Detergent Structure and Associated Lipid as Determinants in the Stabilization of Solubilized Ca\(^{2+}\)-ATPase from Sarcoplasmic Reticulum*

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The properties of detergents required to substitute the lipid environment of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase with retention of good functional properties were determined by the use of a large number of diverse detergents and delipidated enzyme. Detergents having an intermediate chain length (≥C\(_{12}\)) and a polyoxyethylene glycol or carbohydrate polar group were optimal for Ca\(^{2+}\)-ATPase function and stabilization, while detergents with short alkyl chain (C\(_{6}\)) or bulky head groups and many zwitterionic detergents led to rapid inactivation. Under optimal conditions (including solubilization in the E\(_{1}\) state), stability of delipidated Ca\(^{2+}\)-ATPase approximated that obtained by solubilization of Ca\(^{2+}\)-ATPase with a layer of bound lipid. Some detergents (in particular long chain members of the Tween family) were characterized by an inadequate interaction with delipidated Ca\(^{2+}\)-ATPase, resulting in biphasic inactivation. According to analytical ultracentrifugation and high performance liquid chromatography experiments, the rapid and slow components of biphasic inactivation were due to the formation of monomeric and oligomeric Ca\(^{2+}\)-ATPase, respectively.

It is concluded that both hydrophobic and polar interactions are important for the detergent effect and that solubilizing detergents of intermediate and short chain length may be bound as a monolayer, differently than the membrane lipid. Long chain detergents cause protein aggregation and, despite their resemblance to natural lipids, are inferior in their activity-retaining properties. The previous use of such detergents to prepare oligomeric Ca\(^{2+}\)-ATPase with long term retention of activity (cf. Møller, J. V., Anderson, J. P., and le Maire, M. (1988) \textit{Methods Enzymol.} 157, 261–270) is shown to depend on the presence of residual lipid in these preparations.

Elucidation of protein-protein interactions and their functional role is often difficult in the natural lipid bilayer habitat of membrane proteins (1–7). A useful approach is to solubilize membrane proteins or membrane protein complexes in a native-like state to study functional properties under well defined conditions. Detergents which can be used for this purpose are broadly categorized into two groups: (i) nonionic (polyoxyethylene glycol or alkyl glucosides), and, in some instances, zwitterionic detergents (e.g. dodecyl(dimethylamino)oxide, lysolecithins), and (ii) detergents belonging to the group of bile salts or other steroid-based detergents such as Chaps\(^{\dagger}\) and digitonin. The last mentioned group and octylglucoside have been used extensively for purification of membrane receptors (e.g. 8–13), while alkyl polyoxyethylene glycols, and in particular C\(_{12}\)E\(_{6}\), have been used in the study of transport ATPases (14–23). For bacteriorhodopsin Triton X-100 has been the detergent of choice (24, 25), and for cytochrome oxidase dodecyl maltoside is an efficient solubilizer with retention of functional properties (26–30).

Irrespective of the detergent used, it is quite often found that solubilized membrane proteins are inactivated at high detergent to protein ratios (8, 31) which either suggests dissociation of solubilized membrane protein to smaller entities as a function of detergent concentration or progressive removal of residual lipid attached to the solubilized detergent membrane protein complexes. The use of detergents does not only comprise a consideration of desirable features of the detergent such as a nonabrasive hydrophilic head group, a suitable size of the hydrophobic moiety, cmc, etc., but also one has to take into account the associated state and interplay of the detergent with the membrane lipid that may reside on the protein after detergent solubilization. In this communication we report a study on these aspects of detergent solubilization. Our study includes the use of detergents with different head groups and short, intermediate, and long hydrocarbon chains. The membrane protein studied, sarcoc...
plasmic reticulum Ca\(^{2+}\)-ATPase, is characterized by being vulnerable to the detergent-solubilized state. Nevertheless it has been possible to prepare active preparations with a retention of long term activity by the use of C\(_{12}E_8\) (15, 16, 20), myristoylphosphoglycerolcholine (32, 33), and Tween 80 (15, 34). Furthermore, high activities have been reported after addition of sparingly water-soluble detergents with a low HLB (hydrophile/lipophile balance number) to delipidized Ca\(^{2+}\)-ATPase (35). The present study compares the effect of these and other detergents as substitutes of membrane lipid on delipidized Ca\(^{2+}\)-ATPase. For a large number of different detergents we systematically measured the rate at which solubilized Ca\(^{2+}\)-ATPase becomes irreversibly inactivated when incubated with solutions containing the specified detergent. This inactivation rate was measured under various conditions, and the monomeric or oligomeric state of the Ca\(^{2+}\)-ATPase was established in parallel experiments when necessary. We find that C\(_{12}E_8\) detergents, despite their shorter alkyl chain than membrane lipids, are generally preferable to all other detergents tested in regard to retention of functional properties. The basis for this somewhat surprising result is discussed in relation to different modes of interaction of detergents with the Ca\(^{2+}\)-ATPase.

