ELSEVIER

International Journal of Pharmaceutics 177 (1999) 137–159

international journal of pharmaceutics

Invited review

Surfactants in membrane solubilisation

M.N. Jones *

School of Biological Sciences, University of Manchester, Manchester M13 9PT, UK

Received 12 October 1998; accepted 14 October 1998

Abstract

An understanding of the action of many drugs requires a knowledge of how the drug reaches the site of action in a cell. A detailed knowledge of the structure and function of cell membranes is often required to understand the transport of drugs across the plasma membrane To obtain this information proteins must be isolated. The isolation and characterisation of cell membrane proteins usually requires the solubilisation of the membrane and a method of separation of the various membrane proteins and glycoproteins. The starting point for such an investigation is the choice of a suitable surfactant (detergent) to solubilise the membrane. This review considers the range of surfactants that are available for membrane solubilisation, how surfactants interact with membranes, the part they play in the separation of integral membrane proteins and in the reconstitution of membrane proteins for functional studies. The solubilisation of specific membrane proteins and glycoproteins including the human erythrocyte anion transporter, mitochondrial porin, sarcoplasmic reticulum Ca^{2+}-ATPase, the ATPase-active multidrug transporter P-glycoprotein, bacteriorhodopsin and rhodopsin are also discussed.

Keywords: Membrane protein reconstitution; Membrane solubilisation; Surfactant–bilayer interaction; Surfactant–membrane interaction; Surfactants

1. Introduction

To reach the site of action many drugs must be transported across the plasma membranes of cells either through the lipid bilayer or by interaction with membrane transporters or receptors. A detailed knowledge of the function of membrane proteins and their ability to facilitate membrane processes is essential to an understanding of drug delivery mechanisms. The preparation of biological membranes and their disruption and solubilisation are the first steps in the isolation and characterisation of the structural and functional components of membranes. Membrane solubilisa-

* Tel.: +44-161-275-9093; fax: +44-161-275-5082.

0378-5173/99/$ - see front matter
PII: S0378-5173(98)00345-7

tion is, in most cases, achieved by the use of surfactants and the choice of surfactant for the isolation of a particular membrane component such as a transporter protein or membrane receptor can be critical in its effective isolation, especially if the protein is to be isolated in a pure and functional form.

The most commonly used surfactant in biochemical applications is sodium *n*-dodecyl sulphate (SDS) which effectively solubilises most membranes down to protein or glycoprotein–SDS complexes and mixed membrane lipid–SDS micelles. It is for this reason that SDS finds widespread uses as the surfactant of choice in polyacrylamide gel electrophoresis (PAGE) for the analysis and molecular mass determination of membrane proteins and glycoprotein subunits (Weber and Osborn, 1975). SDS-PAGE is one of the most widely used biochemical techniques and depends on the formation of protein/glycoprotein SDS saturated complexes being separated by a sieving action through cross-linked polyacrylamide in an electric field. Generally, this is done under reducing conditions by treatment of the samples with β-mercaptoethanol, so that the complexes are formed from protein/glycoprotein subunits. SDS denatures most proteins so it is of little value in the isolation of functional proteins/glycoproteins.

There are now available a very large number of surfactants of various types—anionic, cationic, zwitterionic and non-ionic—which in principle could find application in membrane studies. In practice the number that have been used is relatively small. In this review the principle aspects of the solubilisation of biological membranes, the use of surfactants and the factors to be considered in the reconstitution of some functional proteins, will be discussed. Particular aspects of the area of membrane solubilization by surfactants and related work have been the subject of a number of previous reviews to which attention is drawn for the earlier studies in the field (Helenius and Simons, 1975 Lichtenberg et al., 1983 Lichtenberg, 1985 Levitzki, 1985 Jones et al., 1987a Jones, 1992 Jones and Chapman, 1995a Jones and Chapman, 1995b; Jones, 1996). Practical aspects of the isolation and reconstitution of membrane proteins have been described by Jones et al. (1987a). The theoretical aspects of protein–surfactant interactions have also been reviewed (Jones, 1992 Jones and Chapman, 1995a Jones and Chapman, 1995b; Jones, 1996).

1.1. General considerations

The structure of biological membranes consists of a bilayer of lipids in which are embedded proteins and glycoproteins, the so called intrinsic or integral proteins/glycoproteins, and to which are adsorbed, largely on the cytoplasmic side of the membrane, other proteins, the so-called extrinsic or peripheral proteins. The essential features of biological membranes were layed down by Singer and Nicholson (1972) in their now classical 'fluid mosaic' model of cell membranes.

