Detergent Properties of Water-soluble Choline Phosphatides

SELECTIVE SOLUBILIZATION OF ACYL-CoA:LYSOLECITHIN ACYLTRANSFERASE FROM THYMOCYTE PLASMA MEMBRANES

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Several analogs of lysolecithin were found to solubilize human erythrocyte ghosts comparably or even better than other detergents. Derivatives with aliphatic chains of 12 to 14 carbons were most effective. The phosphorylcholine detergents apparently possess low protein-denaturing properties, since they, for the first time, allowed the solubilization of enzymatically active acyl-CoA:lysolecithin acyltransferase from thymocyte plasma membranes. The solubilized enzyme was not sedimented at 177,000 x g for 60 min and penetrated into Sepharose 6B gels. Low detergent concentration resulted in a selective extraction of the acyltransferase (about 70%) as compared to alkaline phosphatase, nucleotide pyrophosphatase, γ-glutamyltransferase or Mg2+-ATPase (30 to 40%). The selectivity was reflected in sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of soluble and sedimentable membrane fractions; three bands of approximately 53, 84, and 94 x 10^6 daltons were enriched in the supernatants, whereas one band of about 68 x 10^6 daltons was concentrated in the pellet. The preferential extraction of acyltransferase may be related to particularly high affinity of lysolecithin analogs for this enzyme, which at higher concentrations was competitively inhibited by these detergents. The inhibitor constants ranged from 1400 M for the C₆ analog (ET-10-II) to 80 M for the compound with 16 carbons (ET-16-II) per aliphatic chain.

Solubilization of membrane-bound enzymes without loss of activity has in the past been achieved by use of non-denaturing detergents such as Triton X-100. Lipid-metabolizing acyltransferases, however, which transfer long chain fatty acids to lipid precursors, are usually completely inactivated by these detergents. One possible explanation for such inactivation would be that the detergents replace membrane phospholipids, the association of which with the enzyme might be essential for enzymatic activity. It has therefore been suggested by Tanford and Reynolds (1) that natural detergents of phospholipid structure, such as lysophospholipids, might possess distinct advantages for the isolation of particularly labile membrane components. The development of procedures suitable for the large scale preparation of synthetic lyso-phosphatides and structural analogs (2-6) might thus provide us with a hitherto almost unexplored potential of new detergents for membrane research. In the present study, we have examined a variety of synthetic analogs of lysophosphatidylcholine with regard to their membrane-solubilizing properties, using human erythrocyte ghosts as a model membrane. We further describe a first successful solubilization of the acyl-CoA:lysolecithin acyltransferase from calf thymocyte membranes without loss of enzymatic activity.

MATERIALS AND METHODS

**Chemicals**

Synthetic choline phosphatides were obtained according to published procedures (2-6). They were derivatives of glycerylphosphorylcholine or propanediol-(1,3)-phosphorylcholine or phosphorylcholine esters of fatty alcohols (see Table I).

Labeled lysolecithin (1-[14C]palmitoyl-sn glycerol-3-phosphorylcholine was prepared by enzymatic acylation of unlabeled 2-acyl-sn glycerol-3-phosphorylcholine with [1-14C]palmitic acid and subsequent cleavage of the 2-acyl group with phospholipase A₂ according to Ferber and Resch (7). Arachidonyl-CoAₙ was prepared according to Reitz and Lands (8) and Okuyama et al. (9) with slight modifications. The starting compound was arachidonyl chloride, obtained from Nu-Chek-Prep. (Bast, Copenhagen, Denmark) and instead of Santorin as antioxidant, butylated hydroxytoluene was added directly after the termination of the reaction. The total amount of added butylated hydroxytoluene was 50 mg/120 μmol of arachidonoyl-CoA in 20 ml of phosphate buffer, pH 7.4. The purity of the compound was checked by gas-liquid chromatography. The quantitative determination of arachidonoyl-CoA was performed with the photometric acyl-CoA:lysolecithin acyltransferase assay according to Lands and Hart (10) using an excess of microsomal enzyme. 5,5'-Dithiobis(2-nitrobenzoic acid) was obtained from Boehringer, Mannheim, Germany and Hepes from Serva, Heidelberg, Germany. All other chemicals were analytical grade reagents.

