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SOLUBILIZATION OF YEAST PLASMA MEMBRANES AND MITOCHONDRIA BY DIFFERENT TYPES OF NON-DENATURING DETERGENTS

ROSA NAVARRETE and RAMÓN SERRANO *

Instituto de Enzimología y Patología Molecular del C.S.I.C., Facultad de Medicina de la Universidad Autónoma, Calle Arzobispo Morcillo 4, Madrid 34 (Spain)

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A comparative study of the solubilization of yeast plasma membranes and mitochondria by different types of non-denaturing detergents has been performed. Zwittergent-14 (3-[tetradecyldimethylammonio]-1-propanesulfonate) at low concentrations (3–4 mM) produced maximum solubilization of both membranes. However, this detergent may inactivate enzymes at high concentrations. Taurodeoxycholate (in the presence of salt) and Triton X-100 were also effective in mitochondria but not in the plasma membranes. Octylglucoside only solubilized these membranes at very high concentrations (20 mM). CHAPS (3-[cholamidopropyldimethylammonio]-1-propanesulfonate) only achieved partial solubilization even at high concentrations. Our results suggest that Zwittergent-14 at low concentrations is one of the most powerful detergents for the general solubilization of native membrane proteins.

Introduction

The study of intrinsic membrane proteins requires their previous solubilization with detergents [1]. Sodium dodecyl sulfate is the standard detergent used to analyze, isolate and characterize the individual polypeptide chains of membrane proteins in their denatured state [2]. However, milder detergents are required for the solubilization of membrane proteins with preservation of their native structure and biological activity (catalysis, transport, binding). In the past the non-ionic detergent Triton X-100 and the bile salts have been extensively used for this purpose [1–3]. More recently new types of mild detergents have been introduced in membrane biochemistry [2]. The non-ionic detergent octylglucoside contains a glucosyl group as a novel type of polar head [4]. Dodecyl octaethyleneglycol monoether ($C_{12}E_8$) mainly differs from Triton X-100 in having a linear hydrophobic chain [5]. The Zwittergents constitute an homologous series of zwitterionic detergents with a sulfobetaine polar head [6]. Finally, the detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) was designed to combine the polar head of zwittergents with the hydrophobic part of bile salts [7].

Very few studies have been performed comparing these novel detergents with those previously employed in membrane solubilization [4,6,8,9]. In addition all previous studies utilized a single type of biological membrane and one or two types of

* To whom correspondence should be addressed.

Abbreviations: Octylglucoside, *n*-octyl-β-D-glucopyranoside; Triton X-100, *p-tert*-octylphenyl nona(deca)oxyethylene ether; C_nE_x, alkyl polyoxyethylene ether, where the alkyl chain contains *n* carbon atoms and *x* is the number of oxyethylene groups; Zwittergent-*n*, 3-(alkyldimethylammonio)-1-propanesulfonate, where *n* is the number of carbon atoms in the alkyl chain; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate.

non-denaturing detergent (non-ionic, bile salts and zwitterionic). In the present work we have compared the effectivity of these three types of detergent on the solubilization of plasma membranes and mitochondria from yeast. These studies were prompted by the failure of all the usual detergents to solubilize the yeast plasma membrane ATPase [10]. Our results support the view [6,10] that Zwittergent-14 may be one of the most powerful detergents for the general solubilization of native membrane proteins.

Methods

Chemicals. The Zwittergents -8, -10, -12, -14 and -16 were donated by A. Seeley (Calbiochem AG, Luzerne, Switzerland) and are now available from Calbiochem. CHAPS was a gift of Dr. L.M. Hjelmeland (National Institutes of Health, Bethesda, MD, U.S.A.) and is now available from Polysciences. Lysophosphatidylcholine (type I, from egg yolk), octylglucoside, dodecyl nonaethyleneglycol monoether ($C_{12}E_9$, 9 lauryl ether), sodium taurodeoxycholate, ATP (grade I), cytochrome *c* (type III) and crude soybean phospholipids (phosphatidylcholine type II-S) were obtained from Sigma. Triton X-100 was from Packard. The phospholipids were washed with acetone [11], suspended at 2% in water and sonicated to clarity in a bath sonicator (Laboratory Supplies Co., Hicksville, NY, U.S.A.) operated under nitrogen. Cytochrome *c* was reduced as described previously [12].