The detailed structure of particular membranes differ; for example, the ratio of protein to lipid by weight varies from around 0.3 in myelin membranes, which function only as a pathway for nerve impulse transmission, to 3.0 in the inner mitochondrial membrane, the location of the numerous enzymes involved in oxidative phosphorylation. Some general points to be noted about membrane structure are as follows:

- The bilayers consist of a wide range of lipid types—e.g. phospholipids, sphingolipids, glycolipids and, except for bacterial membranes, sterols (cholesterol).
- The lipids are asymmetrically distributed, some lipids being found predominantly on the extracellular surface of the bilayer—e.g. phosphatidylcholine and sphingomyelin—while others with smaller head groups are generally on the cytoplasmic side—phosphatidylserine and phosphatidylethanolamine (Roelofsen and Denkamp, 1994).
- The integral membrane proteins/glycoproteins are generally anchored to the bilayer by hydrophobic domains often in the α-helical conformation. Many transporters and receptors have several *trans*-membrane α-helical sequences of approximately 20 amino acids (the number required to cross the hydrophobic core of the bilayers, ≈ 3 nm thick). Numerous integral membrane proteins have seven α-helix span-

Table 1
Surfactants used in membrane solubilisation[a]

Name	Molecular mass (monomer)	CMC (mM)[b]	Aggregation number	Denaturant
Sodium *n*-dodecyl sulphate (SDS)	288.4	8	60	Yes
Sodium cholate	430.6	13	10	No
Sodium deoxycholate	432.6	5	10	No
3-[(3-Cholamidopropyl)-dimethylammonia]-1-propane sulphate (CHAPS)	614.9	1.4	10	No
n-Octyl-β-D-glucopyranoside (OBG)	292.4	23	30	No
n-Octyl-β-D-thioglucopyranoside[c]	308.4	9	–	No
Polyethylglycol *p-t*-octylphenol $n = 9$–10, Triton X-100	650	0.3	140	No
Triton X-100, reduced	656	–	–	No
Polyethylene glycol *n*-dodecanol $n = 9$–10, Lubrol PX	582	0.1	110	No
Polyethylene glycol sorbitan monooleate $n = 20$, Tween-80	1310	0.012	60	No
Octaethylene glycol dodecyl ether ($C_{12}E_8$)	538.8	0.087	120	No

[a] Data from Jones et al. (1987a) and Jones and Chapman (1995c), except where indicated.
[b] The CMCs for ionic surfactants decrease with increasing ionic strength; the values quoted are those for water at 25°C.
[c] Thomas (1986).

ning domains—e.g. bacteriorhodopsin and the β-adrenergic receptor (Jones and Chapman, 1995c).

- The glycoproteins generally have their glycosylated regions orientated on the extracellular bilayer surface which forms the so-called 'glycocalyx' of the cell. They often carry negatively charged groups—e.g.such as sialic acids—which give the cell surface a negative charge.
- Extrinsic membrane proteins are adsorbed to the bilayer surface by ionic and/or hydrophilic interactions or in some cases by hydrophobic covalently linked anchors. These may be acyl residues of myristic or palmitic acid or complex lipids (Sefton and Buss, 1987).
- The structure is dynamic; the lipids can laterally diffuse (Tocanne et al., 1994) and exchange from one bilayer surface to the other (flip–flop). Lateral diffusion is generally faster than flip–flop although for some lipids flippase proteins aid flip–flop. The proteins/glycoproteins can laterally diffuse and rotate but cannot flip–flop (Kapitza and Jacobson, 1986 Thomas, 1986). It should be noted that although the lipids can laterally diffuse the bilayer is not homogeneous but can undergo lateral phase separation; also some membrane proteins have an affinity for specific lipids. It is with reference to the above general features of cell membranes that the interaction of surfactants with the various membrane components must be considered. In summary, membranes are heterogeneous which means that there are numerous possible surfactant–protein/glycoprotein and surfactant–lipid interactions to consider in the solubilisation process. The nature of these interactions will be determined by the surfactant properties, such as hydrophobic/hydrophilic balance (HLB) and critical micelle concentration (CMC) and the properties of the membrane components. In general terms solubilisation involves hydrophobic interactions between the surfactant chains and the lipid/protein in the case of non-ionic surfactants, when ionic surfactants are used the interaction is modified by the ionic interactions.

2. Surfactant used in membrane solubilization

The properties of some of the most commonly used surfactants for membrane solubilisation are summarised in Table 1. As mentioned above, SDS will solubilise most cell membranes but is a strong denaturant at millimolar concentrations. There are, however, some proteins which resist denatu-

ration such as the proteolytic enzymes papain and pepsin (Nelson, 1971), glucose oxidase at pH 6 (Jones et al., 1982a) and bacterial catalase (Jones et al., 1982b). In contrast, the fungal (*Aspergillus niger*) catalase is activated by SDS at pH 6.4 but not in acid or alkaline solution (Jones et al., 1987b). It is interesting that some proteins can undergo changes in secondary structure when incorporated into protein–SDS complexes (Parker and Song, 1992). This takes the form of an increase in α-helical content at the expense of loss of antiparallel β-sheet. Thus pepsin, alcohol dehydrogenase, carbonic anhydrase and immunoglobulin increase their α-helical content by 9, 9, 15 and 25%, respectively, when complexed by SDS. The natural anionic surfactants cholate and deoxycholate are generally non-denaturing. The conformation of the ring structures leads to molecules which have a hydrophobic and a hydrophilic side bearing the hydroxyl groups. A consequence of this 'sidedness' is that they form small micelles with low aggregation numbers; also they form mixed micelles with membrane phospholipids in which the cholates probably locate peripherally around small bilayer fragments as has been found in the case of their derivatives, the bile salts (Mazer et al., 1980). Unlike most membrane proteins/glycoproteins the multidrug transporter P-glycoprotein is deactivated by 1 mM deoxycholate (Doige et al., 1993), while the phospholipases are activated by deoxycholate (El-Sayert and Roberts, 1985).