**Erythrocyte Ghosts**

Citrate-stabilized fresh human blood, blood group O, Rh-positive, was obtained from the local blood bank. The cells were washed and lysed, and the membranes were isolated and purified as described by Dodge et al. (11). The ghosts were stored in small samples in 0.25 M sucrose, 10 mM Hepes buffer, pH 7.2, at -20°C.

**Preparation and Solubilization of Thymocyte Plasma Membranes**

Plasma membranes from calf thymus were prepared as described previously (12) with the modifications described later (13). Suspensions of plasma membranes in 10 mM Hepes, pH 7.2, containing 0.25 M sucrose were kept at -20°C for 3 to 4 weeks without loss of enzyme activities. Before solubilization, the membranes were washed once with 10 mM Hepes, pH 7.2, at 177,000 x g, for 60 min (80,000 rpm, rotor 60 Ti, Beckman) because large amounts (up to 18% of total

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membrane protein) of peripheral proteins could be removed. However, these soluble proteins did not exhibit any activity of those enzymes tested. For solubilization, washed plasma membranes (1.5 to 3.0 mg) were incubated for 20 min at 0°C with 0.75 to 1.5 mg of ET-12-H (see Table I for abbreviations of detergents) in 10 mM Hepes, pH 7.2. After 10 min incubation at 37°C, the reaction was stopped by addition of 0.2 ml of 5 M NaOH. Liberated nitrophenol in supernatants was measured at 405 nm and calculated using a molar extinction coefficient of 17,840 liters·mol⁻¹·cm⁻¹. We determined a Michaelis constant for the alkaline phosphatase in plasma membranes from calf thymus to be $K_m = 75 \mu M$.

**Nucleotide Pyrophosphatase (Phosphodiesterase I) (EC 3.1.4.1)**—The determination of the activity of the nucleotide pyrophosphatase was carried out under conditions identical with those described for the assay of alkaline phosphatase, with the exception that $\gamma$-nitrophosphoryl $5^\prime$ thymidylate (3.0 mM) was used as substrate. We determined a Michaelis constant for the nucleotide pyrophosphatase in plasma membranes from calf thymocytes to be $K_m = 1.2 \mu M$.

**Polyacrylamide Gel Electrophoresis**

Polyacrylamide gel electrophoresis (data not shown) of 100,000 × g supernatants and pellets after solubilization with ET-12-H and ET-16-H showed that all membrane proteins can be extracted by this procedure. No principal differences between the action of the long and short chain derivative are obvious.

**Inhibition of Acyl-CoA:Lysolecithin Acyltransferase by Lysolecithin Analogs**—To test the proposed “mildness” of phosphorylcholine detergents for sensitive membrane components, we planned to test acyl-CoA:lysolecithin acyltransferase from the plasma membrane of calf thymocytes. Since all phosphatides used here were substrate analogs for the acyltransferase, it was reasonable to assume that the analogs might be inhibitors of the enzyme. Fig. 2 shows for the series of alkyl-deoxy lysophosphatides (ET-n-H) that this was, in fact, the case. The degree of inhibition increased with the number of alkyl atoms. ET-18-H exhibited a significantly lower inhibition than ET-16-H. To investigate the mechanism of this inhibition, we determined the inhibitor constants from Dixon plots (22) using a Lineweaver-Burk plot (21) of a kinetic experiment using palmitoyl-sn-glycerol-3-phosphorylcholine as substrate.