**EXPERIMENTAL PROCEDURES**

**Materials**—Sarcoplasmic reticulum Ca\(^{2+}\)-ATPase was prepared from rabbit skeletal muscle (36) and purified by extraction with a low concentration of deoxycholate (37). Monomeric and delipidated Ca\(^{2+}\)-ATPase (containing less than 1 mol of lipid/mol of protein) was prepared by solubilization of 8 mg of protein by 80 mg of C\(_{12}E_8\) in 1 ml of the buffer used for HPLC and removal of insoluble residue by centrifugation on the Beckman Airfuge. HPLC was performed on a TSK G3000SW, 7.5 x 600-mm column (Toyo Soda, Tokyo) in the presence of 1 mg of C\(_{12}E_8\)/ml, 20 mM Tes/Tris (pH 7.0), 100 mM KCl, 0.5 mM Mg\(^{2+}\), and 1 mg/ml C\(_{12}E_8\). The eluting delipidated Ca\(^{2+}\)-ATPase monomer was present at around 1.2 mg of protein/ml together with 1.6 mg of C\(_{12}E_8\)/ml in the peak fraction. In some experiments, when it was necessary to reduce the C\(_{12}E_8\) concentration, we prepared oligomeric Ca\(^{2+}\)-ATPase by solubilizing 8 mg of protein with 20 mg of C\(_{12}E_8\), followed by HPLC on a TSK G4000SW, eluting with 0.1 mg of C\(_{12}E_8\)/ml instead of 1 mg of C\(_{12}E_8\)/ml. The resulting oligomeric (dimeric-trimeric) peak contained about 0.8 mg of protein/ml and 0.3 mg of C\(_{12}E_8\)/ml.

The sources of the detergents used were: Octaethyleneglycol monooctyl ether (C\(_{12}E_8\), Nikko Chemical Co., Tokyo; dodecyl-\(\beta\)-maltoside (C\(_{12}G_2\), Boehringer or Calbiochem; Triton X-100, Serva, Heidelberg; myristoylphosphoglycerolcholine, Calbiochem; dodecyl-dimethylamino ether, C\(_{12}N(CH_3)_2\), DDAO, Commissariat à l'Énergie Atomique, Paris; dodecyl-\(\beta\)-dimethylamino-3-propylsulfonate (Zwittergent 3-12), C\(_{12}N(CH_3)_2\)SO_3H, Serva, Heidelberg; tetraethylene glycol mono-octyl ether (C\(_{12}E_8\)), Bachem, Basele; octyl-\(\beta\)-D-glucoside (C\(_8G\), Boehringer or Calbiochem; Triton X-100, Serva, Heidelberg; decyl-dienyl 6-D-maltoside (C\(_{12}G_2\)), Boehringer or Calbiochem; Triton X-100, Serva, Heidelberg.

**Stability Assays**—In a first type of test, the suitability of the detergents was evaluated from their ability to maintain enzyme activity of Ca\(^{2+}\)-ATPase incubated in a medium containing 0.1 mM Ca\(^{2+}\), 1 mM Mg\(^{2+}\), 10 mM Tris/Tris (pH 7.5), 100 mM KCl, and the specified detergent at concentrations of 1 mg/ml unless otherwise stated. The presence of Ca\(^{2+}\) in this medium is known to confer on the Ca\(^{2+}\)-ATPase the E\(_{2}\) conformation, according to the generally accepted nomenclature (39, 40), and hence inactivation rates are denoted as k\(_{B2}\). Delipidated Ca\(^{2+}\)-ATPase, prepared by HPLC, was assayed for ATPase activity at concentrations of 0.05-0.06 mg/ml (between 10 and 12 mg/ml protein/ml). The detergent tested was present in a large excess relative to the C\(_{12}E_8\) contributed by the HPLC-delipidation procedure (about 500 times). In some experiments the Ca\(^{2+}\)-ATPase inactivation rate was measured in the E\(_{2}\) state in the presence of 0.2 mM EGTA, 10 mM Mg\(^{2+}\), 10 mM Tes/Tris (pH 7.5), 100 mM KCl, and detergent (specified in the figure legend). ATPase activity remaining after various incubation periods was measured under standard conditions with a coupled enzyme assay by further diluting the ATPase to 4 mg/ml in a medium containing 100 mM KCl, 10 mM Tes/Tris (pH 7.5, 20 °C), 1 mM Mg\(^{2+}\), 0.1 mM Ca\(^{2+}\), 5 mM MgATP, 1 mM phosphonopyruvate, 0.15 mM NADH, 0.1 mg/ml pyruvate kinase, and 0.1 mg/ml lactate dehydrogenase and 1 mg/ml C\(_{12}E_8\) (under these conditions, C\(_{12}E_8\) during ATPase activity measurement is in large excess compared to the detergent used during the incubation period; we have verified that there is no effect of C\(_{12}E_8\) on the other detergents used on the capability of the coupled assay system to follow ATP hydrolysis).