Non-ionic surfactants such as OBG and octylthioglucoside are favoured for membrane solubilisation because of their high CMC's which makes their removal from solubilised membrane extracts by dialysis easier than from surfactants with low CMCs. In dialysis it is the diffusion of the monomeric surfactant through the dialysis membrane which is the rate determining step so that surfactants with low CMCs diffuse very slowly. The octylthioglucoside is considerably cheaper than OBG although its CMC is lower at 9 mM (Saito and Tsuchiya, 1984) but still high enough for rapid dialysis.

The synthetic non-ionics, the Tritons, Lubrols and Tweens, offer a wide range of HLB. They are cheap but have low CMCs; they are non-denaturing and there are examples of activation by Triton X-100 in the case of the multidrug transporter P-glycoprotein (Doige et al., 1993) and glucose-6-phosphatase (Beyhl, 1986). It has also been found that it is possible to refold the sulphur transferase enzyme, rhodanese, at high concentration in mixed Triton X-100–phospholipid mixed micelles after its denaturation in urea or guanidinium chloride (Zardeneta and Horowitz, 1994).

A potential disadvantage of the Triton class of non-ionics is the presence of the aromatic ring which absorbs in the UV at 280 nm where the aromatic side-chains of proteins/glycoproteins also absorb. This prevents UV detection of membrane proteins/glycoproteins in the presence of Tritons. To avoid this problem reduced Tritons have been produced (Tiller et al., 1984). The interference by surfactants in protein assays is a problem which must often be addressed (Thorne, 1978 Pande and Murthy, 1994).

Some novel surfactants have been described for the reconstitution of membrane proteins. One such compound is ammonium perfluorooctanoate which forms vesicle-like structures called 'surfactosomes' (Reviakine et al., 1996). The surfactosomes have been used for the solubilisation, reconstitution and 2-D crystallisation of the 16 000 molecular mass membrane sector of the V-type H^+-ATPase. Another class of solubilising agents based on sulphoxide derivatives of THAM (Tris(hydroxymethyl)-acrylamidomethane) has been synthesised (Barthélémy et al., 1998). The structure of these materials is as follows:

$$R - SO - CH_2CH_2CO - NH - CH(CH_2OH)_3$$

where R is octyl, decyl, dodecyl, palmitoyl or C_6F_{13} (CH_2). The CMCs of the alkyl derivatives of chain length C_n obey the relationship:

$$\log(\mathrm{CMC/mM}) = -0.71C_n + 6.87$$

so that for the decyl compound the CMC is 0.59 mM. This compound shows the best efficacy as a solubiliser of protein from the nuclear ($\approx$ 57%), mitochondrial ($\approx 71\%$) and microsomal ($\approx 69\%$) membranes of rat liver.

A series of polymeric surfactants has also been synthesised by the esterification of dextran with fatty acids (Bauer et al., 1997). These promise to

replace the block copolymers based on polyoxyethylene–polyoxypropylene (Polyoxamers, Pluronics and Pluroils) which have poor solubility. The dextran fatty acid esters prepared from dextran and lauric acid at mole ratios of 10:1 and 15:1, of molecular mass approximately 30 000, have a haemolytic activity of approximately 10^{-3} of that of SDS towards erythrocytes, but compared to block copolymers improved solubilisation capacities.

2.1. Surfactant interactions with lipid bilayers

The mechanism of solubilization of a lipid bilayer by a surfactant is directly relevant to membrane solubilisation. Considering the process as the surfactant concentration is increased, the initial interaction is between the surfactant monomer and the bilayer. The monomer penetrates into the bilayer until saturation occurs after which mixed micelles of surfactant and lipid can form. Lichtenberg et al. (1983) and Lichtenberg (1985) considered the process in terms of the effective molar ratio (R_e) of surfactant (S) to bilayer lipid (L), defined by the equation:

$$R_e = \frac{[S] - [S]_{monomer}}{[L]} \quad (1)$$

where [S], $[S]_{monomer}$ and [L] are the molar concentration of total surfactant, monomeric surfactant and lipid, respectively. The solubilisation process can be described by the following sequence of processes:

Lipid bilayer (L)

$\downarrow\uparrow + S$

Surfactant saturated bilayer $(LS_n) - R_e^{sat}$

$\downarrow\uparrow + S$

LS_n + mixed micelles (LS_m)

$\downarrow\uparrow + S$

Mixed micelles (LS_p) + surfactant

Micelles (S_x) R_e^{sol}

The surfactant saturated bilayer, mixed micelles and surfactant micelles will always be in equilibrium with 'free' monomeric surfactant at the CMC. Above R_e^{sat} some of the bilayer will disrupt with the formation of mixed micelles (LS_m); further addition of surfactant will result in progressive bilayer disruption until only mixed micelles enriched in surfactant (LS_p, where $p > m$) and surfactant micelles (S_x) are present at R_e^{sol}.