**RESULTS**

Solubilization of Human Erythrocyte Ghosts by a Variety of Phospholipid-containing Detergents—A number of phosphorylcholine-containing water-soluble lipids, available in the laboratory, have been screened for their ability to clear turbid suspensions of human erythrocyte membranes. Ghosts were isolated as described by Dodge et al. (11) and suspended in Hepes buffer, pH 7.2, at 2 mg protein/ml. Samples (0.1 ml) of these suspensions were mixed with increasing amounts of the various phosphatides, diluted to a total volume of 0.6 ml, and kept for 20 min at 0°C and the extinction of the solution was determined at 541 nm. In the absence of detergent, this extinction was approximately 0.4 cm (control).

We have found that the degree of solubilization under these conditions is primarily determined by the ratio of detergent to membrane protein and not so much by the absolute detergent concentration. Therefore, in Table I we have compiled the magnitude of this ratio necessary for a 50% decrease in turbidity. The data reveal that a considerable number of compounds, particularly those with C₁₂ and C₁₄ aliphatic chains, are comparable to or even more active in this test than Triton X-100 or SDS. Shorter as well as longer hydrophobic chains result in less potent detergents. In fact, all saturated C₁₄ compounds (with the noteworthy exception of ET-18-OCH₃) increase rather than decrease the turbidity. The importance of relatively small structural changes for the detergent properties of these phospholipids is emphasized by the finding that an exchange of saturated for unsaturated C₁₄ aliphatic chains leads to rather potent long chain detergents. Moreover, the benzylation of the hydroxyl function in ET-12-OH resulted in the most active membrane solubilizer of our series (ET-12-OCH₂CH₃). A more detailed impression of the solubilization properties of a selection of compounds is given in Fig. 1a.

Since turbidity decrease may not necessarily coincide with true solubilization of membrane material, we have, for some of the compounds employed, compared these data with the percentage of membrane protein remaining in ultracentrifugation supernatants (last column of Table I, and Fig. 1b). The data reveal that, in general, good agreement exists between the two methods but that some quantitative variations will have to be considered. Regarding the importance of the length of the hydrophobic chain for the solubilization power of phosphocholine detergents (Fig. 1a), it is interesting to note that the poorest detergents in this assay (ET-18-H, ET-16-H) are the hemolytically most active substances of the series (19, 29).

SDS-polyacrylamide gel electrophoresis (data not shown) of 100,000 × g supernatants and pellets after solubilization with ET-12-H and ET-16-H showed that all membrane proteins can be extracted by this procedure. No principal differences between the action of the long and short chain derivative have been observed.

**DISCUSSION**

Enzyme Assays

**Alkaline Phosphatase (EC 3.1.3.1)**—The activity of the alkaline phosphatase was followed with $\beta$-nitrophosphorylphosphate as substrate. The reaction mixture contained in a total volume of 1.0 ml, 100 µmol of diethanolamine, pH 9.5, 1.0 µmol of MgCl₂, 5 mg of Triton X-100, 2.5 µmol of $\beta$-nitrophosphorylphosphate, and 3 to 10 µg of membrane protein. After 10 min incubation at 37°C, the reaction was stopped by addition of 0.2 ml of 5 M NaOH. Liberated nitrophenol in supernatants was measured at 405 nm and calculated using a molar extinction coefficient of 17,840 liters·mol⁻¹·cm⁻¹. We determined a Michaelis constant for the alkaline phosphatase in plasma membranes from calf thymus to be $K_m = 75 \mu M$.

**Nucleotide Pyrophosphatase (Phosphodiesterase I) (EC 3.1.4.1)**—The determination of the activity of the nucleotide pyrophosphatase was carried out under conditions identical with those described for the assay of alkaline phosphatase, with the exception that $\beta$-nitrophosphoryl $5^\prime$ thymidylic acid (3.0 mM) was used as substrate. We determined a Michaelis constant for the nucleotide pyrophosphatase in plasma membranes from calf thymocytes to be $K_m = 1.2 \mu M$.