In a second type of test the effect of the various detergents on Ca\(^{2+}\)-ATPase during enzymatic cycling was tested by direct addition of the HPLC-delipidated ATPase to an ATPase assay medium identical to that described above except that the test detergent, and not C\(_{12}E_8\), was present at a concentration of 1 mg/ml. The rate of NADH oxidation as a function of time was followed on an Aminco DW2a spectrophotometer, operated in the double-beam mode, with 345 nm as the measuring and 470 nm as the reference wavelength. The medium was supplemented with new NADH as required, and inactivation was ordinarily followed until at least 90% complete. Activities after given time periods were tested in two ways: from successive tangents of the NADH oxidation curve; this allowed us to estimate inactivation rate constants (denoted as k\(_B\)) and initial activities (denoted as V\(_{0}\)) from logarithmic plots of activity as a function of time. In these assays C\(_{12}E_8\), originating from the delipidated preparations, only present in amounts too low to conflict with the test detergent, usually at a weight ratio of 1:200 up to 1:20.

The question whether the low amount of C\(_{12}E_8\) present in these assays might have affected the results in the presence of the test detergent was considered by comparison of inactivation rates at various ratios, obtained by increasing the test detergent concentration to 10 mg/ml, and by performing supplementary experiments in which Ca\(^{2+}\)-ATPase was solubilized directly in test detergent at a high detergent to protein ratio (500:1). These variations in procedure usually led to the same inactivation rate constants, except for the most sensitive detergents. The test results obtained on each detergent were taken as bona fide values for inactivation by test detergent, except for the few which exhibited atypical features. Direct solubilization assays could not be used with detergents that are inefficient solubilizers of lipids and biological membranes. In these cases we sometimes employed DEAE-anion exchange chromatography [41] in the following way. C\(_{12}E_8\)-solubilized Ca\(^{2+}\)-ATPase was first bound to DEAE-cellulose column (1 x 2 cm), then delipidated with 35 ml of 2 mg of C\(_{12}E_8\)/ml, 50 mM NaCl, 10 mM Tes/Tris (pH 7.4), and 0.5 mM Ca\(^{2+}\); this was followed by exchange of C\(_{12}E_8\) with test detergent in the same electrolyte medium, and elution of Ca\(^{2+}\)-ATPase by raising the concentration of NaCl in the medium to 400 mM.

**Aggregational State**—The aggregational state of the solubilized Ca\(^{2+}\)-ATPase was examined by HPLC and sedimentation velocity in the analytical ultracentrifuge (Beckman Model E, equipped with a photoelectric scanner) as previously described [42]. Time courses for monomeric proteins on TSK G3000SW columns were established by reference to the analytical ultracentrifuge experiments and on the basis of previous standardization of HPLC columns [42]. For long chain detergents, agarose gel chromatography after initial solubilization of Ca\(^{2+}\)-ATPase was sometimes used in preference to HPLC according to procedures previously described for Tween 80 (15, 34). Delipidated Ca\(^{2+}\)-ATPase Delipidation—To evaluate the extent of Ca\(^{2+}\)-ATPase delipidation when SR membranes, and not HPLC-delipidated Ca\(^{2+}\)-ATPase, were directly solubilized in detergent, we measured the fluorescence quenching of ATPase tryptophans by a small amount of added brominated phospholipids, in the presence of various concentrations of detergent. Brominated phospholipid (dibromooleophosphatidylethanolamine) was a kind gift from Dr. A. G. Lee, South-
ampton, United Kingdom (East and Lee (43)). Fluorescence was measured in a SLM 400 S fluorometer (excitation and emission wavelengths were 290 and 335 nm, respectively).

