Biochimica et Biophysica Acta, 733 (1983) 210–215
Elsevier

BBA 71793

DETERGENT EFFECTS ON ENZYME ACTIVITY AND SOLUBILIZATION OF LIPID BILAYER MEMBRANES

MARY D. WOMACK *, DEBRA A. KENDALL ** and ROBERT C. MACDONALD ***

Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60201 (U.S.A.)

(Received March 28th, 1983)

Key words: Detergent; Solubilization; Critical micelle concentration; (Bilayer)

Over 50 detergents were tested to establish which would be most effective in releasing proteins from membrane-bounded compartments without denaturating them. Various concentrations of each detergent were tested for two activities: (1) solubilization of egg phospholipid liposomes as measured by reduction of turbidity and (2) effect of detergent concentration on the activities of soluble, hydrolytic enzymes. Those detergents most effective in solubilizing 0.2% lipid and least detrimental to enzymes were five pure, synthetic compounds recently introduced: CHAPS, CHAPSO, Zwittergents 310 and 312, and octylglucoside. Industrial detergents were generally much inferior, insofar as they solubilized membranes inefficiently and/or inactivated certain hydrolytic enzymes readily. The five detergents were characterized by (a) an unusually high critical micelle concentration and (b) a preference for forming mixed micelles with lipids instead of forming pure micelles, as indicated by an ability to solubilize lipid at concentrations of detergent significantly below the critical micelle concentration. This characteristic permits solubilization of high concentrations of membrane below the critical micelle concentration of the detergent so that protein denaturation is minimized. A generally applicable guideline that emerged from this study is that detergents should be used at approximately their critical micelle concentration which should not be exceeded by the concentration of membrane. Similar considerations should apply to the use of detergents in purifying and reconstituting intrinsic membrane proteins.

Introduction

It has long been desirable to be able to disrupt both natural and artificial membrane compartments and release the contained molecules undamaged. Cellular membranes constitute barriers between compartments and the accurate assay of enzyme activity within those compartments requires the release of those activities intact. Similarly, liposomes to be used for microinjection of otherwise impermeant enzymes into cells must be disrupted to permit assay of encapsulated enzyme activity without reducing that activity. Detergents have the ability to disrupt structures such as membranes that are held together by hydrophobic interactions [1]. For this reason detergents have been used to liberate molecules trapped within membrane-bounded compartments. Unfortunately, many detergents that disrupt lipid bilayers also disrupt hydrophobic interactions that contribute to the stability of many globular proteins. Thus, the release of undenatured proteins from either vesicles or membranes calls for a detergent that is capable of solubilizing lipid bilayers without alter-

* Present address: Department of Neurobiology, Harvard University, Boston, MA 02115, U.S.A.
** Present address: Rockefeller University, New York, NY 10021, U.S.A.
*** To whom correspondence should be addressed.

ing the structure of proteins.

We have had a need to encapsulate an enzyme in liposomes, and, therefore, a need to know how much active enzyme had been incorporated within the vesicles. For simplicity, disruption of the vesicles with detergent was the method of choice. Although a number of common detergents released enzyme activity, the mere appearance of free protein was no guarantee that the released enzyme was fully active. Specific activity was not a reliable guide because the enzyme was not pure and there was the possibility of selective encapsulation. Uncertainties are even greater in investigations of natural membrane systems where specific activities of native proteins are often unknown. We therefore decided to survey a variety of detergents with respect to their ability to solubilize lipid bilayers and with respect to their effect on enzymes, the encapsulation of which within liposomes might be desirable. Decrease in turbidity proved an accurate and simple measure of the disruption of liposomes, and since this is simultaneously a measure of solubilization of lipid membranes, it appeared that such information would be useful in attempts to solubilize natural membranes and to purify their component proteins and to reconstitute membranes by dialysis of detergent from a solution of lipid and protein [2].

More than 50 detergents were surveyed. Of those (about 35) that gave sufficiently clear solutions to warrant testing on lipid dispersions, most inactivated the test enzymes to a significant extent and only a few were capable of completely micellizing a liposome suspension without affecting the enzyme activities. Pure, synthetic compounds were far superior to industrial detergents and natural products. Some reasons for the efficacy of the former became apparent and are discussed.