**Extraction of Membrane Structures**

Membranes from calf thymocytes to be $K_m = 1.2 mM$. $\gamma$-Glutamyltransferase (EC 2.3.2.2) was assayed using a modification of the technique of Crane and Lipmann (15) using $[\gamma-^{32}P]ATP$ and adsorption of nucleotides on activated charcoal. The reaction mixture contained in a total volume of 1.0 ml: 100 µmol of imidazole buffer, pH 7.4, 5 µmol of MgCl₂, 30 µmol of KCl, 150 µmol of NaCl, 600 µmol of ATP containing 0.4 µCi of $[\gamma-^{32}P]ATP$, and 10 to 40 µg of membrane protein. After an incubation time of 10 to 30 min at 37°C, 2 µl of bovine serum albumin was added to facilitate membrane protein precipitation, and the reaction was stopped by addition of 4 ml of 5% (w/v) ice cold trichloroacetic acid. The extract was centrifuged, and 3 ml of the supernatant were adsorbed on approximately 2 g of trichloroacetic acid-washed activated charcoal (Norton & Serva, Heidelberg). After centrifugation, 1 ml of the extract was mixed with 10 µl of Aquasol (New England Nuclear) and counted in a Packard TriCarb scintillation counter.

Acyl-CoA:1-Acylglycerol-3-phosphocholine O-acyltransferase (EC 2.3.1.23)—The activity of the acyltransferase was determined by reaction of arachidonyl-CoA with ¹⁴C-labeled lysolecithin (1-[¹⁴C]palmitoyl-sn-glycerol-3-phosphocholine) as described earlier (7).

**Polyacrylamide Gel Electrophoresis**

SDS-polyacrylamide gel electrophoresis (data not shown) of 100,000 × g supernatants and pellets after solubilization with ET-12-H and ET-16-H showed that all membrane proteins can be extracted by this procedure. No principal differences between the action of the long and short chain derivative have been observed.

**Inhibition of Acyl-CoA:Lysolecithin Acyltransferase by Lysolecithin Analogs**—To test the proposed “mildness” of phosphorylcholine detergents for sensitive membrane components, we planned to test acyl-CoA:lysolecithin acyltransferase from the plasma membrane of calf thymocytes. Since all phosphatides used here were substrate analogs for the acyltransferase, it was reasonable to assume that the analogs might be inhibitors of the enzyme. Fig. 2 shows for the series of alkyl-deoxy lysophosphatides (ET-n-H) that this is, in fact, the case. The degree of inhibition increased with increasing chain length up to an optimal length of 16 carbon atoms. ET-18-H exhibited a significantly lower inhibition than ET-16-H. To investigate the mechanism of this inhibition, we used the most active compound ET-16-H. Fig. 2 shows the Lineweaver-Burk plot (21) of a kinetic experiment using palmitoyl lysolecithin as substrate and ET-16-H as inhibitor. The results from these studies are consistent with a competitive mechanism. The dissociation constants determined were $K_m = 65 \mu M$ for palmitoyl lysolecithin and $K_i = 100.5 \mu M$ for ET-16-H. In another series of experiments, we determined the inhibitor constants from Dixon plots (22) using increasing concentrations of the inhibitor. These studies also revealed that ET-16-H exhibited the highest and ET-10-H...
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TABLE I