The process of bilayer solubilisation has been followed in liposomal systems by loading the liposomes with 6-carboxyfluorescein at a concentration where its fluorescence is quenched and following the increase in fluorescence on addition of surfactant (Alonso et al., 1987 Urbaneja et al. 1987 Ruiz et al., 1988). The decrease in quenching (increase in fluorescence) when the liposomes become leaky and the extent of solubilisation, by separating intact liposomes from disrupted ones, can be followed as a function of surfactant concentration. Leakage occurs before solubilization and follows a steeper curve as a function of surfactant concentration. The concentration of surfactant required to 50% solubilise the liposomes ($R_e^{50\%}$) increases with the CMC of the surfactant. Generally, the more hydrophobic the surfactant and the lower its CMC, the greater will be its tendency to penetrate into lipid bilayers at low concentrations. Thus for Triton X-100 (CMC 0.3 mM) $R_e^{50\%}$ is 1.7 mM while for OBG (CMC 23 mM) $R_e^{50\%}$ is 20 mM.

The fine details of surfactant interaction with bilayers will vary considerably with the lipid composition of the bilayer and its physical state—e.g. mesophase structure and temperature. A further consideration is the effect that a surfactant binding protein can have on the state of aggregation and phase transformations of the phospholipid. If the protein binds surfactant strongly then the value of R_e will be reduced which might in turn effect the surfactant concentrations at R_e^{sat} and R_e^{sol}. An example of such behaviour is the effect of albumin on phosphatidylcholine (PC)–sodium cholate mixtures (Meyuhas and Lichtenberg, 1995). Albumin binds very strongly seven cholate molecules regardless of the PC concentration. In the PC–cholate system the concentration of albumin alters the size of the PC vesicles formed such that at low albumin concentrations the vesicles are smaller whereas at high albumin concentrations the vesicle size increases appreciably.

Albumin can also induce changes in phase in dioleoylphosphatidylcholine–oleic acid (DOPE–OA) vesicles by binding OA, resulting in the transformation from the lamellar phase to the hexagonal II phase (inverted micellar phase) (Straubinger et al., 1985). PEs commonly form hexagonal II phases, but the bilayer phase is found in the presence of OA so the removal of the OA by binding to albumin triggers the phase transformation back to the hexagonal II phase.

2.2. Surfactant interactions with membranes

The interaction of surfactants with cell membranes resembles that with lipid bilayers, with the additional implications of the interaction of surfactant monomers with the membrane proteins and glycoproteins. The sequence of solubilisation can be described as follows:

Membrane

$\downdownarrows + S$

Membrane S_n Saturation

$\downdownarrows + S$

Membrane S_n Lysis

$\downdownarrows + S$

$\text{Lipid} - \text{protein} - S_m$ complexes

$+ \text{Lipid} - S_p$ mixed micelles

Disruption and solubilisation

$\downdownarrows + S$

$\text{Protein} - S_p$ complexes

$+ \text{Lipid} - S_r$ mixed micelles

$+ S_s$ surfactant micelles

As with lipid bilayers the first step involves saturation of the membrane with monomeric surfactant, the membrane may then lyse with release of the cytoplasmic contents. Membrane breakdown occurs initially into lipid–protein–surfactant complexes and mixed lipid–surfactant micelles. Further addition of surfactant generally causes the lipid–protein–surfactant complexes to become richer in surfactant as more mixed micelles are formed. The final state may ideally be a mixture of protein (and glycoprotein)–surfactant complexes, mixed micelles and surfactant micelles, which will be in equilibrium with free surfactant at the CMC of the system. In practice complete delipidation of the protein complexes may not occur, particularly if the protein has a strong affinity for a particular lipid. As for lipid bilayers, the total amount of surfactant required to solubilise the membrane will increase with the CMC of the surfactant.

Choice of surfactant can influence the mode of solubilisation in other ways apart from the value of the CMC. For example, sodium cholate which forms mixed micelles with a high lipid to cholate molar ratio, due to the unusual stereochemistry of the cholate molecule, will remove lipid from cell membranes preferentially at low cholate concentrations, while still leaving the membrane unlysed. The ratio of solubilised protein to solubilised lipid initially falls below unity as lipid is removed. Such an effect has been observed on interaction of cholates with the human erythrocyte membrane (Kirkpatrick et al., 1974) human platelets (Shiao et al., 1989) and trout gill epithelial cells (Partearroyo et al., 1991).

2.3. Separation of integral membrane proteins

The separation of integral (intrinsic) membrane proteins from peripheral (extrinsic) membrane proteins has been achieved by several general methods. Early studies depended on the use of salts or chaotropic agents which weakened the ionic interactions between the bilayer surface and peripheral proteins, or disrupted the hydrophobic interactions between transmembrane domains of integral proteins or glycoproteins. For example, the strength of attachment by erythrocyte membrane proteins to the bilayer was demonstrated by membrane extraction with increasing concentrations of sodium iodide (Steck, 1974) which resulted in removal of the peripheral proteins leaving only Band 3, the anion transporter, at the highest concentration of sodium iodide. The chaotropic agent lithium diiodosalicylate was used by Marchesi and Andrews (1971) to release glycophorin A, the major sialoglycoprotein of the human erythrocyte membrane, prior to isolation

by partitioning in the aqueous phase of a phenol–water two-phase system.

