Some Observations on the Choice of Detergent for Solubilization of the Human Erythrocyte Membrane

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Solubilization of the human erythrocyte membrane by seven detergents is described. Components released into the supernatant or retained in the residue were identified by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Two non-ionic detergents exhibiting little u.v. absorption were more efficient than u.v.-absorbing Triton X-100. Evidence is presented of an interchange between protein PAS 1 and protein PAS 2.

The architecture of the erythrocyte membrane has been extensively studied in recent years. It has been established that the polypeptide components designated band 3, PAS 1 (also called glycophorin or the major sialoglycoprotein) and PAS 2 [nomenclature of Steck (1974)] span the membrane (Bretscher, 1973; Boxer *et al.*, 1974; Segrest *et al.*, 1972; Mueller & Morrison, 1974). In addition, it is proposed that the band-3 polypeptide has various transport properties (Ho & Guidotti, 1975; Brown *et al.*, 1975; Lin & Spudich, 1974). Without exception the individual polypeptides were isolated in a denatured form after solubilization of the membrane with sodium dodecyl sulphate.

More recently some non-ionic detergents have been used to isolate components in non-denatured form, and this is an essential prerequisite to any functional study. Yu *et al.* (1973) described the selective solubilizing properties of Triton X-100 towards particular membrane polypeptides and have subsequently reported the purification of band-3 protein in a 20% yield (Yu & Steck, 1975*a,b*). Kahlenberg (1976) has described the preparation of this protein in the same detergent with an 80% yield. Kasahara & Hinkle (1976) demonstrated glucose transport in reconstituted liposomes containing a Triton X-100 extract of the erythrocyte membrane. Liljas *et al.* (1974) have described the selectivity of Tween 20 under various conditions of ionic strength and pH, and the same workers have also purified protein PAS 1 with this detergent (Liljas *et al.*, 1976).

The present paper reports a study of seven detergents (other than sodium dodecyl sulphate) with a view to assessing their potential for functional studies on the proteins of the erythrocyte membrane.

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Experimental

Triton X-100 was obtained from Rohm and Hass, Philadelphia, PA, U.S.A.; Tween 20SD, Tween 80SD and the detergent G3707 were from Atlas Chemicals, Everberg, Belgium; Mulgofen BC-720 was from GAF Corporation, Stockholm, Sweden; Empigen BB was from Allbright and Wilson, Whitehaven, Cumbria, U.K.; sodium deoxycholate was from Merck, Darmstadt, Germany. The structure and HLB value† (where known) for each detergent are shown in Fig. 1.

Erythrocyte membranes were prepared by the method of Dodge *et al.* (1963). Membrane suspension (0.1 ml; 8–9 mg of protein/ml) was diluted to 1 mg/ml with detergent (final concn. 1%) in an appropriate buffer and incubated at 4°C overnight (see the legend to Table 1). After centrifugation at 220000*g*_{max} for 45 min, the residue was re-extracted for 1 h with 0.5 ml of 1% detergent. The supernatants were pooled and any residue was suspended in 0.5 ml of water. Portions were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis in gradient gels (Pharmacia PAA4/30).

Protein concentrations in the presence of detergent were determined by the method of Wang & Smith (1975). Phospholipids were determined (as phosphate) as described by Bartlett (1959). The u.v. spectra of 1% solutions of each detergent (except Triton X-100, which was 0.1%) were measured from 220 nm to 350 nm on an LKB-Beckman 25 spectrophotometer.

Results and Discussion

The behaviour of amphiphatic membrane proteins in solution is dependent on a wide variety of variables,

† The HLB value is the hydrophile/lyophile balance proposed by Griffin (1949) for classifying non-ionic surfactants.

Structure and chemical name	Trade name	HLB value
<p>Poly(ethylene glycol) <i>p</i>-iso-octylphenyl ether</p>	Triton X-100	13.5
<p>$a + b + c + d = n$</p> <p>Polyoxyethylene sorbitan monolaurate, $n_{av.} = 20$</p> <p>Polyoxyethylene sorbitan mono-oleate, $n_{av.} = 80$</p>	Tween-20 Tween-80	16.7 15.0
<p>Polyoxyethylene isoalcohol, $n_{av.} = 9.75$</p>	Mulgofen BC-720	13.8
<p>Polyoxyethylene alcohol, $n = ?$</p>	G3707	12.8
<p>Alkyldimethylbetaine</p>	Empigen BB
<p>Sodium deoxycholate</p>		

Fig. 1. Structure, chemical name, trade name and HLB value (see the text) of each detergent tested

the most important being the detergent/protein ratio (Clarke, 1975), the ionic strength, temperature and pH (Hjertén, 1973) and the position of the buffer constituents in the Hoffmeister series (Påhlman, *et al.*, 1977).