RESULTS

Effect of Detergent Concentration on Lipid Retention and Stability of Solubilized Ca**+-ATPase

It is a common observation that solubilized membrane proteins often become inactivated at high detergent to protein ratios. This is illustrated for SR Ca**+-ATPase in Fig. 1 which shows that the inactivation rate of membraneous Ca**+-ATPase, solubilized at a protein concentration of 0.2 mg/ml with various concentrations of C12Es is dependent on detergent concentration (open triangles and circles). At 10 mg of C12Es/ml, which is appreciably higher than the minimal concentration at which full solubilization of the Ca**+-ATPase membranes occurs, the inactivation rate approaches a maximum. An increased inactivation rate is especially obtained if the protein is incubated in the E2 state (presence of EGTA) where enzyme lability is 2 orders of magnitude higher than in the E3 state (presence of Ca**+). The protective effect of Ca**+ may reflect stabilization of a Ca**+ binding domain, close to the hydrophobic, membrane spanning region of Ca**+-ATPase (44). However, a mere unspecific effect of Ca**+ is not excluded, since Pikula et al. (45) found that addition of 20 mM Ca**+ exerted an even stronger activity retaining effect.

It is seen from the remaining data of Fig. 1 that if lipid-depleted Ca**+-ATPase is used (closed circles and triangles), or if membraneous Ca**+-ATPase is solubilized at low protein concentrations (open squares), no effect of C12Es concentration is observed on the inactivation rate which is maximal under these conditions. The range of inactivation rates at 0.2 mg of protein/ml together with endogenous lipid (approximately 0.1 mg/ml) occurs under conditions where most of the Ca**+-ATPase is in monomeric form (16, 46). Therefore the variable inactivation rates, observed especially in the E2 state, suggest displacement of stabilizing lipid from the Ca**+-ATPase as the detergent/lipid ratio is raised (see also Refs. 46 and 47). In agreement with this view, addition of exogenous PC to a C12Es-containing inactivation medium reduced the inactivation rate of HPLC delipidated ATPase (data not shown).

A direct demonstration of lipid retention by detergent-solubilized Ca**+-ATPase was performed by measuring the extent to which Ca**+-ATPase fluorescence could be quenched by brominated phospholipids (Fig. 2). The experiment was performed in the following way: to native SR vesicles (suspended at 0.05 mg of protein/ml and containing about 0.025 mg/ml endogenous phospholipid, i.e. about 30 μM lipid) C12Es was gradually added up to a concentration of 0.2 mg/ml. This resulted in complete solubilization as judged from clarification and drop in the intensity of scattered light (open symbols in Fig. 2). At this point, the Ca**+-ATPase intrinsic fluorescence level was increased 3-5% by addition of detergent to the native membrane as the result of perturbation of tryptophan groups by C12Es (cf. Refs. 48 and 49). Then, a stock solution of mixed micelles of C12Es and brominated lipids (triangles) or dioleoyllecithin (squares), were added, resulting in the addition of 0.02 mg of C12Es/ml and 10 μM exogenous lipids to the sample. (This represents only a small increase in detergent and lipid concentration of the sample.) After addition of brominated lipids the Ca**+-ATPase fluorescence was quickly quenched by 15-16% (▴), while the addition of dioleoyllecithin lipid had little effect (▪), compared to that of the control sample without added lipid (○). The decrease in fluorescence caused by the addition of brominated lipid indicates close proximity of brimaine atoms and tryptophan residues of the Ca**+-ATPase (43), i.e. insertion of the brominated lipid into the first lipid shell surrounding the Ca**+-ATPase. When more C12Es was added quenching by brominated phospholipids was relieved, and fluorescence reverted toward almost the same level as observed in the presence of dioleoyl-phosphatidylcholine. Thus, progressive delipidation of Ca**+-ATPase occurred following the increase in the detergent/protein concentration ratio.

Figs. 1 and 2 demonstrate that lipid retention is a significant determinant of enzyme stability and that a high detergent/protein ratio is required to displace lipid from the protein. In order to study the true effect of the various detergents themselves, in the rest of this study we therefore measured inactivation rates of Ca**+-ATPase samples which previously had
been thoroughly delipidated by HPLC so that less than 1 of the 100 lipids originally present per mol of Ca\(^{2+}\)-ATPase was left.