Membrane preparations. The plasma membranes and mitochondria of baker's yeast (*Saccharomyces cerevisiae*) were obtained by slight modifications of described methods [13]. Briefly, the yeast cells were washed with water and broken by vibration with glass beads (Vibrogen Cell Mill, E. Bühler, Tubingen, F.R.G.) in a medium containing 0.15 M Tris-HCl (pH 8.5), 10 mM EDTA and 5 mM mercaptoethanol. After centrifugation of debris at $100 \times g$ for 10 min, the supernatant was centrifuged for 45 min at $20000 \times g$. The pellet was homogenized in glycerol buffer and applied to a discontinuous sucrose gradient. The glycerol buffer contained: 20% glycerol (v/v), 10 mM *N*-tris(hydroxymethyl)methylglycine, 1 mM EDTA and 1 mM dithioerythritol (pH 7.5). After overnight centrifugation at $50000 \times g$ the mitochondria corresponded to the 30/46% sucrose (w/w) interface and the plasma membranes to the 46/57% sucrose (w/w) interface. The bands were collected, diluted with three volumes of water and centrifuged for 30 min at $70000 \times g$. The pellets were homogenized in glycerol buffer and stored at −70°C.

Membrane solubilization. Plasma membranes and mitochondria were diluted in glycerol buffer to 1.1–1.3 mg protein/ml in aliquots of 2.5 ml. After 5 min at room temperature (17–19°C), 0.5 ml of detergent solution in water were added to obtain the final concentrations indicated in the figures. After 10 min at room temperature the turbidity of the suspensions was estimated by their absorbance at 650 nm (Spectronic 20, Baush& Lomb). The incubation mixtures were centrifuged at 10°C during 30 min at $100000 \times g$ (40000 rev./min in a Beckman 50 rotor). The pellets were homogenized in 3 ml of glycerol buffer.

ATPase assay. The plasma membrane ATPase was assayed in 1 ml of medium containing: 50 mM 2-(*N*-morpholino)ethanesulfonate (Mes) (pH 5.7 with Tris), 10 mM $MgSO_4$, 0.2 mM ammonium molybdate (to inhibit acid phosphatase), 5 mM sodium azide (to inhibit mitochondrial ATPase) and 0.4 mg/ml phospholipids. The volume of samples was 50 μl. The assay medium for the mitochondrial ATPase contained: 50 mM Tris (pH 8.7 with HCl), 2 mM $MgSO_4$, 0.1 mM sodium orthovanadate (to inhibit plasma membrane ATPase) and 0.1 mg/ml phospholipids. The volume of samples was 5 μl. In both cases the reaction was started with 2 mM ATP and the P_i liberated after 10 min at 30°C determined as described [13]. One unit of activity corresponded to 1 μmol P_i/min.

Cytochrome oxidase assay. The assay medium (0.5 ml) contained: 50 mM potassium phosphate (pH 7.0), 25 μM reduced cytochrome *c* and 0.4 mg/ml phospholipids. Samples of 5–25 μl were added and the decrease in absorbance at 550 nm determined in a Zeiss PM 6 spectrophotometer operated at 30°C. One unit of activity corresponded to 1 μmol cytochrome *c*/min.

Protein determination. The measurement of protein concentration in the presence of detergents was performed by a modification of the Lowry procedure which utilizes sodium dodecyl sulfate to

avoid the precipitation of the other detergents [14]. The standards were made with bovine serum albumin and to correct for the interferences of glycerol and dithioerythrytol they received the same amount of glycerol buffer contributed by the samples.

Phospholipid determination. Aliquots of 0.5 ml of the pellets obtained after solubilization were extracted by the method of Bligh and Dyer [15] and the washed chloroform phase was dried and ashed as described [16]. Phosphate determination was as described in Ref. 13.

