTERACTIONS

THE EFFECT OF TRITON X-100 ON SARCOPLASMIC RETICULUM VESICLES

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The effect of Triton X-100 on purified sarcoplasmic reticulum vesicles has been studied by means of chemical, ultrastructural and enzymic techniques. At low detergent/membrane ratios (about 1 Triton X-100 per 60 phospholipid molecules) the only effect observed is an increase in vesicle permeability. Higher surfactant concentrations, up to a 1:1 detergent/phospholipid ratio, produce a large enhancement of ATPase activity. Membrane solubilization occurs as a critical phenomenon when the surfactant/phospholipid molar ratio reaches a value around 1.5:1, corresponding to 2 μmol Triton X-100/mg protein. At this point, the suspension turbidity drops, virtually all the protein and phospholipid is solubilized and every organized structure disappears. Simultaneously, a dramatic increase in the specific activity of the solubilized ATPase is observed. The sudden solubilization of almost all the bilayer components at a given detergent concentration is attributed to the relative simplicity of this membrane system. Solubilization takes place at the same surfactant/membrane ratio, at least between 0.5 and 4 mg membrane protein/ml. The non-solubilized residue seems to consist mainly of delipidized aggregated forms of ATPase.

Introduction

Many surface active molecules interact with biomembranes, eventually disrupting their structure and solubilizing the bilayer components. Advantage has been taken from this fact in order to purify amphipathic intrinsic proteins [1]. These proteins may then be reconstituted with pure phospholipids [2] or even kept in suspension in the active form as detergent-protein mixed micelles [3]. The techniques of intrinsic protein solubilization and reconstitution are extremely important for our understanding of the molecular biology of membranes, and hence the interest of the study of membrane-surfactant interactions.

Membrane solubilization by detergents has often been carried out in an empirical way. Systematic studies carried out on simple systems, such as viral membranes [4], have led to important generalizations [1]. These investigations, however, did not take into account the functional alterations of the membrane as much as the structural impairment caused by the surfactant. Previous studies from this laboratory [5–7] have dealt with the interaction of the non-ionic detergent Triton X-100 with mitochondrial membranes. Triton X-100 had very complex effects on mitochondrial enzyme activities: some were inhibited, while others were stimulated. However, the intrincate architecture

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and multiplicity of enzyme activities in this membrane make difficult any generalization concerning the effects of detergents on intrinsic enzyme activities.

To overcome this difficulty, we have considered a much simpler membrane, namely sarcoplasmic reticulum. It contains a simple intrinsic protein, the Ca^{2+}-ATPase (115 kDa), which has been the object of many investigations, in native as well as in reconstituted form [3,8,9]. In addition, sarcoplasmic reticulum contains some extrinsic proteins, such as calsequestrin. A Ca^{2+}-independent ATPase activity that is often found in sarcoplasmic reticulum preparations has recently been attributed to an impurity [10]. The native membrane contains about 70 phospholipids per ATPase molecule.

The present study deals with the effects of Triton X-100, a non-ionic detergent well known for its membrane-solubilizing properties, on sarcoplasmic reticulum vesicles. We have explored the alterations in membrane architecture, the changes in enzyme activity, and the relationship between both effects.

Methods and Materials

Membrane preparations and detergent treatments

Sarcoplasmic reticulum vesicles were prepared from rabbit back and leg white muscle, according to the method of Nakamura et al. [11]. Sarcoplasmic reticulum vesicles were then purified in a 20–60% continuous sucrose gradient containing 1 M KCl, 5 mM ATP, 2 mM dithiothreitol and 50 mM Tris-HCl (pH 8), washed twice in a 0.25 M sucrose/1 M KCl/50 mM Tris-HCl buffer (pH 8), and resuspended in the same buffer at a final concentration of 1 mg protein/ml, unless otherwise stated. These preparations did not contain any calcium-independent ATPase activity. The average calcium-dependent ATPase specific activity, assayed as described below, was 2.6 ± 0.4 U/mg protein (mean $\pm$ S.D., $n = 6$). Aliquots of the sarcoplasmic reticulum vesicle suspension (0.9 ml unless otherwise stated) were treated with 0.1 ml of the appropriate Triton X-100 solutions in order to obtain final surfactant concentrations ranging from 10^{-5} to 10^{-2} M. Equilibration was allowed to take place for 5 min at room temperature. Preliminary turbidimetric studies carried out with crude sarcoplasmic reticulum preparations [12] had shown that equilibration was complete after about 1 min. When required, the detergent-treated vesicle suspensions were centrifuged at $150000 \times g$ for 60 min at 4°C. The supernatant was considered to contain the solubilized membrane fraction.