**Effect of Detergent Structure on Stability and Turnover of Delipidated Ca\(^{2+}\)-ATPase**

Table I summarizes the effect of the wide variety of detergents that were tested to establish features that are essential for maintenance of activity by lipid depleted Ca\(^{2+}\)-ATPase in the E\(_1\) state (second and third columns) and during turnover (fourth and fifth columns). In most cases inactivation was a monoeponential process and therefore could be characterized by a single inactivation rate constant. The third column shows the intersection with the ordinate of logarthmic plots of Ca\(^{2+}\)-ATPase activity versus incubation time in the E\(_1\) state. Usually this extrapolation occurred at 100 ± 15%, relative to that of C\(_{12}\)E\(_6\) which served as the reference detergent, but sometimes the extrapolation was observed far below this level. In some instances this was probably an artifact, arising because of a very rapid inactivation rate by the detergent under investigation which resulted in ill-defined incubation periods (dodecylldimethylamine oxide and Zwittergent 3-12, Table 1, Section E). Alternatively, a low extrapolation indicated that decay was a truly biphasic process, consisting of a rapid (<1 min) and a slow inactivation phase. This is the case for most Tween detergents (Table I, Section G), Brij 76 (Table I, Section A), and dilution of Ca\(^{2+}\)-ATPase with detergent-free medium (Table I, Section H, and Fig. 5). This particular situation is analyzed in detail at the end of “Results.”

**Overall Survey of the Data**—The first important, general conclusion which accrues from the data in Table I is that although inactivation rates vary widely among detergents, the inactivation rate constant is always considerably higher during turnover than in the presence of Ca\(^{2+}\). Excluding the steroid-based detergents there is an inverse, but not strict correlation (r = 0.68) between the inactivation rate constant (k\(_{I0}\)) and initial rate during turnover (V\(_i\), see Fig. 3A); there is also a weak correlation between k\(_{I0}\) and k\(_{I}\) (r = 0.58), where k\(_{I}\) represents the inactivation rate constant in the E\(_1\) state (Fig. 3B).

The higher degree of liability of Ca\(^{2+}\)-ATPase during turnover is evidently related to the fact that the various intermediates in the catalytic cycle have different stabilities. For nine out of our detergents we have consistently found an increased liability of solubilized Ca\(^{2+}\)-ATPase in the E\(_1\) form, as already documented for C\(_{12}\)E\(_6\) (Fig. 1 and Ref. 16); we have also found that Ca\(^{2+}\)-ATPase phosphorylated with inorganic phosphate (E\(_{2P}\) form) is inactivated at even higher rates. Since the relative proportion of the various intermediates during turnover in the stationary state probably depends on the detergent used, this factor will probably contribute to the lack of strict correlation.

**Effect of Hydrophobic Chain Length**—By comparing the effect of nonionic detergents with different head groups (Table I, Sections A, B, and C) it is seen that the short chain (C\(_{10}\)) detergents octyl glucoside and C\(_{12}\)E\(_6\) rapidly produce inactivation. This is also the case for a short chain phosphatidylcholine (Table I, Section H). Inactivation occurs with a rate constant which is 80-200 times faster than that observed with C\(_{12}\)E\(_6\). In general detergents with a C\(_{10-14}\) alkyl chain are required for optimal solubilization and preservation of activity (inactivation rates in the 0.001-0.003 min\(^{-1}\) range in the E\(_1\) state, and in the 0.04-0.12 min\(^{-1}\) range during turnover).

Extending the hydrocarbon chain length beyond C\(_{12}\) does not reduce the rate of inactivation further and, in fact, in some cases leads to rapid inactivation. Even in cases where the inactivation rate in E\(_1\) is slow, the inactivation rate during turnover is increased (compare C\(_{10-12}\)E and C\(_{12}\)E\(_{<10}\) with

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C12E10 (Table I, Section A). The presence of double bonds in the aliphatic portion has different effects according to the number of carbon atoms considered, consistent with the idea that this alternative to the simple saturated chain leads to shorter micelle or monolayer dimensions due to kinking. For instance C12E2G2 has a shorter effective chain length and causes a stronger inactivation than C12G2. On the other hand, C18E1C12 is less inactivating than C12E12 (Table I, Section A), consistent with a stabilizing effect of either effective decrease of hydrophobic chain length or of disordering. Furthermore, it can be seen that the presence of many double bonds and chain branching in farnesyl maltoside leads to appreciable destabilization.

Among the detergents with good protective activity, only polyoxyethylene glycol detergents having an intermediate (C12) chain length maintain the same high turnover rate as in the membrane state. Dodecyl glucosides (Table I, Section C), Triton X-100 (Table I, Section B), and myristoylphosphatidylglycerol (Table I, Section D) exhibit activities less than half of those observed with C12Es. Shortening the alkylic chain to C10 results in markedly reduced activities. Vanishingly small activities are measured in the presence of bile salts and Chaps (Table I, Section E). This contrasts with the retention of activity observed immediately after solubilization of membrane Ca2+-ATPase by deoxycholate (50, 51), attesting to the importance of retention of membrane lipid by Ca2+-ATPase under these conditions.