The partitioning of membrane proteins between two phases, one of which is hydrophobic and the other hydrophilic, was brought about by Bodier (1981) using the Triton non-ionic surfactants. The Tritons form a homogeneous micellar solution which on heating passes through a cloud point and separates into two phase, one rich in micellar surfactant and an aqueous phase depleted in surfactant. The micellar phases, contain large micelles which are possibly formed by association of smaller micelles at the cloud point and it is hence capable of solubilizing hydrophobic membrane proteins. The temperature of the cloud point depends on the number of oxyethylene units (n) in the surfactant. Thus for Triton X-100 ($n=9$ or 10) the cloud point is 64°C which is generally too high for membrane protein partitioning if the possibility of thermal denaturation is to be avoided. Triton X-114 ($n=1$ or 8) has a cloud point of 20°C and is thus ideal for partitioning. Bodier (1981) demonstrated that hydrophilic globular proteins such as serum albumin, catalase, ovalbumin, concanavalin A, myoglobin and cytochrome *c* partition predominantly into the Triton-depleted (upper) aqueous phase whereas hydrophobic membrane proteins such as acetylcholinesterase, bacteriohodopsin and cytochrome *c* oxidases from several sources partitioned predominantly into the Triton-rich (lower) micellar phase. In the case of bacteriorhodopsin the partitioning between the phases reflected the partitioning of the Triton X-114 itself. Specifically, approximately 95% of the Triton was in the micellar phase which held 80% of the bacteriorhodopsin. In contrast, for a globular protein such as haemoglobin over 95% partitioned into the aqueous Triton-depleted phase. This is a valuable general method for the separation of intrinsic and extrinsic membrane proteins and it has been used to isolate and purify integral membrane protiens of the mammalian visual transduction cascade (Justice et al., 1995). Care must, however, be taken if other surfactants are present in the system, other surfactants, particularly those more hydrophilic than Triton X 114 will raise the cloud temperature and can prevent phase separation altogether.

3. Surfactants in reconstitution of membrane proteins

A common aim in the study of membrane proteins/glycoproteins is to reconstitute the molecule of interest into a bilayer system, frequently a phospholipid vesicle or a bilayer lipid membrane (BLM) formed across an orifice in a hydrophobic support such as a Teflon partition. The attraction of such a reconstituted system is that the function of the protein can be studied in isolation without complications from other membrane components. The function of transport proteins in phospholipid vesicles is very conveniently followed by monitoring the uptake of substrate by the vesicles, for example. There are several possible mechanisms suggested for the reconstitution of membrane proteins which have been discussed in detail by Helenius et al. (1981); all depend on the initial solubilisation of the membrane protein by surfactant followed by removal of the surfactant.

The simplest is the progressive removal of surfactant from a solubilized mixture of lipid–surfactant–protein and lipid–surfactant mixed micelles by dialysis. This results in the reformation of a bilayer phase which on further surfactant removal can result in the formation of sealed vesicles with the protein incorporated in the bilayer more or less symmetrically, if the protein has a recognisable asymmetry with an outward and inward facing orientation in the natural membrane. Another method of reconstitution is to mix surfactant–protein complexes with preformed lipid vesicles, whereupon the gradual removal of the surfactant by dialysis will result in the insertion of the protein into the bilayer. In this case, because the vesicles are preformed and the protein can only be inserted from the outside of the vesicles the protein orientation will be predominantly asymmetric. Finally, lipid–surfactant mixed micelles can form lipid–surfactant vesicles in the presence of protein–surfactant complexes, when the surfactant to lipid ratio is reduced, resulting in lipid vesicles plus lipoprotein complexes. The reconstitution process in vesicles can often be improved by the introduction of a freeze–thaw step. Freeze–thawing results in the formation of larger

vesicles by fusion of smaller ones. While the mechanism of the fusion processes is not fully understood, it has been suggested that on freezing, water molecules crystallise on the charged phospholipid interfaces and form planes between bilayers of adjacent vesicles. The bilayers are then easily fractured and the exposed hydrophobic cores fuse to give rise to larger vesicles during slow thawing (Pick, 1981).

The choice of surfactant for reconstitution studies has been discussed by Jones and Chapman (1995d) with reference to the reconstituion of membrane transporters and receptors in a variety of phospholipid systems. The choice of surfactant for a particular reconstitution study depends on the following factors.