Results

The solubilization by detergents of yeast plasma membranes and mitochondria was studied by four different criteria: decrease in turbidity of the suspensions and solubilization of protein, phospholipid and specific enzymes, like the plasma membrane ATPase and the mitochondrial ATPase and cytochrome oxidase. The relevant properties of the detergents used are presented in Table I.

The effect of different sulfobetaine detergents on the plasma membrane is shown in Fig. 1. The most effective detergents of this group were Zwittergent-16 and Zwittergent-14, which at concentrations of 3–5 mM produced a 90% decrease in turbidity (Fig. 1A) and solubilized 75% of the protein (Fig. 1B) and 85% of the phospholipid (Fig. 1C). Zwittergent-12 was slightly less effective and Zwittergent-10 only solubilized at high concentrations (10–20 mM). Zwittergent-8 was completely ineffective by all the studied criteria. CHAPS did not solubilize any significant amount of protein and its effects on turbidity and phospholipid solubilization were partial even at concentrations greater than 10 mM.

With the exception of Zwittergent-8 and Zwittergent-10, all these detergents activated the plasma membrane ATPase up to 3-fold (Fig. 1D). A likely explanation is that the detergents destroy a permeability barrier between the enzyme and its

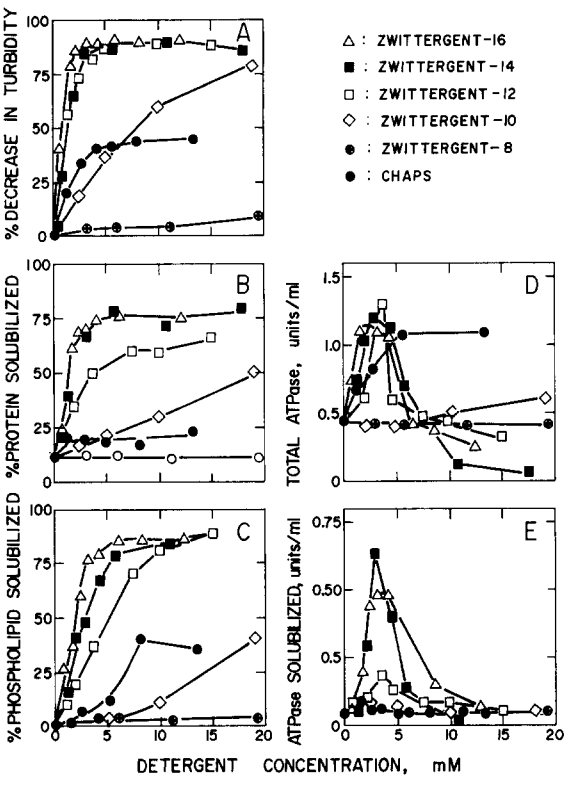

Fig. 1. Effect of sulfobetaine detergents on the yeast plasma membrane. Turbidity (A) and solubilization of protein (B) and phospholipid (C) are presented as percent of the values of the respective parameters in the suspension of membranes. These absolute values were: turbidity, 0.80 units of absorbance; protein, 0.90 mg/ml; phospholipid, 0.38 μmol/ml. The total ATPase activity (pellets plus supernatant, D) and the ATPase solubilized (E) are also indicated.

TABLE I

PROPERTIES OF THE DETERGENTS USED IN THE PRESENT WORK

Data on the Zwittergents are taken from Ref. 6 and on CHAPS from Ref. 7. Other data are taken from Ref. 2. Triton X-100 and lysophosphatidylcholine are mixtures of related compounds and average values of their properties are shown. CMC, critical micelle concentration.

Detergent	Mol. wt.	CMC (mM)
Zwittergent-8	279.6	390
Zwittergent-10	307.6	39
Zwittergent-12	335.6	3.6
Zwittergent-14	363.6	0.3
Zwittergent-16	391.6	0.03
CHAPS	614.9	4–6
Lysophosphatidylcholine	510	0.02
Triton X-100	626	0.3
$C_{12}E_9$	582	0.1
Octylglucoside	292.2	25
Taurodeoxycholate (sodium salt)	521.7	1.7–3