Electron microscopy

Electron microscopy observations were carried out by the negative staining method with ammonium molybdate as described in Ref. 23. For thin-cut observations, the pellets were processed as indicated by Gurtubay et al. [5].

Analytical techniques

The turbidity of the membrane suspensions was measured as absorbance at 550 nm in a Beckman UV-5260 spectrophotometer. Proteins were determined by the method of Lowry et al. [13]; determinations in the presence of Triton X-100 were made following the modification of Wang and Smith [14]. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate was carried out according to Fairbanks et al. [15]. Gel cylinders were stained with Coomassie brilliant blue and scanned at 550 nm with a UV-5260 Beckman spectrophotometer, equipped with a densitometry accessory. Lipid extraction, phospholipid and fatty acid analysis were carried out as described by Gurtubay et al. [5].

Ca^{2+}-dependent ATPase activity was assayed at 37°C with an ATP-regenerating system containing 10 mM ATP, 1.5 mM phospho*enol*pyruvate, 0.2 mM NADH, 4 U/ml pyruvate kinase, 6 U/ml lactate dehydrogenase, 100 mM KCl, 5 mM $MgCl_2$, 0.05 mM EGTA, 100 mM triethanolamine-HCl (pH 7.4). After adding the membrane suspension (10 μl) the reaction was started with $CaCl_2$. The optimum concentration of the latter was determined in preliminary experiments for each sarcoplasmic reticulum preparation.

Results

Membrane solubilization

Membrane solubilization by detergents is easily monitored by means of turbidity measurements. Changes in turbidity have been expresed as 'per-

centage turbidity', i.e., percentage decrease in turbidity with respect to the untreated sarcoplasmic reticulum suspension. The effects produced by Triton X-100 on sarcoplasmic reticulum membranes are not linearly related to detergent concentrations (Fig. 1a). It is apparent that virtually the whole solubilization process takes place between $8 \cdot 10^{-4}$ and $4 \cdot 10^{-3}$ M Triton X-100. The detergent concentration producing a 50% decrease of the original turbidity, $[\text{Triton}]_{50}$, is a useful parameter with which to compare the solubilization conditions in the membrane preparations of varying protein concentrations. The turbidity changes in the presence of Triton X-100 were studied for membrane preparations ranging from 0.5 to 4 mg protein/ml. A plot of $[\text{Triton}]_{50}$ versus membrane protein concentration shows a linear relationship, corresponding to about 2 μmol detergent/mg protein (Fig. 1b).

The study of the solubilization of membrane components provides a further insight into our system. Detergent-treated membrane suspensions are centrifuged, as described under Methods and Materials, and the supernatants are considered to contain the solubilized fraction. In this way, the percentage solubilization of protein and lipid phosphorus can be evaluated. Results are shown in Fig. 2a. They demonstrate clearly that, in agreement with the turbidimetric observations, the solubilization is a 'critical' process occurring at Triton X-100 concentrations around 10^{-3} M for a membrane protein concentration of 1 mg/ml. At low surfactant concentrations (below 10^{-3} M), the percentage solubilization of protein is slightly higher than that of lipid. Polyacrylamide gel electrophoresis studies failed to reveal preferential solubilization of any protein component at this stage. Above the critical level of 10^{-3} M, the bulk of the phospholipid is solubilized and, consequently, the intrinsic protein becomes also part of the aqueous phase. These results are confirmed and complemented by the study of the phospholipid/protein molar ratios in the supernatants and pellets obtained after centrifugation of the detergent-treated membrane suspensions (Fig. 2b).

Some degree of selectivity in the solubilization of the different phospholipid classes by detergents is commonly found in membranes [1,4,17,18]. In our case, perhaps because of the simplicity of the sarcoplasmic reticulum membrane, all the examined supernatants and pellets contain essentially the some phospholipid composition and fatty acid distribution than the native membrane. Note, however, that for practical reasons only super-