Solubilization of human erythrocyte membranes

| Systematic name | Abbreviation* | 50% turbidity decrease* | 50% protein solubilization' |
|-----------------|---------------|------------------------|-----------------------------|
| 1-Decyl propanediol-3-phosphorylcholine | ET-10-H | 4.0                        | 5.0                          |
| 1-Dodecyl propanediol-3-phosphorylcholine | ET-12-H | 0.5                        | 0.8                          |
| 1-Tetradecyl propanediol-3-phosphorylcholine | ET-14-H | 0.5                        | 1.0                          |
| 1-Hexadecyl propanediol-3-phosphorylcholine | ET-16-H | 1.0                        | 3.0                          |
| 1-Octadecyl propanediol-3-phosphorylcholine | ET-18-H | —                          | >5.0                         |
| 1-Lauroyl propanediol-3-phosphorylcholine | ES-12-H | 0.9                        | n.d.                         |
| 1-Stearyl propanediol-3-phosphorylcholine | ES-18:0-H | —                          | n.d.                         |
| 1-Oleoyl propanediol-3-phosphorylcholine | ES-18:1-H (cis) | 1.0                        | n.d.                         |
| 1-Elaidyl propanediol-3-phosphorylcholine | ES-18:1-H (trans) | 0.8                        | n.d.                         |
| 1-Linoleyl propanediol-3-phosphorylcholine | ES-18:2-H | 0.5                        | n.d.                         |
| rac-1-Octadecyl glycerol-3-phosphorylcholine | ET-18-OH | —                          | n.d.                         |
| rac-1-Octadecyl 2-methylglycerol-3-phosphorylcholine | ET-18-OCH3 | 2.0                        | n.d.                         |
| rac-1-Octadecyl 2-benzylglycerol-3-phosphorylcholine | ET-18-OC6H5 | —                          | n.d.                         |
| rac-1-Dodecyl glycerol-3-phosphorylcholine | ET-12-OH | 0.8                        | n.d.                         |
| rac-1-Dodecyl 2-methylglycerol-3-phosphorylcholine | ET-12-OCH3 | 0.8                        | n.d.                         |
| rac-1-Dodecyl 2-benzylglycerol-3-phosphorylcholine | ET-12-OC6H5 | 0.25                     | n.d.                         |
| Dodecyl phosphorylcholine | Alk-12 | 1.6                        | 1.5                          |
| Tetradecyl phosphorylcholine | Alk-14 | 0.65                        | 1.0                          |
| Octadecyl phosphorylcholine | Alk-18 | —                          | 5.0                          |
| Polyethylene glycol p-isooctylphenyl ether | Triton X-100 | 0.8                        | n.d.                         |
| Sodium dodecyl sulfate | SDS | 1.6                        | n.d.                         |

* Abbreviations relate to chemical structure (17). The symbols ES or ET indicate whether aliphatic chains are linked via ether (ET) or ester (ES) linkages. Figures indicate the number of carbons per aliphatic chain, if necessary, with an indication of the number of double bonds in the chain. The following symbols represent the chemical structure of the substitution for the sn-2-hydroxyl group in glycerol (the symbol H thus refers to 2-deoxy-glycerol- or propanediol-1,3 derivatives).

Fig. 1. Concentration dependence of solubilization of human erythrocyte membranes. a, turbidity determinations at 514 nm; b, protein determination in 100,000 x g supernatants. For details, see footnotes to Table I.

the lowest inhibition of the acyl-CoA:lysolecithin acyltransferase (Table II).

However, it should be kept in mind that the system used is not a homogeneous one since most of the added substrates and detergents is adsorbed to the membrane (20). Therefore, the capacity of the detergent to inhibit the enzyme and to solubilize membrane proteins depends within the first approximation not on the concentration but on the ratio of detergent to the membrane mass. This will be demonstrated also in the series of experiments using ET-12-H as detergent to solubilize thymocyte plasma membranes.

Solubilization of Membrane-bound Enzymes—In previous experiments not shown here, we found that treatment of membranes even with nondenaturing detergents leads to a complete loss of lysolecithin acyltransferase activity. In these experiments, the following detergents were tested in concen-
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only this compound in concentrations ranging from 0.5 to 2.5 μmol/mg of membrane protein (corresponding to 0.2 to 1.0 mg of ET-12-H/mg of membrane protein).

Table III shows that ET-12-H solubilizes the acyltransferase without loss of activity, which is documented by a recovery of 90 to 100%. The activities of the alkaline phosphatase, nucleotide pyrophosphatase, and the γ-glutamyltransferase were also not impaired whereas, surprisingly, the Mg²⁺-ATPase was inhibited to an extent of 50 to 70%.