- The surfactant must readily solubilise the protein/glycoprotein from the cell membrane without initiating denaturation. Non-ionic surfactants such as Tritons, and octylglucoside and the anionic surfactants sodium cholate and deoxycholate are very frequently chosen.
- Since once solubilized the surfactant must be progressively removed by dialysis, surfactants with high CMCs are preferable, other factors being equal, since the higher the CMC the more easily the surfactant monomer can be dialysed out of the system. Octylglucoside CMC (23 mM) is thus a favourite choice although its cost is high. Octylthioglucoside is cheaper and also has a relatively high CMC (9 mM) (Saito and Tsuchiya, 1984).
- During a reconstitution study it is useful to follow the solubilization and reconstitution by assaying for the amount of protein/glycoprotein in the system. The ratio of protein/glycoprotein to lipid is a convenient measure of the level of reconstitution that has been attained in vesicles in relation to the functional activity of the reconstituted system. It is thus important that the surfactant used does not interfere with the protein/glycoprotein assay or the assay of functional activity. In this respect the Tritons which absorb strongly at 280 nm, the wavelength frequently used to monitor protein concentration, are far from ideal. However, some reduced Tritons in which the phenol group has been replaced by the cyclohexyl group are now available and have the added advantage of a 12% higher CMC (Tiller et al., 1984).
- The stability of the surfactant is also of importance, especially as a reconstitution may take several days to complete. Unsaturated surfactants and those prone to hydrolysis and/or autooxidation are best avoided.

Other factors which might need to be considered, such as surfactant purity, toxicity, ease of radiolabelling or availability in radiolabelled form, have been discussed by Jones et al., (1987a).

4. Solubilisation of specific membrane proteins and glycoproteins

Table 2 shows some examples of specific membrane proteins and glycoproteins which have been solubilised and the surfactants which have been used in their isolation. As most of these studies have as their objective the isolation of the membrane constituents in an active form, the surfactants used are predominantly non-denaturing. In many of these studies the objective has been to isolate a particular membrane protein or glycoprotein and then to incorporate it into a bilayer system such as phospholipid vesicles, in order to assess functional characteristics of the material in the absence of other complicating cellular processes. Such reconstitution studies are of individual membrane processes involving transporters and receptors and as well as identifying which particular membrane component can be associated with a particular function, can also be used to assess the role of the lipid environment and in some cases specific lipid involvement on function.

As can be seen from Table 2 the range of materials which can be solubilised by surfactants is very wide. Perhaps one of the most studied systems is the human erythrocyte plasma membrane from which the anion, and glucose transporters have been extensively studied. The sialoglycoproteins particularly the glycophorins which are responsible for most of the negative charge on the membrane surface have also been frequently isolated and reconstituted in phospholipid vesicles. As shown in Table 2 several differ-

Table 2
Surfactants used in the isolation of functional membrane proteins/glycoproteins

Tissue source	Membrane protein/glycoprotein	Surfactants used	Reference
Human erythrocyte plasma membrane	Glycophorin A	Sodium deoxycholate	Segrest et al., 1979
Human erythrocyte plasma membrane	Band 3 (anion transporter)	$C_{12}E_9$, Triton X-100, Brij 58	Wong, 1993 Schuck et al., 1995
Human erythrocyte plasma membrane	Glucose transporter (GLUT 1)	OBG, Triton X-100	Baldwin et al., 1982 Englund and Lundahl, 1993
Human erythrocyte plasma membrane	Kell protein and glycophorin C	$C_{12}E_9$, $C_{13}E_{15}$, $C_{12}E_{23}$	Khamlichi et al., 1994
Human erythrocyte plasma membrane	All integral proteins/glycoproteins	SDS	Lundahl et al., 1992
Human erythrocyte plasma membrane	Mg^{2+}-ATPase	C_{12},E_9	Zimmerman and Daleke, 1993
Bovine heart muscle	Glucose transporter (GLUT 4)	Sodium cholate	Wheeler and Hauck, 1985
Bovine heart mitochondria membrane	Cytochrome oxidase	Triton X-100	Scotto and Zakin, 1986
Bovine retina	Visual pigment (rhodopsin)	OBG	Jackson and Litman, 1982
Bovine kidney brush border membrane	Sodium phosphate symporter	CHAPS	Vachon et al., 1995
Rabbit small intestine brush border vesicles	Na^+-dependent α-amino acid transporter	$C_{12}E_8$, CHAPS	Nakanishi et al., 1993
Rabbit small intestine brush border	Sucrose isomaltase	Triton X-100, sodium cholate	Brunner et al., 1978
Rabbit liver	β-Adrenergic receptor	Lubrol PX	Pederson and Ross, 1982
Rabbit skeletal muscle sarcoplasmic reticulum	Ca^{2+}-ATPase	$C_{12}E_8$	Lévy et al., 1992
Rat adipocyte	Insulin receptor	Sodium cholate	More and Jones, 1983
Rat ascitic fluid	Immunoglobulin E receptor	Triton X-100, CHAPS OBG, sodium cholate	Klausner et al., 1980
Rat liver mitochondrial membrane	Plasma membrane fatty acid binding protein, aspartate amino transferase	Triton X-100	Stump et al., 1993
Rat liver liposomal membranes	Sulphate transporter	Triton X-100	Koetters et al., 1995
Rat brain	γ-Hydroxybutyrate receptor	Triton X-100	Cash et al., 1996
Rat liver	Trihydroxycoprostanoyl-CoA synthase	Triton X-100	VanVeldhoven et al., 1996
Rat adipocyte plasma membrane	Glucose transporter (GLUT 4)	Sodium cholate	Gorga and Lienhard, 1984
Pig-kidney brush border membrane	Nitrobenzylthioinosine binding site	CHAPS	Ciruela et al., 1994
Pig brain stem	Na^+–Cl^- coupled glycine transporter	CHAPS, sodium cholate	Lopez Corcuera et al., 1993
Pig liver microsomes	UDP glucuronosyltransferase	Triton X-100	Lopez Corcuera et al., 1993
Ehrlich ascites tumour cell plasma membrane	Nucleoside transporters	OBG	Hammond, 1994
Brain capillaries	Transporters	Triton X-114	Pouliot and Beliveau, 1994
Ascaris suum muscle	Membrane aminopeptidase	Triton X-100	Sayid et al., 1997
Escherichia coli	Lactose transporter	OBG	Garcia et al., 1983
Escherichia coli	Maltose-binding protein dependent maltose transporter system	Triton X-100	Panagiotidis et al., 1993