Experimental procedures

Detergents and enzymes

Detergents were from the following sources: sodium dodecyl sulfate, sodium deoxycholate, sodium cholate, Tween 20 and 80 (polyoxyethylene sorbitan alkyl esters), Brij 35, 36T and 58 (polyoxyethylene alkyl ethers), and octylglucoside from the Sigma Chemical Co. (St. Louis, MO); CHAPSO (3-[(3-cholamidopropyl)dimethylammonio]-1-[2-hydroxy-1-propane]sulfonate) and all of the Zwittergent series (*N*-alkyl-*N*,*N*-dimethyl-3-ammoniopropane sulfonates, designated as 3*n*, where *n* is the number of carbon atoms in the alkyl group) from CalBiochem-Behring (La Jolla, CA); KYRO EOB (polyoxyethylene alkyl ether) from Proctor and Gamble (Cincinnati, OH); Triton CF54, WR1339, X-100 (alkylaryl polyoxyethylene alcohols), Myrj 59 (polyoxyethylene stearate), Lubrol WX (polyoxyethylene alkyl ether) and Cutscum from Supelco (Bellefonte, PA); CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate) from Polyscience (Warrington, PA); Lodyne S107, S100, S110 (fluorocarbon surfactants) from CIBA-GEIGY (Ardsley, NY); sucrose monomyristate from Pfalz-Bauer (Flushing, NY); Crodesta SL-40, F160, F20, S10, F10, F110, F50, A20, A10 and SL-40 (sucrose esters) from Croda, Inc. (New York, NY); C_{12-14} alkyl 9-glucoside, oleyl 6-glucoside and oleyl 4-glucoside (alkyl polyglucosides) from ICI America (Wilmington, DE); Pluronic L43, F48, P105, F87, F108, F68, L64, and L35 (ethylene oxide/propylene oxide block polymers) from BASF (Wyandotte, MI). All detergents are commercially available except KYRO EOB and those from ICI America. The latter were experimental compounds prepared as described by Hughes and Lew [3]. KYRO EOB is similar to Lubrol WX, which is commercially available.

With the exceptions of octylglucoside and Lodyne S107, all of the nonionic detergents were deionized. About 0.5 g detergent was dissolved in about 30 ml water and the resultant solution treated with well-washed Amberlite MB-3 (Mallinckrodt) ion exchange resin until the resistivity was above $3 \cdot 10^5\ \Omega \cdot$ cm. The detergent was recovered by lyophilization. All detergents were made up as 10% stock solutions, and, as necessary, adjusted to about pH 7.

Sulfatase (type H-2) and alkaline phosphatase (type III) were from Sigma. These were used without further treatment.

Wheat esterase was purified according to a method similar to that reported by Stauffer and Glass [4]. Fresh wheat kernels, obtained locally, were homogenized in a blender with 5 vol. distilled water. Following clarification by centrifugation ($12\,000 \times g$ for 10 min) the supernatant solution

was adjusted to pH 5.5 with dilute acetic acid. The precipitate was spun down (12000 × *g* for 20 min) and discarded. The supernatant was then subjected to ammonium sulfate fractionation, the 40–70% fraction being retained and dialysed to remove the ammonium sulfate. These steps resulted in about 5-fold purification. The sample was then chromatographed on DEAE-Sephadex. Elution with a 0–0.3 M NaCl linear gradient resulted in an additional 10-fold purification. By SDS-polyacrylamide gel electrophoresis, the preparation contained about four major bands. A product similar to the low pH precipitate is sold by Sigma Chemical Co. (wheat germ lipase, type I).

Enzyme assays

Esterase, at a concentration convenient for determination of the reaction rate, was added to 9 vol. of detergent at 0.1, 0.3, 1 and 5% in Mops (3-morpholinepropanesulfonic acid) at 0.05 M and pH 6.5. After 1.0–1.5 h 45 μl of this solution were transferred to a fluorometer cuvette containing 2 ml of 10^{-5} M diacetylfluorescein (Eastman) in 0.05 M Mops buffer (pH 6.5). Fluorescence was measured with a Zeiss PMQII and rates were obtained from chart recordings. An interference filter with peak transmission at 450 nm was used for excitation and a Corning 3-70 cutoff filter was substituted for the emission monochromator. All detergents were tested at 5% to assure that none catalyzed the hydrolysis of the substrate. Many nonionic detergents contain polyethoxy groups that may give rise to peroxides with potentially detrimental effects on enzymes [5]. This is not a concern with wheat esterase; 0.1% H_2O_2 had no effect on enzyme activity.

Sulfatase and phosphatase activities were measured by release of 4-methylumbelliferone from the corresponding sulfate and phosphate. Aside from fluorometer filters, which were appropriate for umbelliferone, the procedure was analogous to that described for the esterase.