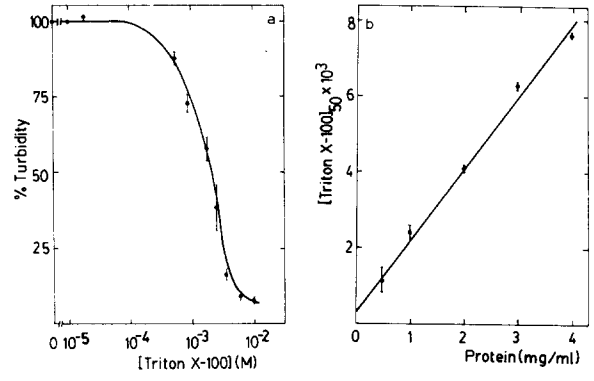


Fig. 1. (a) The effect of Triton X-100 on the turbidity of sarcoplasmic reticulum suspensions measured as percentage turbidity vs. [Triton X-100]. 100% turbidity corresponds to A_{550} of a membrane suspension containing 1 mg protein/ml. (b) The relationship between the Triton concentration producing a 50% decrease in turbidity, $[\text{Triton}]_{50}$, and membrane protein concentration. Data represent mean values of six experiments ± S.E.

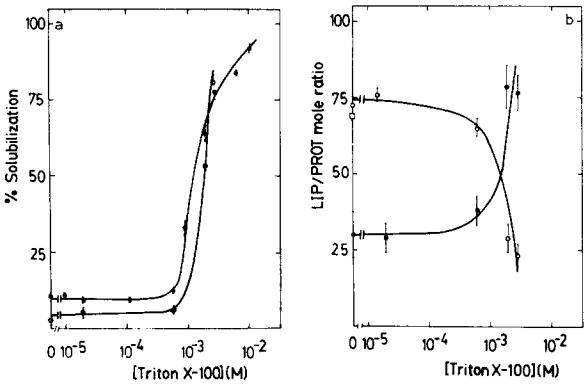
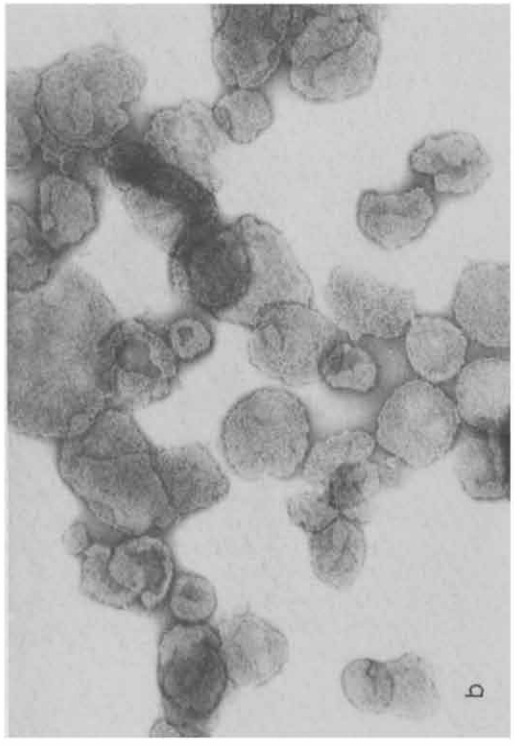