Effect of the Polar Head Group—In general polyoxyethylene glycol and glucoside detergents are best suited for retention of activity. This is true also for alklypoloxylene glycol detergent with very small head groups (Table I, Section F) which do not form well defined micelles, but lamellar or other microscopic phases (52, 53). However, rapid inactivation and low enzymatic activities are observed for polyoxyethyleneglycol detergents with very bulky head groups (see the bottom of Table I, Section A). Moreover, these detergents have a tendency for biphasic inactivation of Ca2+-ATPase as discussed below in connection with the Tween family.

The crucial importance of a proper choice of a hydrophilic group is seen from the fact that dodecyltrimethylammonium oxide and Zwittergent 3-12 produce immediate inactivation, despite the presence of many double bonds and chain branching in farnesyl maltoside.

Stability of Detergent-solubilized Ca2+-ATPase

As noted above inactivation of Ca2+-ATPase by the Tween family (except Tween 20 which is discussed below) is a distinctly biphasic process, the majority of Ca2+-ATPase being instantaneously inactivated (within 1 min), while the remainder is inactivated at a slow rate (Table I, Section G). Biphasic inactivation with Tween 80 is shown in Fig. 5 which furthermore demonstrates that this characteristic decay mode is also observed after dilution of C12Es-ATPase into a detergent-free medium under conditions where the final detergent concentration has the same head group as Zwittergent 3-12.

A specific interaction of the polar head with Ca2+-ATPase, even for "harmless" detergents which maintain activity well, is illustrated by the data in Fig. 4 in which we have compared the inactivation rates of Ca2+-ATPase in the E2 state in the presence of three different detergents as a function of pH. It is seen that the optimal stabilizing pH is about 6.5 for C12Es, 6.5-7.0 for Tween 20, and about 8.0 for dodecyl maltoside. Since none of the detergents are ionized at pH 6.5-8, these data on detergents with the same length of the hydrocarbon chain and similar cmc (31) indicate a detergent-protein specificity which presumably arises from differences between the interaction of the detergent head groups with polar amino acid residues on the Ca2+-ATPase.

Biphasic Inactivation of Ca2+-ATPase by Tween Detergents

As noted above inactivation of Ca2+-ATPase by the Tween family (except Tween 20 which is discussed below) is a distinctly biphasic process, the majority of Ca2+-ATPase being instantaneously inactivated (within 1 min), while the remainder is inactivated at a slow rate (Table I, Section G). Biphasic inactivation with Tween 80 is shown in Fig. 5 which furthermore demonstrates that this characteristic decay mode is also observed after dilution of C12Es-ATPase into a detergent-free medium under conditions where the final detergent concentration

![Graph](image-url)
Stability of Detergent-solubilized Ca\(^{2+}\)-ATPase

It has previously been found that monomeric Ca\(^{2+}\)-ATPase is less resistant than oligomeric Ca\(^{2+}\)-ATPase toward inactivation with detergent (14-16). To account for the phenomenon of biphasicity illustrated in Fig. 5 we propose that the rapid phase represents inactivation of monomeric Ca\(^{2+}\)-ATPase at the low final C\(_{12}E_8\) concentration, and the slow phase inactivation of oligomeric Ca\(^{2+}\)-ATPase that is quickly formed after dilution with a detergent-free or Tween 80-containing medium. In order to study this possibility further we performed supplementary experiments on the aggregated state of Ca\(^{2+}\)-ATPase by sedimentation in the analytical ultracentrifuge. Since for these experiments a higher protein concentration (about 0.05-0.1 mg/ml) was required to follow accurately the sedimentation of the protein in the analytical ultracentrifuge cell, some modifications in the preparative procedure were required. Accordingly, delipidated Ca\(^{2+}\)-ATPase was prepared with a lower concentration of C\(_{12}E_8\) than in the standard procedure as described under "Experimental Procedures." The resultant preparation containing 0.6 mg of Ca\(^{2+}\)-ATPase/ml and 0.3 mg of C\(_{12}E_8\)/mg was oligomeric (dimeric/trimeric) as judged from the elution pattern (35). However, after 10 times dilution we found that about half of the protein sedimented slowly with a sedimentation coefficient of 5 S, corresponding to monomeric Ca\(^{2+}\)-ATPase (16), and the use of Lubrol WX is required to maintain good functional properties of Ca\(^{2+}\)-ATPase. The slow inactivation rates shown in Table I, Section G, for the Tween family are mainly inactivation rates of oligomers, not monomers.