Assay for solubilization of lipid bilayers.

A 0.2% dispersion of egg phospholipid (Sigma egg phosphatidylcholine, type IX-E) in 0.05 M Mops (pH 6.5) was sonicated with a probe apparatus until the absorbance was 0.75 at 375 nm. Stock detergent solution was added to aliquots of the lipid to yield detergent concentrations of 0.1, 0.3, 1.0% and when sufficient detergent was available, 5%. The absorbance of the mixture was then recorded for 1 min. If there was a change over that time, the solution was held for 5 h at room temperature and then measured again. A few cases of slow equilibration were seen, in which the maximum difference between the short and long-time values was 30%. Slow equilibration was not exhibited by detergents that scored well in our test. Absorbance was corrected for dilution by the detergent solution. Except in a few cases at the highest concentration, the absorbance of the detergent solution alone was negligible.

Results

All detergents shown were first tested with esterase. Those that gave least inactivation of the esterase were then tested against aryl sulfatase and alkaline phosphatase. There were nine such detergents. Up to an incubation concentration of 5%, none of these had any effect on alkaline phosphatase, so those data are not shown.

Fig. 1 presents the data obtained on detergents that gave sufficiently clear solutions to warrant testing. The enzymes were incubated with detergent solutions of the concentrations shown and assayed at concentrations 1/44 those shown. Activities are given as percent, normalized to 100% in the absence of detergent.

Discussion

The tested detergents vary dramatically in their effects on enzyme activity and their abilities to reduce the turbidity of a liposome suspension. Some, such as Tween 80, Triton WR1339, and Pluronic F38, were totally ineffective in solubilizing lipid, yet they drastically inhibited esterase activity at the higher concentrations. A few, such as Pluronic P105 and Myrj 59, had little effect on enzyme activity, but were also weak solubilizing agents. Nearly a third of the detergents tested, for example, the Brij series and Lubrol WX, caused a roughly parallel reduction in turbidity and enzyme activity.