Fig. 2. (a) Percentage of protein (●) and lipid (○) solubilization after Triton X-100 treatment. Protein concentration was 1 mg/ml. (b) Lipid/protein ratios after Triton X-100 treatment and centrifugation. Pellets (○) and supernatants (●) are represented. Protein concentration was 1 mg/ml. The lipid/protein ratio for native sarcoplasmic reticulum was 71 mol lipid/mol protein. For these calculations, it was assumed that the average relative molecular weights of protein and phosphlipids were, respectively 115000 and 775. Data represent mean values of six experiments ± S.E.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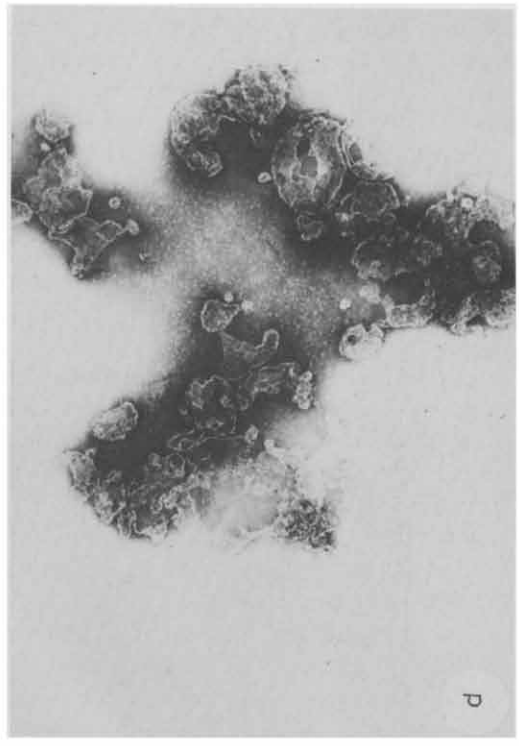
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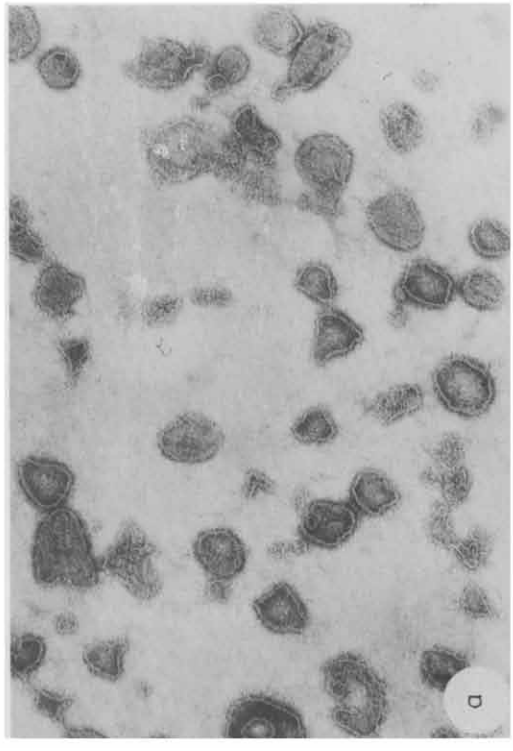
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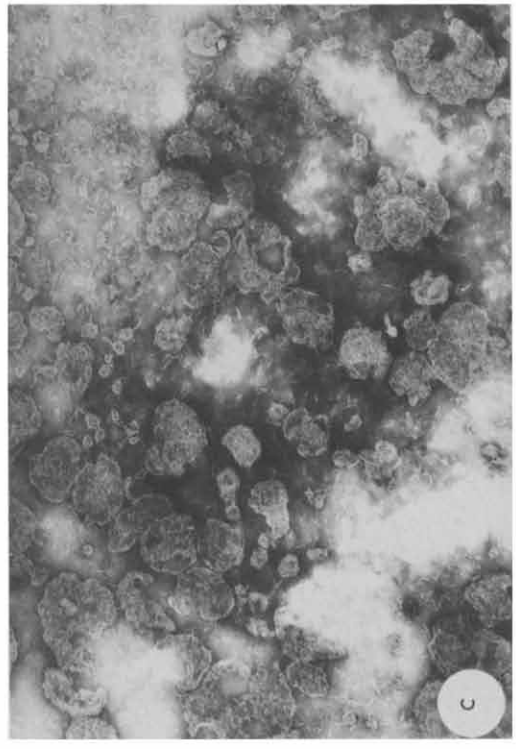
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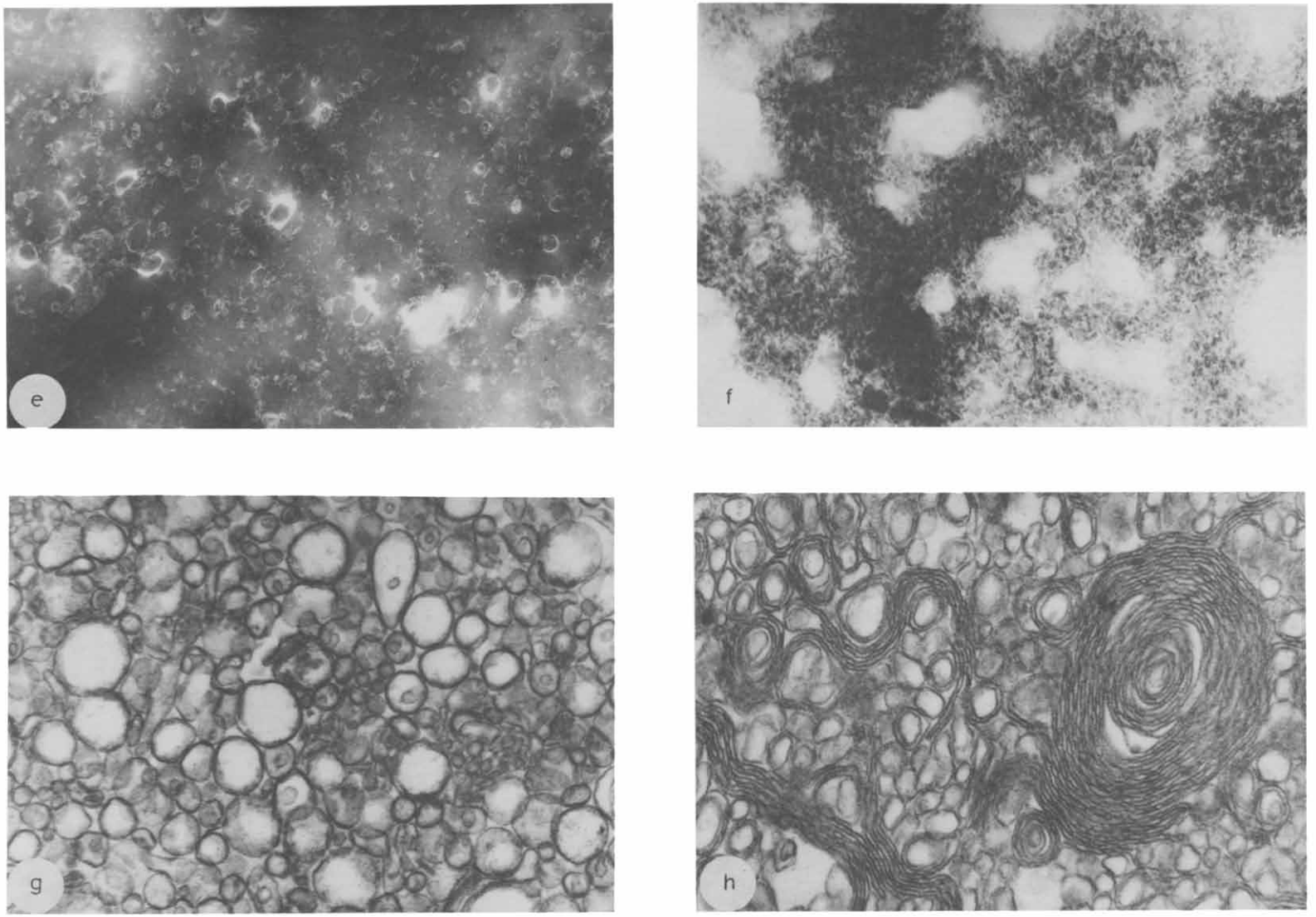