Washing of plasma membranes with buffer (Hepes, 10 mM, pH 7.4) removed appreciable amounts of protein (16%). However, these proteins did not contain any activity of the membrane-bound enzymes tested.

It is of particular interest that ET-12-H extracts the acyltransferase with preference since optimal concentrations of
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**Table III**

| Protein | Specific activity | Total activity | Specific activity | Total activity | Specific activity |
|---------|------------------|---------------|------------------|---------------|------------------|
| Alkaline phosphatase | 8651 | 114372.2 | 84 | 81600 | 107 |
| Nucleotide phosphatase | 3061 | 139421.6 | 5 | 30400 | 107 |
| Lysolecithin acyltransferase | 91.4 | 91 | 90.3 | 90.3 | 90.3 |
| Lysolecithin | 91.4 | 91 | 90.3 | 90.3 | 90.3 |

**DISCUSSION**

Lysophosphatidylcholine has occasionally been used as a detergent, and particularly with mitochondrial membranes, a certain selectivity in the solubilization of membrane components has been reported (23-28). These studies have led to the notion that lysolecithin may combine the solubilizing properties of detergents with enzyme stabilization or even activation properties of membrane lipids (25, 29). We have taken advantage of the availability of the great variety of physicochemically characterized synthetic derivatives of lysophosphatidylcholine (6, 19, 20) and have screened these compounds for their membrane solubilizing properties.

As has been demonstrated in this study, a fair number of these analogs compare favorably with established detergents such as Triton X-100 or SDS. In general, our data reveal that lysolipids with aliphatic chains of 12 to 14 carbons or unsaturated long chain compounds are the most potent solubilizers (Table I, Fig. 1). This is in remarkable contrast to the cytolytic activities of these substances. In this respect, the saturated C16/C18 lysophosphatides are by far the most active ones (20).

As mentioned above, the qualities of a detergent are, however, not simply determined by its solubilization power but more so by its ability to protect natural protein conformation in micellar solutions, i.e. to leave enzymatic activities unimpaired. Only this property will enable the investigator to use it for purification and characterization of membrane-bound enzymes. With regard to lysophosphatides, we have demonstrated that, at least for the case of acyl-CoA:lysolecithin acyltransferase, these surfactants possess distinct advantages over all other detergents tested.

Only a few successful solubilizations of long chain acyltransferases have been reported in the literature (30-32). In all cases, however, the enzyme (glycerophosphate acyltransferase of various sources) was inevitably inactivated by the detergents (Triton X-100 or cholate) and could only be determined upon reactivation by a large excess of membrane lipids. Moreover, all reports consistently show that these preparations exhibit no activity for the acylation of monoacyl-sn-glycero-3-phosphate. The latter compound was the only product during the acylation of glycerophosphate. This failure can be explained either by an irreversible inactivation of the lysophosphatidate acyltransferase by the detergent treatment or by the fact that the lipid-soluble acceptor molecule (lysophosphatidate) becomes inaccessible because it is partitioned into the excess of liposomal lipid. Thus, to our knowledge, the extraction of membranes with analogs of lysolecithin is the first procedure which leads to an active and soluble lysophosphatidate acyltransferase.

We have defined effective membrane solubilization as re-
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leaching membrane components not sedimented at 177,000 x g, for 60 min. Moreover, we tested extracts from thymocyte plasma membranes by chromatography on Sepharose 6B in ET-12-H containing buffer. These experiments revealed that the acyl-CoA:lysolecithin acyltransferase activity was included into the gel and eluted significantly later than the void volume.