Only five of the detergents tested had no or

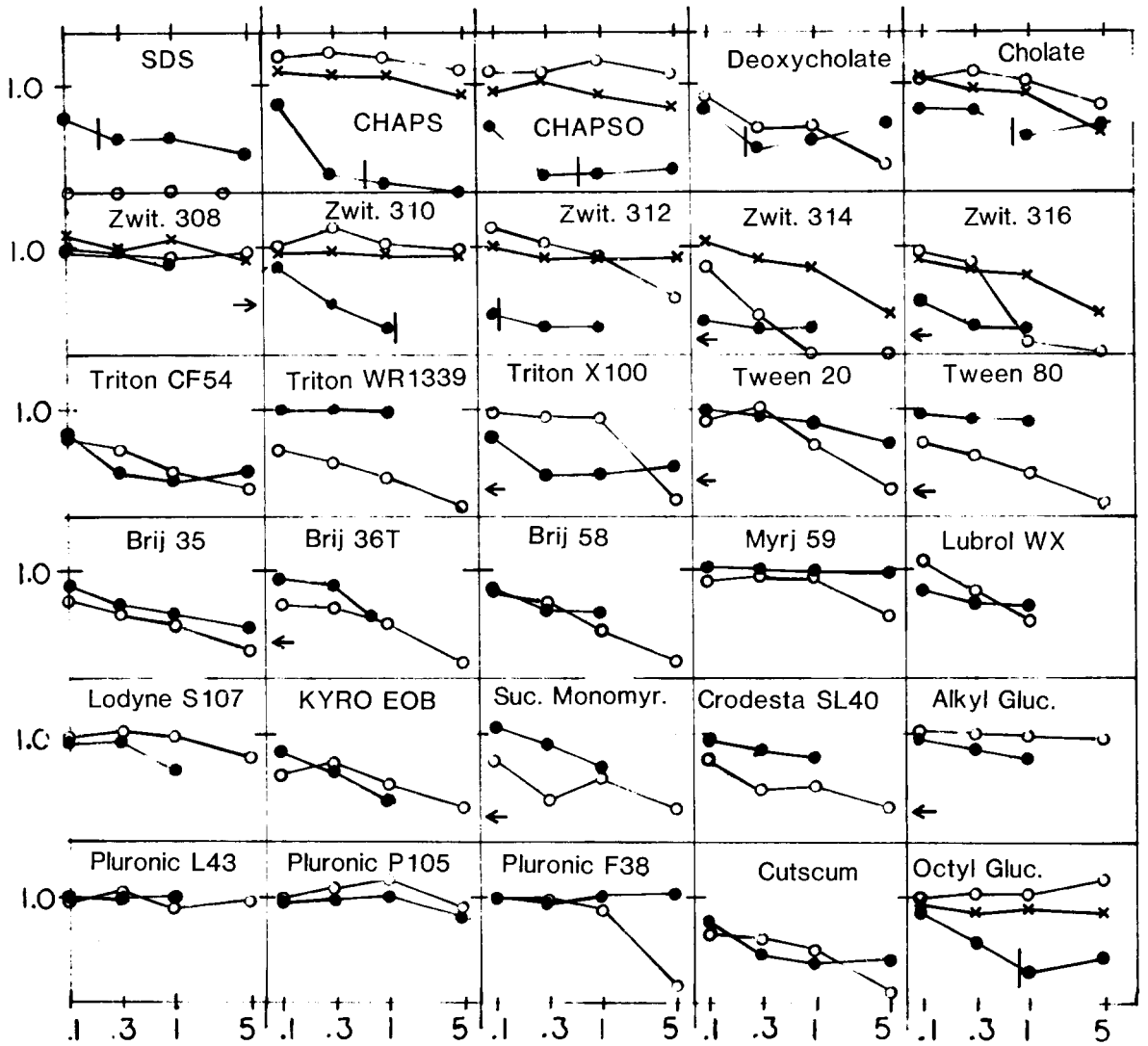

Fig. 1. Solubilization of lipids and activity of enzymes in detergent solutions. The turbidity of 0.2% egg phospholipid dispersed in detergent solution of the indicated concentrations is represented by the closed circles, normalized to the original absorbance which was 0.75. The critical micelle concentration, when available, is given by the short vertical line, or if off scale, by the horizontal arrow. The enzymes, esterase (○) and sulfatase (×), were incubated with the indicated concentrations of detergent and then diluted 44-fold for assay of hydrolytic activity remaining. Activity is expressed as a percent of the activity measured in the absence of detergent.

very little effect on esterase, sulfatase or phosphatase activity at an incubation concentration as high as 5% and were also able to reduce the turbidity of the lipid dispersion to a low level at either 1 or 5%. These detergents are CHAPS, CHAPSO, Zwittergent 310, octylglucoside, and Zwittergent 312. These differed significantly with respect to the lowest concentration that was able to solubilize lipid. If, for purpose of comparison, the ratio of normalized esterase activity to normalized absorbance for incubations at 1% detergent is taken as an arbitrary figure of merit, the values for

the five detergents in the order given in the previous sentence are, 16.2, 7.3, 4.1, 3.6 and 3.5. The corresponding values, when reckoned on the basis of sulfatase activity, are, to within 15%, the same.

These five detergents are all pure compounds that were specifically synthesized for applications in biochemistry. They are notable for having unusually high CMC values; all are above 0.1% [6–11]. CHAPS [8,12] and octylglucoside [13,14] have previously been found to be unusually effective in solubilizing membrane proteins in active form.

The following general recommendations emerge from these studies. For effective solubilization of lipids with minimum damage to proteins, detergents should be used at concentrations at or, preferably, slightly below their CMC values. The consequences of such a practice are particularly clear in the case of the Zwittergent series, where the CMC falls by a factor of 10 from one homolog to the next longer one [7]. As may be seen from the second row of Fig. 1, lipid solubilization increases with increasing CMC. (316 does not follow this pattern because of its low Krafft temperature [7].) Enzyme inactivation increases in parallel although it trails lipid solubilization. Thus, in general, preservation of enzyme activity requires the use of a lower concentration of detergent, the lower its CMC. This relationship was predicted by Reynolds ([15]; see also Tanford, Ref. 11) who pointed out that because binding of detergent to protein is generally weaker and the cooperative number smaller than for formation of micelles, saturation of sites on proteins by detergent usually requires a concentration of detergent in excess of its CMC. Depending somewhat on the characteristics of the particular detergent, an amount of detergent similar to the amount of lipid is required for complete micellization [16]; we used 0.2% lipid, and few detergents had significant effects on turbidity at concentrations less than 0.3%. Thus, the CMC of the detergent provides not only a guide for the appropriate concentration of detergent, but also an approximate upper limit to the concentration of membrane to be solubilized.