Fig. 3. Micrographs corresponding to sarcoplasmic reticulum treated with Triton X-100 at the following concentrations: (1) no Triton; (b) $1 \cdot 10^{-5}$ M; (c) $1 \cdot 10^{-4}$ M; (d) $8 \cdot 10^{-4}$ M; (e) $1 \cdot 10^{-3}$ M; (f) supernatant from the previous preparation. Sarcoplasmic reticulum centrifuged and thin cuts obtained from the pellets after treating it with (g) no Triton and (h) $2 \cdot 10^{-3}$ M Triton. Magnification: 42000× in all cases.

natants from treatments with [Triton] > $3 \cdot 10^{-3}$ M, and pellets from treatments with [Triton] < $8 \cdot 10^{-4}$ M were examined in this respect.

Ultrastructural studies

Negative-staining electron micrographs of our sarcoplasmic reticulum preparations (Fig. 3a) show more-or-less spherical vesicles about 120 nm in diameter, quite impermeable to molybdate, and with a characteristic outer 'fuzzy layer', corresponding probably to the Ca^{2+}-ATPase polar moieties [19]. The addition of 10^{-5} M Triton X-100 produces important changes (Fig. 3b): an increase in permeability is apparent, since the stain is now more evenly distributed between the inside and outside of the vesicles: there is also an increase in size, perhaps because of osmotic reasons. The outer 'fuzzy layer' is less clear, if at all present, either because of the entry of stain, or because of a rearrangement of the bilayer and symmetrical distribution of the protein.