Oligomers of Ca\(^{2+}\)-ATPase Prepared by Gel Chromatography

The poor performance demonstrated here for detergents with bulky head groups as protecting agents contrast with the previous use of Tween 80 to prepare active and stable oligomers of Ca\(^{2+}\)-ATPase (14, 15), and the use of Lubrol WX to purify active Na\(^+\), K\(^+\)-ATPase in an oligomeric form (58). With respect to the gel-chromatographically prepared oligomer of Ca\(^{2+}\)-ATPase with long term activity in Tween 80 an essential difference from the present experiments is that membrane phospholipid was partially retained on Ca\(^{2+}\)-ATPase after chromatography (14, 15). Fig. 6 shows the results of similar experiments on agarose 1.5m, performed with Lubrol WX instead of Tween 80. It is seen that enzymatically active Ca\(^{2+}\)-ATPase elutes between the void volume and mixed micelles of lipid and detergent at a position which on this column corresponds to a trimeric-tetrameric aggregational state (34). A similar observation was made with another long chain detergent, namely C\(_{12}E_8\)clay. (not shown).

Insert in Fig. 6 shows that remaining activity in the fractions is correlated with lipid content. Activity can be further increased (about 100%) by the addition of C\(_{12}E_8\) instead of Lubrol WX, although not to the level of the original Ca\(^{2+}\)-ATPase membrane preparation. For Tween 80 the irreversible decrease in activity corresponds to a decrease in active enzyme concentration as indicated by a decreased phosphorylation capacity after column chromatography (34). In the present...
buffer plus 0.2 mg of Lubrol WX/ml. Enzyme activities of the eluted agarose (Bio-Rad) column, equilibrated and eluted with the same other membrane proteins (8, 9, 54, 56). In the absence of lipid, plasmic reticulum Ca²⁺-ATPase to inactivation by many de-useful for delipidation of Ca²⁺-ATPase and solubilization in Tween detergents and Lubrol WX) led to rapid inactivation DTT, shows enzyme activity (expressed as rmol/mg/min) in the presence Ca²⁺-ATPase in the presence of Lubrol WX. The present study documents the vulnerability of sarco-plasmic reticulum Ca²⁺-ATPase to inactivation by many de-tergents such as octyl β-D-glucoside and dodecyl dimethyldi-amine oxide which have been useful in the study of many other membrane proteins (8, 9, 54, 56). In the absence of lipid, detergents with intermediate chain length (=C₁₂) and having a polyoxyethyleneglycol or carbohydrate hydrophilic head group, are optimal for maintenance of activity of delipidated Ca²⁺-ATPase. Interestingly these kinds of detergents are also useful for delipidation of Ca²⁺-ATPase and solubilization in monomeric form, in contrast to long chain detergents. The use of long chain detergents with bulky head groups (e.g. Tween detergents and Lubrol WX) led to rapid inactivation of delipidated Ca²⁺-ATPase. A closer examination of this finding indicated that the previous evidence for long term stability of oligomeric Ca²⁺-ATPase, prepared by gel chromato-graphy in the presence of these detergents (14, 15, 58), is contingent upon retention of a layer of lipid bound to the hydrophobic region of Ca²⁺-ATPase. In agreement with this view we have observed that after initial solubilization of SR membranes with C₁₂E₈ or dodecyl maltoside, the addition of detergents with bulky head groups failed to delipidate Ca²⁺-ATPase further, even when present in a large excess (59). Another example of preferential interaction with Ca²⁺-ATPase was that observed here with C₁₂E₈ when this detergent was present in low amounts together with Tween 20.

A plausible explanation for the instability and low activity of Ca²⁺-ATPase in the presence of long chain detergents with bulky head groups is that these detergents are unable to establish adequate contact with the hydrophobic region, due to steric restrictions caused by the bulky head group. In this connection one may recall that a detergent with 10 oxyethyl-ene units has an average length of around 17 Å as a random coil which is the more probable conformation in a polyethyleneglycol detergent (60). In agreement with this view several long chain detergents with very short polyethyleneglycol head group are better suited for interaction with delipidated Ca²⁺-ATPase (Ref. 36 and Table I, Section F). However, these detergents do not form well defined micelles (52, 53), and it is probable that the protein in these cases is inserted in lamellar or other elongated phases in a similar way as in the lipid bilayer as illustrated schematically in Fig. 7A.