substrate. Accordingly, the plasma membranes contained an space inaccessible to ATP which is greatly reduced by the detergents (Navarrete, R. and Serrano, R., unpublished data). However, the possibility of a conformational change induced by the detergents can not be discarded. CHAPS did not inactivate the ATPase even at high concentrations but the Zwittergents become inhibitory at concentrations greater than 3–5 mM. Concerning this inactivation it must be recalled that the solubilization was performed in the presence of glycerol, EDTA and erythritol to protect the enzyme [10] and that the assay included 0.5 mM phospholipid to counteract the reversible inactivation produced by the detergents [1,10]. Accordingly, the ATPase activity of the pellets resulting from solubilizations at high detergent concentration was stimulated up to 10-fold by the phospholipids. The inactivation observed at high detergent concentration may reflect either denaturation of the enzyme or reversible inactivation which would require higher concentrations of phospholipid to be reverted. In any case, only Zwittergent-14 and Zwittergent-16 solubilized the ATPase, although the maximum solubilization achieved was only 40–50% (Figs. 1D and 1E).

The effect of other types of detergents on the plasma membrane is shown in Fig. 2. Lysophosphatidylcholine was the most effective and at a concentration of 5 mM resulted in 75% decrease of turbidity and 75% solubilization of protein. Phospholipid solubilization was not measured with this detergent because it contained phosphorus and therefore interferes with our analytical method.

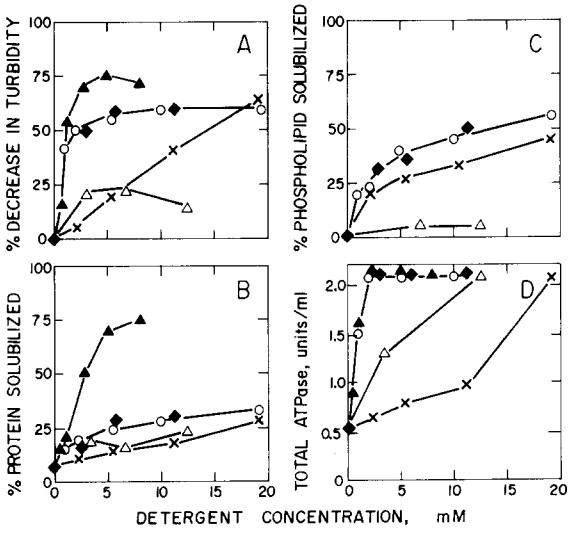

Fig. 2. Effect of non-ionic detergents and bile salts on the yeast plasma membrane. Triton X-100 (○), lysophosphatidylcholine (▲), $C_{12}E_9$ (◆), octylglucoside (×) and taurodeoxycholate (△) corresponded to the indicated symbols. Turbidity (A) and solubilization of protein (B) and phospholipid (C) are presented as percent of the values of the respective parameters in the suspension of membranes. These absolute values were: turbidity, 0.90 units of absorbance; protein, 1.0 mg/ml; phospholipid, 0.44 μmol/ml. The total ATPase activity (pellets plus supernatants, D) is also indicated.