$1 \cdot 10^{-4}$ M Triton X-100 produces scarcely any solubilizing effect, according to our physical or chemical studies (Figs. 1 and 2) yet it causes some disruption of the membranous material (Fig. 3c), giving rise to fibrillar or filamentous structures, which nevertheless sediment with the non-solubilized material in the centrifugation procedure. (An alternative explanation is that the fibrillar structures appear as a consequence of the electron microscopy preparation.) No asymmetric distribution of bilayer components is appreciable in the vesicles at this step. The morphological appearance of the membrane suspensions does not vary substantially with detergent concentrations between $1 \cdot 10^{-4}$ and $8 \cdot 10^{-4}$ M; the vesicles just become more irregular in size (Fig. 3d). Above 10^{-3} M surfactant, solubilization takes place, and the suspensions consist mostly of the fibrillar material described above (Fig. 3e). When the corresponding supernatants are examined, no structure is visible at Triton X-100 concentrations below $8 \cdot 10^{-4}$ M. At this and higher concentrations, a fine filamentous material gives rise to more or less complex reticular or spongy structures (Fig. 3f). The morphological appearance of these supernatants is the same, in spite of their different lipid/protein ratios (Fig. 2b).

The examination of ultra-thin cuts of the sedimented material after detergent treatment confirms the negative-staining observations. A remarkable feature, however, is the presence of convoluted structures and myelin-like figures in the pellets of the sarcoplasmic reticulum suspensions treated with Triton X-100 at concentrations around 10^{-3} M, i.e., when most of the membranous material has been solubilized (Fig. 3g, h). These structures may arise from a reassembly of the solubilized bilayer components.

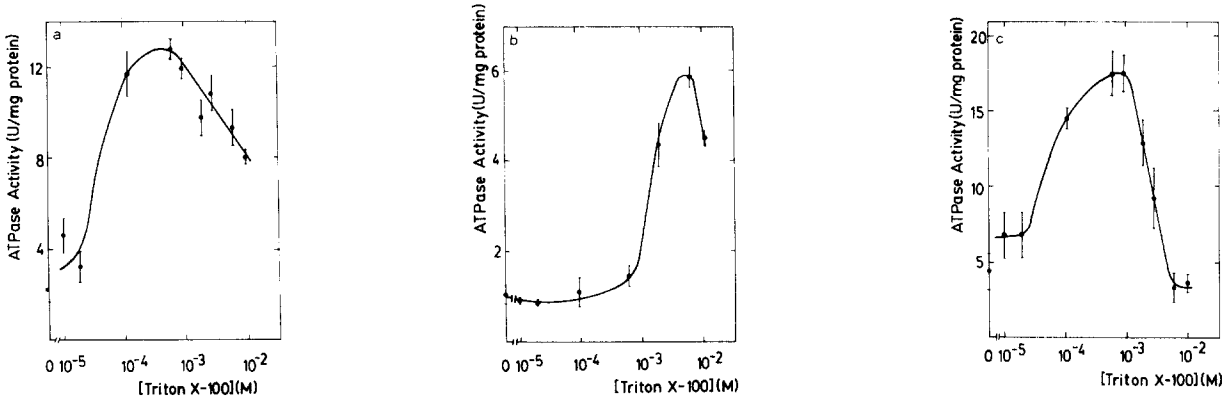

Fig. 4. Effect of Triton X-100 on sarcoplasmic reticulum ATPase specific activity. The activity was measured as described in Methods and Materials. Native sarcoplasmic reticulum had a specific activity of 2.6 U/mg protein (1 enzyme unit will liberate 1.0 μmol P from ATP per min under the assay conditions described in the Methods and Materials section). The activities were measured in: (a) sarcoplasmic reticulum without centrifugation; (b) supernatants; and (c) pellets after centrifugation. Data represent mean values of four experiments $\pm$ S.E.

Effects on enzyme activity

Our preparations did not contain any appreciable Ca^{2+}-independent ATPase activity. Assays of the Ca^{2+}-dependent activity is suspensions of sarcoplasmic reticulum containig various amounts of detergents (Fig. 4a) show that the specific activity in the preparations increases with increasing Triton X-100 concentrations up to $8 \cdot 10^{-4}$ M. Higher detergent concentrations decrease the ATPase specific activity, but even at the highest surfactant concentrations tested (10^{-2} M) the activity is 3–4-times the original one.

An examination of the Ca^{2+}-dependent ATPase specific activities in supernatants and pellets after centrifugation of the detergent-treated sarcoplasmic reticulum vesicles reveals interesting differences between both fractions. In supernatants (Fig. 4b) the activity remains unchanged by Triton X-100 concentrations up to $8 \cdot 10^{-4}$ M, and then increases following closely the pattern of membrane solubilization (Fig. 2). The specific activity of the pellets increases steadily in the presence of detergent at concentrations up to $8 \cdot 10^{-4}$ M (Fig. 4c). According to protein solubilization data (Fig. 2a), these pellets contain most of the protein previously in suspension, and this explains the similarities of Figs. 4a and 4c below $8 \cdot 10^{-4}$ M detergent, i.e., when the bulk of the the membrane protein has not yet been solubilized. In pellets, opposite to what happens in supernatants, increasing detergent concentration above 10^{-3} M leads to a steep decrease in specific activity, which is virtually abolished at Triton X-100 concentrations around 10^{-2} M.