The attribute that determines the suitability of a detergent for membrane dissolution without protein denaturation is its ability to solubilize lipid at concentrations significantly below its CMC. This means that the monomer concentration in equilibrium with mixed micelles is lower than that in equilibrium with pure micelles. This could occur either because packing in the pure micelle is energetically unfavorable relative to that in mixed micelles or because of specific interactions between detergent and lipid molecules [11]. The latter, in the form of dipole interactions, might occur in the case of the zwitterionic detergents. Relatively poor packing in pure micelles might well be expected of detergents with rigid hydrophobic moieties, which would be better accommodated by fatty acyl portions of the lipid molecules in mixed micelles, as indicated by the accommodation of cholesterol by lipid bilayers [17]. The efficacy of the two choleamide detergents seems to be due to these factors, plus the likelihood that their rigid steroid nucleus adapts poorly to the surface of and is thus relatively impotent at denaturing proteins. The entropy of mixing also contributes to the preferred formation of mixed micelles, although this, of course, applies to all detergents [11]. Finally, it may be pointed out that all five pure detergents would not, unlike industrial detergents, contain minor components that might have atypically low CMC values and/or high affinities for proteins.

Although our primary concern has been the release of soluble enzymes from membrane-bounded compartments, the ability to solubilize membrane-bound proteins is acquiring increasing importance and a comment on the relationship between the two processes is appropriate. The two processes might seem to require detergents with different properties since binding of detergent is to be avoided in the case of release of soluble proteins, but is sought in the case of solubilization of membrane proteins. The requirements are, however, likely to be very similar. Membrane lipid (perhaps mixed with detergent) will probably associate with the portions of intrinsic membrane proteins that penetrate the membrane under any conditions of lipid solubilization. Portions of membrane proteins that extend out of the membrane are probably similar to soluble proteins; these are the portions that must be preserved in the native state and, in contrast to the intramembrane part, should be kept free of detergent. Thus, it is likely that solubilization of membranes for both purpo

ses will require detergents of similar properties. Selective extraction of membrane components, on the other hand, probably requires detergents with different characteristics [17,18] and maintenance of membrane proteins in solution may sometimes be most conveniently accomplished with a low concentration of a detergent with a low CMC.

Acknowledgements

We are grateful to Ciba-Geigy Corp., Croda Inc., BASF Wyandotte Corp., and ICI America Inc., for samples of their detergents and Dr. N.W. Cornell for a sample of KYRO EOB. We thank Dr. L.M. Hjelmeland for sending us a copy of a manuscript before publication and Dr. R.I. MacDonald for invaluable advice and assistance. This research was supported by NIH research grant GM 28404.

References

1 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79
2 Razin, S. (1972) Biochim. Biophys. Acta 265, 241–296
3 Hughes, F.A. and Lew, B.W. (1970) J. Am. Oil Chem. Soc. 47, 162–167
4 Stauffer, C.E. and Glass, R.L. (1966) Cereal Chem. 43, 644–657
5 Chang, H.W. and Bock, E. (1980) Anal. Biochem. 104, 112–117
6 Helenius, A., McCaslin, D.R., Fries, E. and Tanford, C. (1979) Methods Enzymol. 56, 734–749
7 Gonenne, A. and Ernst, R. (1978) Anal. Biochem. 87, 28–38
8 Hjelmeland, L.M. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 6368–6370
9 Hjelmeland, L.M., Nebert, D.W. and Osborne, J.C. (1983) Anal. Biochem., in the press
10 Shinoda, K., Yamaguchi, T. and Hori, R. (1961) Bull. Chem. Soc. Japan 34, 237–241
11 Tanford, C. (1980) The Hydrophobic Effect: Formation of Micelles and Biological Membranes, Wiley Interscience
12 Simonds, 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
13 Baron, C. and Thompson, T.E. (1975) Biochim. Biophys. Acta 382, 276–285
14 Stubbs, G.W., Smith, H.G. and Litman, B.J. (1976) Biochim. Biophys. Acta 425, 46–55
15 Reynolds, J.A. (1979) Methods Enzymol. 61, 58–62
16 Jackson, M.L., Schmidt, C.F., Lichtenberg, D., Litman, B.J. and Albert, A.D. (1982) Biochemistry 21, 4576–4582
17 Yu, J., Fischman, D.A. and Steck, T.L. (1973) J. Supramol. Struct. 1, 233–248
18 MacDonald, R.I. (1980) Biochemistry 19, 1916–1922