The performance of each detergent was measured in terms of the amount of phospholipid and protein solubilized. The buffers were representative of those with buffering capacity in the basic pH range. It was noteworthy that the amount of protein extracted

by the two Tween-series detergents was consistently low in comparison with that extracted by the other non-ionic detergents. At the same time the large amount of phospholipid remaining in the residue (up to 90%) indicated some degree of selectivity.

It has been proposed by several groups of workers that there is an interconversion between glycoproteins PAS 1 and PAS 2 (Marton & Garvin, 1973; Furthmayr & Marchesi, 1976), but it has also been suggested that they are two distinct components

EXPLANATION OF PLATE 1

Analysis of the carbohydrate-staining components of the human erythrocyte membrane extracted in various detergents and buffers at either pH7 or pH8.8 by polyacrylamide-gradient-gel electrophoresis in sodium dodecyl sulphate

Polyacrylamide gradient gels were pre-equilibrated for 1 h with 50 mm-glycine/NaOH, pH9.8, containing 4 mm-sodium dodecyl sulphate. Samples were made 100 mm and 20 mm in sodium dodecyl sulphate and 2-mercaptoethanol respectively. After electrophoresis the gels were initially stained for carbohydrate by the method of Fairbanks *et al.* (1971), and then for protein (see Plate 2). Samples may be identified as follows: Sup, supernatant; Res, residue; Tx, Triton X-100; M, Mulgofen BC-720; G, detergent G3707; Tw80, Tween 80; P, 20 mm-NaH₂PO₄/NaOH, pH7; Gly, 20 mm-glycine/NaOH, pH8.8; Bar, 20 mm-barbituric acid/sodium barbitone, pH7. Thus Sup Tx B indicates the supernatant following extraction of the erythrocyte membrane in 1% Triton X-100 in 20 mm-boric acid/NaOH, pH7. A is whole membrane solubilized in 0.1 M-sodium dodecyl sulphate. Fractions extracted in the same detergents and buffers at either pH8 or pH9.8 were analysed in an identical manner. Protein components are numbered by the nomenclature of Steck (1974).

EXPLANATION OF PLATE 2

Analysis of the protein-staining components of the human erythrocyte membrane
Gels electrophoresed and stained for carbohydrate (see Plate 1) were then stained for protein with 0.5% Amido Schwarz 10B in 7% (v/v) acetic acid. Samples may be identified as described in the legend to Plate 1.

Table 1. *Amounts of protein and phospholipid solubilized from the erythrocyte membrane*

All buffers were made up to a final concentration of 20mM in the first-named constituent after adjustment of the pH by addition of the second constituent. Specific conductance was measured as a function of ionic strength. Each result represents the mean of duplicate determinations from at least two experiments. The standard error is given. Experiments performed in phosphate buffer were not assayed for phospholipid since the buffer could not be completely removed by dialysis.

Detergent	Buffer	Specific conductance at 4°C (mmho)	pH	Phospholipid solubilized (%)	Protein solubilized (%)	Phospholipid (%) Protein (%)
Triton X-100	Boric acid/NaOH	0.01	7	92 ± 7	89 ± 8	1.0
		0.03	8	76 ± 4	91 ± 6	0.8
	Barbituric acid/sodium barbitone	1.04	7	70 ± 8	60 ± 6	1.2
		1.55	8	68 ± 6	71 ± 7	1.0
	NaH ₂ PO ₄ /NaOH	1.15	7		33 ± 4	
		1.50	8		68 ± 4	
Mulgofen BC-720	Glycine/NaOH	0.12	8.8	90 ± 4	70 ± 8	1.3
		0.36	9.8	78 ± 8	75 ± 5	1.1
	Boric acid/NaOH	0.01	7	64 ± 7	64 ± 5	1.0
		0.03	8	80 ± 9	74 ± 6	1.1
	Barbituric acid/sodium barbitone	1.04	7	57 ± 4	56 ± 6	1.0
		1.55	8	72 ± 5	67 ± 8	1.1
Detergent G3707	NaH ₂ PO ₄ /NaOH	1.15	7		40 ± 5	
		1.50	8		75 ± 4	
	Glycine/NaOH	0.12	8.8	90 ± 5	83 ± 4	1.1
		0.36	9.8	92 ± 8	95 ± 4	1.0
	Boric acid/NaOH	0.01	7	52 ± 5	92 ± 5	0.6
		0.03	8	65 ± 5	87 ± 2	0.8
Tween 20	Barbituric acid/sodium barbitone	1.04	7	63 ± 7	79 ± 4	0.8
		1.55	8	85 ± 4	90 ± 3	0.9
	NaH ₂ PO ₄ /NaOH	1.15	7		42 ± 3	
		1.50	8		67 ± 3	
	Glycine/NaOH	0.12	8.8	83 ± 8	90 ± 7	0.9
		0.36	9.8	90 ± 3	96 ± 4	0.9
Tween 80	Glycine/NaOH	0.12	8.8	38 ± 4	60 ± 4	0.6
		0.36	9.8	24 ± 3	53 ± 5	0.5
Empigen BB	Glycine/NaOH	0.12	8.8	10 ± 2	28 ± 2	0.4
		0.36	9.8	11 ± 1	38 ± 8	0.3
Sodium deoxycholate	Glycine/NaOH	0.12	8.8	80 ± 6	92 ± 8	0.9
		0.36	9.8	72 ± 3	82 ± 4	0.9
		0.12	8.8	58 ± 9	49 ± 3	1.2
		0.36	9.8	47 ± 6	57 ± 1	0.8