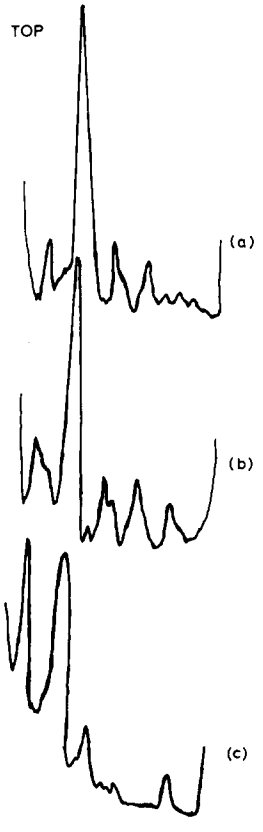

Fig. 5. Densitograms of SDS-polyacrylamide electrophoresis gels obtained from: (a) intact sarcoplasmic reticulum; (b) non-solubilized residue after treating sarcoplasmic reticulum (1 mg protein/ml) with $1 \cdot 10^{-3}$ M Triton X-100; (c) as (b) but with $1 \cdot 10^{-2}$ M Triton X-100. All the gels contained the same amount of protein.

In order to ascertain whether this decrease in specific activity was due to an enrichment of the pellets in proteins other than ATPase, or rather to the detergent-induced aggregation of ATPase, the pellets were examined by SDS-gel electrophoresis (Fig. 5). The gels show that the pellets are not particularly enriched in extrinsic proteins, and that, at high Triton X-100 concentrations, ATPase aggregates are formed that cannot be solubilized even in the presence of SDS.

Discussion

The main observation reported in this paper is that sarcoplasmic reticulum vesicles are solubilized at a critical detergent/membrane ratio. We shall discuss the events taking place at sublytic detergent concentrations, the solubilization pehomenon, and the nature of the non-solubilized residue.

The lowest detergent concentration tested has been 10^{-5} M. This corresponds to about 1 Triton X-100 per 60 phospholipid molecules. At this low detergent/lipid ratio, the detergent should become incorporated into the bilayer in the form of monomers [1]. Its main effect i.e., an increase in membrane permeability at low concentrations (Fig. 3), is well documented [22–24]. Increasing surfactant concentrations up to $6 \cdot 10^{-4}$ M, equivalent to a 1 : 1 detergent/phospholipid ratio, produces and increased incorporation of surfactant molecules

into the bilayer, so that the formation of lipid-detergent mixed micelles is more probable. This in turn makes the vesicles still more permeable, and so an increase in ATPase activity is observed (Fig. 4a), possibly due to uncoupling. In addition, the non-bilayer structures favour the flip-flop movement of the enzyme molecules so that the membrane asymmetry is no longer observed (Fig. 3).

Membrane solubilization occurs as a critical phenomenon in the case of sarcoplasmic reticulum in the presence of Triton X-100 (Figs. 1–3), at about 10^{-3} M detergent when protein concentration is 1 mg/ml. Solubilization is a gradual process in mitochondrial and other complex membranes [5–7] and even in the case of crude sarcoplasmic reticulum preparations [12]. However, a similar critical behaviour was observed in the case of the relatively simple Semiliki-Forest viral membranes [4]. The lytic detergent/membrane ratio remains constant, for membrane protein concentrions ranging from 0.5 to 4 mg/ml, at about 2 μmol detergent/mg protein (Fig. 1b). This shows that the surfactant concentration producing membrane solubilization is independent of its critical micellar concentration, and rather depends on the relative proportions of water, membrane and surfactant. An extrapolation of Fig. 1b to zero protein concentration gives an estimate of the free equilibrium, concentration of Triton X-100; the latter happens to be near its critical micellar concentration (around 0.3 mM), in agreement with previous studies [4,5,16].