Our results further showed that the relatively low lysophosphatidate concentrations used did not impair biochemical analysis and, hence, did not have to be removed. An additional advantage of using short chain deoxy-alkyl analogs of lysolecithin comes from the fact that they do not interfere with the natural substrate of acyl-CoA:lysolecithin acyltransferase and that they may be separated by thin layer chromatography from natural lysolecithin. Moreover, these detergents apparently exhibit a selectivity for the extraction of the acyltransferase, leading, at low detergent concentrations, to about a 2-fold enrichment in the extracts as compared to the original membrane material. This selectivity seems to be rather restricted, since an electrophoretic comparison of extracts and pellets (Fig. 2) revealed only few differences. Three bands in the SDS-polyacrylamide gel electrophoresis, however, appear to be enriched in the extracts: two of 80,000 to 90,000 daltons and one of 50,000 to 55,000 daltons.

The results should not be discussed without recalling the rather special conditions of our system. The detergent is a substrate analog of the enzyme to be isolated and may, at higher concentrations, act as a competitive inhibitor. The inhibitor properties of the various analogs are apparently linked directly to the membrane affinities of these substances (20). Like those, inhibition is most pronounced for the C16 derivative and decreases with shorter as well as with longer aliphatic residues (Fig. 2, Table II). The discrepancy with a report of Wittels and Hulbert (33) who found that ES-18:0-H did not inhibit acyl-CoA:lysolecithin acyltransferase may be resolved by the notion that these authors (a) used a different enzyme source, (b) employed an acyl- instead of an alkyl-deoxy lysolipid, and (c) used maximally 40 μM concentrations, whereas we found 50% inhibition for approximately 200 μM concentration (Fig. 2).

The effective solubilization of thymocyte plasma membranes by ET-12-H contrasts with the findings of Peterson and Deamer (34) who observed that sarcoplasmic reticulum membranes only can be solubilized by lysolecithins of a chain length of 16 to 18 carbon atoms but not by those carrying C16 chains. Thus, this difference can only be explained by the fact that we used a lysolecithin analog with an ether linkage (position 1) and a deoxy group (position 2) or, more likely, one has to assume that the thymocyte plasma membrane is more susceptible to solubilization by these detergents than sarcoplasmic reticulum membranes. Solubilization of membrane-bound enzymes by natural lysolecithins obviously has some advantages. Rydstrom et al. (35) reported that mitochondrial nicotinamide nucleotide transhydrogenase together with succinate dehydrogenase could be preferentially solubilized with lysolecithin, thus leading to preparations of higher specific activities of these enzymes compared with Triton X-100 extracts. In contrast to Triton X-100, even an excess of lysolecithin did not inhibit the transhydrogenase (35). A similar property of this detergent was observed in studies on the Ca2+-ATPase of sarcoplasmic reticulum. Peterson and Deamer (34) found that low concentrations of lysolecithin inhibited this enzyme up to 70% but it was possible to reactivate the ATPase by 3 to 6 times higher concentrations of lysolecithin. However, in these studies, the ATPase was not solubilized but remained enriched in the membranous pellet (25).

The surprising selectivity in the extraction of acyltransferase with ET-12-H might possibly be related to the aforementioned enzyme/inhibitor relation. In fact, this correlation may be encouraging for further attempts to use "custom-made" detergents for special solubilization problems. The use of other lyso compounds of appropriate hydrophobicity such as analogs of lysophosphatidylethanolamine or lysophosphatidylserine, to mention only some possibilities, may be rewarding for solubilization studies of certain lipid-requiring enzymes or surface antigens.