Table 2 (*Continued*)

Tissue source	Membrane protein/glycoprotein	Surfactants used	Reference
Purple membrane *Halobacterium halobium*	Bacteriorhodopsin	Triton X-100	Lévy et al., 1990 Rigaud et al., 1988
Bacillus lichenformis	Penicillinase	OBG	Helenius et al., 1981
Trypanosoma brucei	D-Glucose transporter	OBG, octylthioglucoside	Seyfang and Duszenko, 1993
Torpedo californica	Acetylcholine receptor	Sodium cholate	Jones et al., 1987a
Semliki forest virus	Spike glycoprotein	OBG	Helenius et al., 1981
Vesicular stomatitus viruses	G and M envelope protein	OBG	Paternostre et al., 1997
M13 bacteriophage	Coat protein	Sodium cholate	Florine and Feigenson, 1987
Spinach chloroplasts	H^+-ATPase	Triton X-100	Lévy et al., 1990 Richard et al., 1990
Spinach chloroplasts	Photosystem II	OBG, *n*-dodecyl-β-D-maltoside	Hankamer et al., 1997
Pea photosynthetic system	Chlorophyll *a*/*b* light harvesting complex	Triton X-100	McDonnel and Donner, 1981
Zucchini (zea) plasma membrane	Auxin-binding protein	Triton X-114	Hicks et al., 1993
Castor oil seeds endosperm	Acid lipase	CHAPS	Fuchs et al., 1996 Altaf et al., 1997
Vero cell plasma membrane	Kunjin virus receptor	OBG	Sankaran et al., 1997
Ricinus cotyledons	H^+-ATPase, H^+-PPase	*n*-Dodecyl-β-D-maltoside	Long et al., 1997

ent none denaturing surfactants have been used to isolated erthrocyte membrane proteins. For a number of membrane proteins systematic studies have been carried out to determine what influence choice of surfactant has on the nature of the extract—e.g. its oligomeric state and/or functional characteristics. It is appropriate to consider a few selected examples where comprehensive studies have been made.

4.1. Erythrocyte plasma membrane anion transporter (Band 3)

The anion transporter is an integral membrane protein of molecular mass approximately 95 000. It is responsible for Cl^-/HCO_3^- exchange and comprises approximately 50% by weight of the total integral membrane protein. The amino acid sequence has been deduced from cDNA sequencing (Tanner et al., 1988) and hydropathy analysis predicts that it spans the membrane bilayer 14 times (Anstee, 1990). A carboxyl-terminal 52 000 molecular mass domain produced by mild trypsin treatment mediates anion transport independently of the remaining 43 000 molecular mass amino-terminal cytoplasmic domain. The transporter is oligmeric both in the membrane and in surfactant solutions and exists as a mixture of dimers and tetramers (Jennings, 1984); the tetramers are believed to interact with the cell cytoskelton through ankyrin (Low, 1986). A systematic study of the oligomeric state of the transporter in various surfactant has been reported by Casey and Reithmeier (1993). The transporter was isolated in octaethylene glycol dodecyl ether ($C_{12}E_8$) tagged with [^{14}C]$C_{12}E_8$ and size exclusion high-performance liquid chromatography (HPLC) was used to study the exchange of $C_{12}E_8$ by other surfactants and the oligomeric state of the protein. The $C_{12}E_8$ surfactant could be displaced from the transporter provided that the displacing surfactant concentration was in excess of the CMC; however, for all the non-ionic surfactants studied, the transporter was found to be dimeric, only SDS disperses it in the monomeric state. Table 3 shows some of the data for the C_xE_y non-ionics, Triton X-100 and SDS. The minimum surfactant concentration required to maintain dispersion of the transporter is always in excess of the CMC and for the $C_{14}E_8$ and $C_{16}E_8$ surfactant 100 times the CMC. The model proposed for the transfer of the $C_{12}E_8$-complexed transporter to complexes in other surfactants involves the progressive exchange of the $C_{12}E_8$ from a micellar array around

Table 3
Oligomeric state of the human erythrocyte anion transporter in surfactants[a]