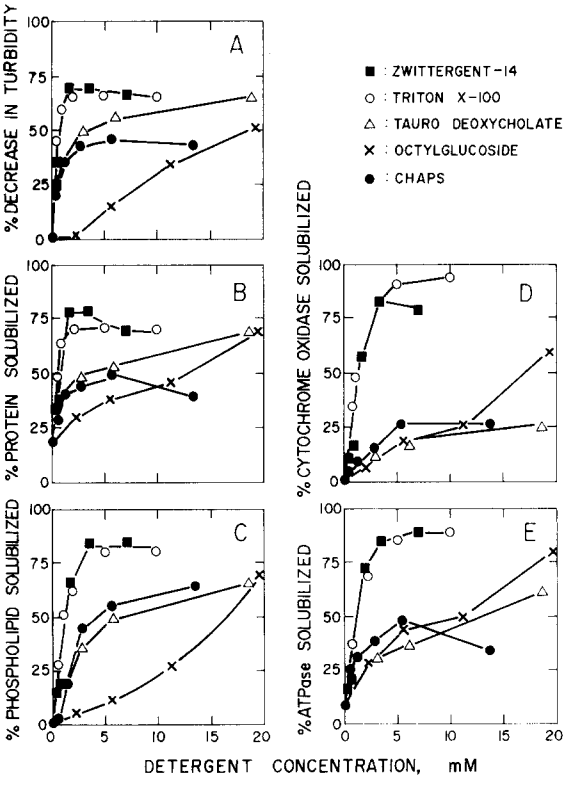

Fig. 3. Effect of different detergents on yeast mitochondria. Turbidity (A) and solubilization of protein (B), phospholipid (C), Cytochrome oxidase (D) and ATPase (E) are presented as percent of the values of the respective parameters in the mitochondrial suspension. Thes eabsolute values were: turbidity, 0.85 units of absorbance; protein, 1.1 mg/ml; phospholipid, 0.29 μmol/ml; cytochrome oxidase 1.2 units/ml; ATPase, 7.6 units/ml. The cytochrome oxidase was slightly activated by the detergents (up to 1.9 units/ml). In addition, the Zwittergent-14 at concentrations greater than 3 mM slightly inactivated the enzyme. These effects were corrected in the calculation of the percent solubilization.

Triton X-100 and $C_{12}E_9$ produced a 60% decrease in turbidity at low concentrations (Fig. 2A) but they solubilized very little protein (Fig. 2B) and required high concentrations for effective phospholipid solubilization (Fig. 2C). Taurodeoxycholate was inefficient by all criteria and octylglucoside required high concentrations to solubilize phospholipid and to reduce the turbidity. However, as occurred with Triton X-100 and $C_{12}E_9$, octyl glucoside solubilized very little protein. All these detergents produced a 3.5–4.0-fold activation of the plasma membrane ATPase (Fig. 2D) but only octylglucoside at 20 mM produced some solubilization (20%, not shown).

These results show a good qualitative agreement between turbidity decrease and phospholipid solubilization. The solubilization of total protein or of the ATPase were not related to the global solubilization of the plasma membrane indicated by the previous parameters.

Similar experiments conducted in mitochondria with a selected group of detergents are depicted in Fig. 3. Here Triton X-100 was as effective as Zwittergent-14 by all the studied criteria. The effect of CHAPS was again partial even at high concentrations and taurodeoxycholate and octylglucoside required very high concentrations to be effective. The solubilizing power of taurodeoxycholate was greatly improved by 0.4 M ammonium sulfate, becoming almost as effective as Triton X-100 (results not shown).

Discussion

An important aspect of the present work is that the three types of non-denaturing detergents (non-ionic, bile salts and zwitterionic) are compared in two different biological membranes: the plasma membranes and mitochondria of yeast cells.

Previous studies with non-ionic detergents have demonstrated that those with hydrophile-lipophile balance around 13 are the most effective [17–20]. Accordingly, we selected Triton X-100 and $C_{12}E_9$ as representatives of this group and no differences were observed between them in the solubilization of the plasma membranes. Octylglucoside corresponds to a novel type of non-ionic detergent. In agreement with earlier work [4] it required very high concentrations for effective solubilization. As detergent micelles are usually required for membrane solubilization [1], this poor performance can be explained by the high critical micelle concentration of octylglucoside (Table I). Accordingly, solubilization by this detergent is improving as the critical micelle concentration (25 mM) is approached (Figs. 2 and 3). Taurodeoxycholate has been advocated as the most useful of the bile salts [2] and therefore it was selected as representative of this group for the present experiments. In the absence of salts it was a very poor detergent. In erythrocyte membranes deoxycholate is also much less effective than Triton X-100 [21]. However, in mitochondria, taurodeoxycholate becomes very effective at high ionic strength. This enhancement of solubilizing power by salts is a general property of bile salts [3], although in the case of plasma membranes this effect was much less pronounced than in mitochondria.