(Fujita & Cleve, 1975). Our present results support the former view. The relative amounts of proteins PAS 1 and PAS 2 found in the supernatants were dependent on both the detergent and buffer used (Plates 1 and 2). Protein PAS 2 was the principal glycoprotein obtained after extraction in borate or barbitone buffer at pH 7 with either Triton X-100, Mulgofen BC-720 or compound G3707 as the detergent. Protein PAS 1 was only poorly represented. However, with the same buffers and detergents at pH 8, approximately equal amounts of proteins PAS 1 and PAS 2 were solubilized in Triton X-100 or Mulgofen BC-720, and protein PAS 2 was almost totally converted into protein PAS 1 in detergent G3707. No trace of these glycoproteins remained

in the residue. In phosphate buffer these detergents released both proteins PAS 1 and PAS 2 at the two pH values tested. Some of the proteins PAS 2 and PAS 3 remained in the residue. With the same three detergents in glycine buffer, pH 8.8, only proteins PAS 2 and PAS 3 were present in the supernatant; no components remained in the residue. At pH 9.8 the solubilized glycoprotein components were proteins PAS 1 and PAS 3; again no major glycoprotein remained in the residue. Tween 20 solubilized equal proportions of proteins PAS 1 and PAS 2 together with protein PAS 3 in glycine buffer at both pH 8.8 and pH 9.8; only a trace of protein PAS 2 remained in the residue. Tween 80 partially solubilized protein PAS 2 under the same con-

Fig. 2. Absorption spectra of detergent solutions A, Tween 80; B, Triton X-100; C, Tween 20; D, sodium deoxycholate; E, Empigen BB; F, detergent G3707; G, Mulgofen BC-720.

ditions, and only proteins PAS 2 and PAS 3 were found in the residue. In sodium deoxycholate at both pH values tested, the solubilized glycoprotein was protein PAS 1, whereas protein PAS 2 was found in the residue. Proteins PAS 1 and PAS 2 were present in equal amounts in the supernatant after Empigen BB treatment; there was no residue.

It would seem that there was negligible alteration to this interconversion during analysis in the presence of sodium dodecyl sulphate. If this were not so, the ratio between proteins PAS 1 and PAS 2 would have been the same for all experiments (rapid equilibrium), or broad zones with tailing (slow equilibrium) would have been observed.

Extraction of the major membrane protein, band 3, was less conclusive (Plates 1 and 2). No combination of buffer and detergent selectively released this polypeptide independently of the other membrane components. Only when the membrane was completely solubilized was there total release of band-3 protein (for example, in 1% detergent G3707 or Mulgofen BC-720 at pH 9.8). Solubilization of the other membrane proteins was similarly non-selective.

The u.v. spectrum of the detergents (Fig. 2) showed that both Triton X-100 and Tween 80 had strongly absorbing chromophores at 280 nm. By contrast, Mulgofen BC-720, detergent G3707 and Empigen BB did not absorb at this wavelength. These detergents would be more advantageous for monitoring fractionation of membrane proteins.

In conclusion, it would appear that the properties of the detergent under test were as significant as the ionic strength and pH of the buffer in determining the

amount and molecular form of each component solubilized. The combination of different separation techniques will enable individual components to be isolated. This is particularly valuable in the case of band-3 protein, since questions concerning its heterogeneity and multiple functions have yet to be clarified.

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