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REFERENCES
1. Tanford, C., and Reynolds, J. A. (1976) Biochim. Biophys. Acta 457, 133-170
2. Arnold, D., Waltzien, H. U., and Westphal, O. (1967) Justus Liebigs Ann. Chem. 709, 234-239
3. Waltzien, H. U., and Westphal, O. (1967) Justus Liebigs Ann. Chem. 709, 244-247
4. Ehl, H., and Westphal, O. (1967) Justus Liebigs Ann. Chem. 709, 234-247
5. Weltzien, H. U., Arnold, B., and Westphal, O. (1973) Justus Liebigs Ann. Chem. 773, 1439-1444
6. Arnold, D., and Weltzien, H. U. (1968) Z. Naturforsch. Teil B Anorg. Chem. Org. Chem. Biochem. Biophys. Biol. 23, 675-683
7. Ferber, E., and Resch, K. (1973) Biochim. Biophys. Acta 296, 335-349
8. Reits, R. C., Lands, W. E. M., Christie, W. W., and Holman, R. T. (1968) J. Biol. Chem. 243, 2241-2249
9. Okuyama, H., Lands, W. E. M., Christie, W. W., and Gunstone, F. D. (1969) J. Biol. Chem. 244, 6514-6519
10. Lands, W. E. M., and Hart, P. (1969) J. Biol. Chem. 240, 1905-1911
11. Dodge, I. T., Mitchell, C., and Hanahan, D. I. (1963) Arch. Biochem. Biophys. 100, 119-130
12. Ferber, E., Resch, K., Wallach, D. F. H., and Imm, W. (1972) Biochim. Biophys. Acta 266, 494-504
13. Brunner, G., Heidrich, H.-G., Golecki, J. R., Bauer, H. C., Suter, D., Plückhahn, P., and Ferber, E. (1977) Biochim. Biophys. Acta 471, 199-212
14. Novogrodsky, A., Tate, S. S., and Meister, A. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 2414-2418
15. Eibl, H., and Westphal, O. (1967) Justus Liebigs Ann. Chem. 709, 240-243
16. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971) Biochemistry 10, 2606-2617
17. Weltzien, H. U. (1975) Z. Naturforsch. Teil C Biochem. Biophys. Biol. Viro 30, 785-792
18. Moore, S., and Stein, W. S. (1948) J. Biol. Chem. 176, 367-388
19. Weltzien, H. U., Arnold, B., and Kalkoff, H. G. (1976) Biochim. Biophys. Acta 455, 56-65
20. Weltzien, H. U., Arnold, B., and Reuther, R. (1977) Biochim. Biophys. Acta 466, 411-421
21. Lineweaver, H., and Burk, D. (1934) J. Am. Chem. Soc. 56, 686-666
22. Dixon, M. (1953) Biochem. J. 55, 170-171
23. Gent, W. L. G., Gregson, N. A., Gammack, D. B., and Raper, J. H. (1969) J. Biol. Chem. 244, 6514-6519
24. Rydstrom, J. (1970) Biochim. Biophys. Acta 455, 24-35
25. Deamer, D. W. (1973) J. Biol. Chem. 248, 5477-5485
26. Sadler, M. H., Hunter, D. R., and Haworth, R. A. (1974) Biochem. Biophys. Res. Commun. 59, 804-812
27. Serrano, R., Kanner, B. L., and Racker, E. (1976) J. Biol. Chem. 251, 2453-2461
28. Komai, H., Hunter, D. R., and Takahashi, Y. (1973) Biochem. Biophys. Res. Commun. 53, 82-89
29. Rydstrom, J. (1975) Fed. Proc. 34, 577.
30. Snider, M. D., and Kennedy, E. P. (1977) J. Bacteriol. 130, 1072-1083
31. Yamashita, S., and Numa, S. (1972) Eur. J. Biochem. 31, 565-573
32. Monroy, G., Chroboczek Kellner, H., and Pullman, M. E. (1973) J. Biol. Chem. 248, 2845-2852
33. Weltzien, H. U., and Hubert, S. (1977) Biochim. Biophys. Acta 469, 67-78
34. Peterson, S. W., and Deamer, D. W. (1977) Arch. Biochem. Biophys. 189, 218-229
35. Rydstrom, J., Hoek, J. B., and Hundal, T. (1974) Biochem. Biophys. Res. Commun. 60, 448-455
Detergent properties of water-soluble choline phosphatides. Selective solubilization of acyl-CoA:lyssolecithin acyltransferase from thymocyte plasma membranes.

H U Weltzien, G Richter and E Ferber

*J. Biol. Chem.* 1979, 254:3652-3657.

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