The zwitterionic detergents studied in the present work included the natural compound lysolecithin and the synthetic sulfobetaine detergents of the Zwittergent series [6] and CHAPS [7]. Our results suggest that Zwittergent-14 may be the most powerful detergent for the general solubilization of native membrane proteins. At concentrations of 3–4 mM produced maximum solubilization of plasma membranes and mitochondria without inactivating the assayed enzymes. Together with Zwittergent-16 were the only detergents which solubilized the yeast plasma membrane ATPase. Lysophosphatidylcholine was very effective in the general solubilization of the plasma membrane but it failed to solubilize the ATPase. This detergent and also Zwittergent-16 have low solubility in the cold and therefore are less convenient that Zwittergent-14. Lower members of this series (Zwittergent-10 and Zwittergent-8) are very poor detergents. As discussed for octylglucoside, this can be explained by the high critical micelle concentration of these detergents (Table I). Recently, Zwittergent-14 has been successfully utilized in the solubilization of the yeast [10,22] and plant [23] plasma membrane ATPases which could not be solubilized by conventional detergents. Although CHAPS has been reported to be effective in the solubilization of rat liver [7] and bovine brain [8] microsomes, in our hands it was a very poor detergent. Apparently, a linear hydro-

phobic chain like in Zwittergent-14 is required for maximum solubilizing power. However, as previously discussed [7–9], this linear chain is responsible for the denaturing properties of high concentrations of Zwittergent-14. This detergent must be used with caution because at high concentrations may inactivate enzymes, as we have observed with the plasma membrane ATPase and, to a minor extent, with the mitochondrial cytochrome oxidase.

A remarkable difference between plasma membranes and mitochondria is their susceptibility to solubilization by Triton X-100. In mitochondria this classical detergent is as effective as Zwittergent-14 but in plasma membranes it produces very little solubilization of protein. This result strength the importance of performing comparative studies of detergents in more than one type of biological membrane.

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References

1 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79
2 Helenius, A., McCaslin, D.R., Fries, E. and Tanford, C. (1979) Methods Enzymol. 56, 734–749
3 Tzagoloff, A. and Penefsky, H.S. (1971) Methods Enzymol. 22, 219–230
4 Baron, C. and Thompson, T.E. (1975) Biochim. Biophys. Acta 382, 276–285
5 Dean, W.L. and Tanford, C. (1977) J. Biol. Chem. 252, 3551–3553
6 Gonenne, A. and Ernst, R. (1978) Anal. Biochem. 87, 28–38
7 Hjelmeland, L.M. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 6368–6370
8 Simons, W.F., Koski, G., Streaty, R.A., Hjelmeland, L.M. and Klee, W.A. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 4623–4627
9 Hjelmeland, L.M., Nebert, D.W. and Chrambach, A. (1978) in Electrophoresis '78 (Catsinpoolas, N., ed.), pp. 29–56, Elsevier, Amsterdam
10 Malpartida, F. and Serrano, R. (1980) FEBS Lett. 111, 69–72
11 Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477–5487
12 Hodges, T.K. and Leonard, R.T. (1974) Methods Enzymol. 32, 392–406
13 Serrano, R. (1978) Mol. Cell. Biochem. 22, 51–63
14 Markwell, M.A.K., Haas, S.M., Bieber, L.L. and Tolbert, N.E. (1978) Anal. Biochem. 87, 206–210
15 Veerkamp, J.H. and Broekhuyse, R.M. (1976) in Biochemical Analysis of Membranes (Maddy, A.H., ed.), pp. 269–270, Chapman and Hall, London
16 Ames, B.N. (1966) Methods Enzymol. 8, 115–118
17 Umbreit, J.N. and Strominger, J.L. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 2997–3001
18 Egan, R.W., Jones, M.A. and Lehninger, A.L. (1976) J. Biol. Chem. 251, 4442–4447
19 Slinde, E. and Flatmark, T. (1976) Biochim. Biophys. Acta 455, 796–805
20 Collins, M.L.P. and Salton, M.R.J. (1979) Biochim. Biophys. Acta 553, 40–53
21 Kirkpatrick, F.H., Gordesky, S.E. and Marinetti, G.V. (1974) Biochim. Biophys. Acta 345, 154–161
22 Malpartida, F. and Serrano, R. (1981) Eur. J. Biochem. 116, 413–417
23 Vara, F. and Serrano, R. (1982) J. Biol. Chem. 257, 12826–12830