In the study of transport ATPases, C₁₂E₈ or very similar detergents have been predominantly used to solubilize the proteins in enzymatically active form. Among the large variety of detergents used in this study dodecyl maltoside or dodecyl maltotrioside were only marginally better for preserving Ca²⁺-ATPase activity and led to a lower turnover in the presence of ATP. Our study suggests that there is no advantage in using more natural detergents like myristoylphosphoglycerolcholine either (cf. Ref. 61). The only detergent which we found to be significantly superior to C₁₂E₈ was Tween 20 in con-junction with residual C₁₂E₈ which noticeably stabilized the enzyme during phosphorylation and turnover (Table I, Section G). However, oligomerization is likely to contribute to stabilization of Ca²⁺-ATPase under these conditions. Since the head group of Tween 20 is as bulky as that of Tween 80 it is pertinent to ask how Tween 20 could sustain the same high degree of activity as observed in the presence of C₁₂E₈.

To discuss this question in more detail we suggest that different modes are available to solubilizing detergents in their interaction with Ca²⁺-ATPase as shown schematically in Fig. 7, B and C. First, the protein might be inserted into a detergent “quasi” micelle (Fig. 7B). From previous studies on the fatty acid chain length dependence of phospholipids, used for re-constitution of Ca²⁺-ATPase, this would require a hydrocarbon chain of around 16 carbon atoms (62, 63). Alternatively,
short chain and intermediate chain length detergents may cover the hydrophilic domain of Ca\(^{2+}\)-ATPase with a monolayer of detergent (Fig. 7C), as previously discussed (31, 41). Binding of Tween 20 in this mode would remove many of the sterically unfavorable interactions of the head moiety with Ca\(^{2+}\)-ATPase, especially if residual C\(_{12}\)E\(_9\) that was shown to play a role in this case is present at the edge of the monolayer. On the other hand, Tween 80 or Lubrol WX may be forced by their long chain to adopt the micellar type of interaction (Fig. 7B), and under these conditions, constraints due to the large head group might lead to lower activity and higher instability of the Ca\(^{2+}\)-ATPase monomer.

Biphasic inactivation, as observed in this study with the long chain Tween detergents and Brij 76 (C\(_{18}\)E\(_{10}\)), probably is an indication of a particularly weak interaction of detergent with Ca\(^{2+}\)-ATPase, since this phenomenon also was observed by dilution of Ca\(^{2+}\)-ATPase into detergent-free medium. It is of interest in this connection that we have found that Lubrol WX (which gave rise to rapid, but monophasic inactivation) is able to slowly solubilize phospholipid vesicles, while Tween 40 is completely inert in this respect (59).

Finally, it should be noted that hydrophilic head group and/or ordering is made plausible by the fact that among the ordinary polyoxyethylene glycol detergents (Table 1, Section A) only Brij 76 (C\(_{18}\)E\(_{10}\)) caused biphasic inactivation. (Note, however, that the more “lipid”-like Brij 72 (C\(_{18}\)E\(_{20}\)) forms lamellar and other phases (Fig. 7A) gives good retention of activity.) Inefficient solubilization and retention of activity of Ca\(^{2+}\)-ATPase by Brij 56 (C\(_{18}\)E\(_{10}\)), and Brij 76 (C\(_{18}\)E\(_{10}\)) as compared to C\(_{12}\)E\(_{10}\), suggests that in the micellar or monolayer mode (Fig. 7, B and C) a shorter hydrocarbon chain, leading to higher mobility, or, alternatively, an unsaturated chain (Brij 96 (C\(_{18}\)E\(_{10}\))) is important for interaction with Ca\(^{2+}\)-ATPase.

In conclusion steric factors at the polar head in conjunction with fluidity of the hydrocarbon chain probably are of prime importance for detergent protein interaction. On the one hand a bulky head group and less mobility of hydrocarbon chains will prevent adequate interaction and protection of the hydrophobic domain of Ca\(^{2+}\)-ATPase against the denaturing effect of the aqueous medium. On the other hand a high chain mobility may lead to destabilization, similar to thermal inactivation; this could be the basis for the rapid inactivation by C\(_{12}\) detergents. In addition short detergent chains might penetrate more readily between the hydrophobic loops of the Ca\(^{2+}\)-ATPase membrane portion, contributing to destabilization of the protein.

REFERENCES


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Stability of Detergent-solubilized Ca\textsuperscript{2+}-ATPase