The main step of membrane solubilization consists fundamentally of the solubilization of membrane phospholipids (Fig. 2b). At lower detergent concentrations, the lipid/protein ratio of the solubilized fraction is about 35 : 1, i.e., the same ratio which is found in Ca^{2+}-ATPase purified in the presence of cholate according to Warren et al. [8]. Above the lytic detergent concentration, the phospholipid/protein ratio is, as expected, similar to the intact sarcoplasmic reticulum (about 70 : 1). Simultaneously with membrane solubilization, a dramatic increase in the solubilized Ca^{2+}-ATPase specific activity is observed (Fig. 4b). Several instances at which enzyme activities increase while membranes are solubilized have been described, for example, in mitochondria [5,7]; however, no simple relationship between the two phenomena has been described up to now, probably due to the complexity of those membranes as compared to the sarcoplasmic reticulum.

The non-solubilized residue is poor in phospholipids (Fig. 2), in accord with the data of Becker et al. [4] for viral membranes. The insoluble material consists mainly of aggregated forms of ATPase (Fig. 5). SDS-polyacrylamide gel electrophoresis of supernatants shows no preferential solubilization of ATPase (data not shown), and therefore the pellets are not enriched in peripheral proteins. The complex myelin-like and other structures that are seen among the non-solubilized material from suspensions treated with $2 \cdot 10^{-3}$ M detergent (Fig. 3h) are produced most probably by detergent-mediated lysis and reassembly of the bilayer components. The same phenomenon has been observed with other detergents [25] and other membranes [5] and even liposomes [23].

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References

1 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79
2 Racker, E. and Stoecknius, W. (1974) J. Biol. Chem. 249, 662–663
3 Dean, W.L. and Tanford, C. (1977) J. Biol. Chem. 252, 3351–3353
4 Becker, R., Helenius, A. and Simons, K. (1975) Biochemistry 14, 1835–1841
5 Gurtubay, J.I.G., Goñi, F.M., Gómez-Fernández, J.C., Otamendi, J.J. and Macarulla, J.M. (1980) J. Bioenerg. Biomembranes 12, 47–70
6 Loizaga, B., Gurtubay, J.I.G., Macarulla, J.M., Goñi, F.M. and Gómez, J.C. (1979) Biochem. Soc. Trans. 7, 148–150
7 Gurtubay, J.I.G., Barbero, M.C., Rial, E., Goñi, F.M. and Macarulla, J.M. (1982) Biochem. Soc. Trans. 10, 43–44
8 Warren, G.B., Toon, P.A., Birdsall, N.J.M., Lee, A.G. and Metcalfe, J.C. (1974) Biochemistry 13, 5501–5507
9 Gómez Fernández, J.C., Goñi, F.M., Bach, D., Restall, C.J. and Chapman, D. (1980) Biochim. Biophys, Acta 598, 502–516
10 Fernández, J.L., Rosenblatt, M. and Hidalgo, C. (1980) Biochim. Biophys. Acta 599, 552–568

11 Nakamura, H., Jilka, R.L., Boland, R. and Martonosi, A.N. (1976) J. Biol. Chem. 251, 5414–5423
12 Villalain, J., Goñi, F.M. and Macarulla, J.M. (1982) Mol. Cell. Biochem. 49, 113–118
13 Lowry, O.H., Rosebrough, H.H., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
14 Wang, C.S. and Smith, R.L. (1975) Anal. Biochem. 63, 414–417
15 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617
16 Helenius, A. and Soderlund, H. (1973). Biochim. Biophys. Acta 307, 287–300
17 MacDonald, R.I. (1980) Biochemistry 19, 1916–1922
18 Barbero, M.C., Rial, E., Otamendi, J.J., Goñi, F.M. and Macarulla J.M. (1982) Int. J. Biochem. 14, 933–940
19 Stewart, P.S. and MacLennan, D.H. (1974) J. Biol. Chem. 249, 985–993
20 Restall, C.J., Arrondon, J.L.R., Elliot, D.A., Jaskowska, A., Weber, W.V. and Chapman, D. (1981) Biochim. Biophys. Acta 670, 433–440
21 Murphy, A.J. (1976) Biochemistry 15, 4492–4496
22 Inoue, K., Katagawa, T. (1976) Biochim. Biophys, Acta 426, 1–16
23 Alonso, A., Saez, R., Villena, A. and Goñi, F.M. (1982) J. Membrane Biol. 67, 55–62
24 Barbero, M.C., Prado, A., Gurtubay, J.I.G., Goñi, F.M. and Macarulla, J.M. (1983) Rev. Esp. Fisiol. in the press
25 Agostini, G., Stroner, M. and Hasselbach, W. (1978) Z. Naturforsch 33c, 426–436