Surfactant(s)	CMC (mM)	Aggregation number	$[S]_{min}$ (mM)[b]	$[S]_{min}$/CMC	State
C_8E_5	6.0	32	30	5	D
$C_{10}E_6$	0.46	76	0.92	2	D
$C_{10}E_8$	0.28	–	2.8	10	D
$C_{12}E_6$	0.065	105	0.33	5	D
$C_{12}E_8$	0.056	120	0.112	2	D
$C_{14}E_8$	0.0052	–	0.52	100	D
$C_{16}E_8$	0.00047	–	0.047	100	D
Triton X-100_{red}	0.21	140	1.4	7	D
SDS	0.58	100	5.8	10	M

[a] From Casey and Reithmeier (1993).
[b] Minimum concentration of surfactant required to maintain the transporter in the dispersed dimeric state (D).

the transmembrane domain of the protein by the second exchanging surfactant until the micellar array consists entirely of the second surfactant. The transporter binds micellar amounts of surfactant, however it is impossible to distinguish between highly cooperative binding of monomeric surfactant from the binding of micelles. Binding of micellar surfactant implies a minimal 1:1 stoichiometry of surfactant micelles to protein so that:

$$[S]_{min} = CMC + [protein] \times (\text{surfactant aggregate number}) \quad (2)$$

Thus at a protein concentration of 1 g/l for $C_{12}E_6$ the minimum concentration of bound surfactant would be $[1/(2 \times 95\,000)] \times 105 = 0.53$ mM, which is approximately 10 times the CMC (see Table 3), and which is larger than the minimum surfactant concentration required for dispersion of the dimer and suggests that a cooperative monomer binding mechanism of dispersion is perhaps more likely.

Although in most of the non-ionic surfactants the transporter retains its native structure, it is found to denature in C_8E_5 and in octylglucoside at high concentrations and hence these surfactants are not suitable for Band 3 isolation.

4.2. Mitochondrial porins

The outer membrane of Gram negative bacteria, mitochondria and chloroplasts are permeable to low molecular mass molecules (≈ 600) due to the presence of a class of integral membrane proteins called porins. Porins have a molecular mass of the order of 36 000 and in, for example, the outer membrane of *Escherichia coli*, are arranged in a hexagonal array of trimers. Structurally, unlike many transmembrane proteins, porins consist almost entirely of β-sheet and β-

Table 4
Surfactants used in mitochondrial membrane and porin solubilisation[a]

Surfactant	HLB[b]	CMC[c](mM)
1. Triton X-35 $n = 3$ or 4[d]	7.8	–
2. Triton X-45 $n = 4$ or 5	10.4	0.1
3. Triton X-114 $n = 7$ or 8	12.4	0.21
4. Triton X-100 $n = 9$ or 10	13.5	0.24–0.3
5. Triton X-102 $n = 12$ or 13	14.6	0.33
6. Triton X-165 $n = 16$	15.8	0.43
7. Triton X-405 $n = 40$	17.9	0.81
8. Brij 30	9.7	–
9. Brij 35	16.9	–
10. Brij 52	5.3	–
11. Brij 58	15.7	0.077–0.0039
12. Brij 78	15.3	–
13. Triton N-57	10.0	–
14. Triton N-101	13.4	0.085
15. Nonidet NP 40	13.1	0.029
16. Lubrol WX	14.9	0.02–0.06
17. Tween-20	16.7	–

[a] From De Pinto et al. (1989).
[b] HLB, hydrophilic/lipophilic balance number.
[c] CMC, critical micelle concentration.
[d] n, number of oxyethylene units.

barrel structures. The pores formed by the porins are non-specific and allow the small hydrophilic molecules to cross the bilayer. A very comprehensive study of the solubilisation of the porin of bovine heart mitochondria has been carried out by De Pinto et al. (1989). These authors studied the solubilisation of the porin and the total membrane protein by almost 50 surfactants covering several different classes of compounds with a wide range of hydrophilic/lipophilic balance (HLB) and CMC (Table 4). Fig. 1 shows the extent of solubilization of total mitochondrial membrane protein and porin as a function of HLB. The data were obtained by radiolabelling the membrane with [^{14}C]dicyclohexylcarbodiimide which, as well as labelling membrane H^+-ATPase, also labels porin. Solubilisation is seen to pass through a maximum and maximum porin solubilisation occurs using a surfactant with an HLB of around 12.5. This result is consistent with previous work which suggests that surfactants most effective in membrane protein solubilisation have HLB values in the range 12–15 (Helenius and Simons, 1975).

The effects of systematic structural changes on solubilisation within a given surfactant class are shown in Fig. 2. For the Tritons, maximum porin solubilisation occurs for Triton X-114. For the alkyldimethylamino *N*-oxide series, total mitochondrial membrane proteins are solubilized with chain lengths of 10–12 carbon atoms. Porin was completely solubilized by dodecyl(dimethyl)-amine oxide (DDAO). Studies on the DDAO extract showed that, if it was extracted with chloroform–methanol, only cholesterol was found in the extract, corresponding to 5 moles of cholesterol per mole of porin (35 000 molecular mass) which were essential for the pore function of porin. The results with the zwittergent series of surfactants were similar to those of the amine oxides in that an optimum alkyl chain length of 12–16 carbon atoms was found for solubilisation in the case of the total membrane protein, while 100% solubilisation of

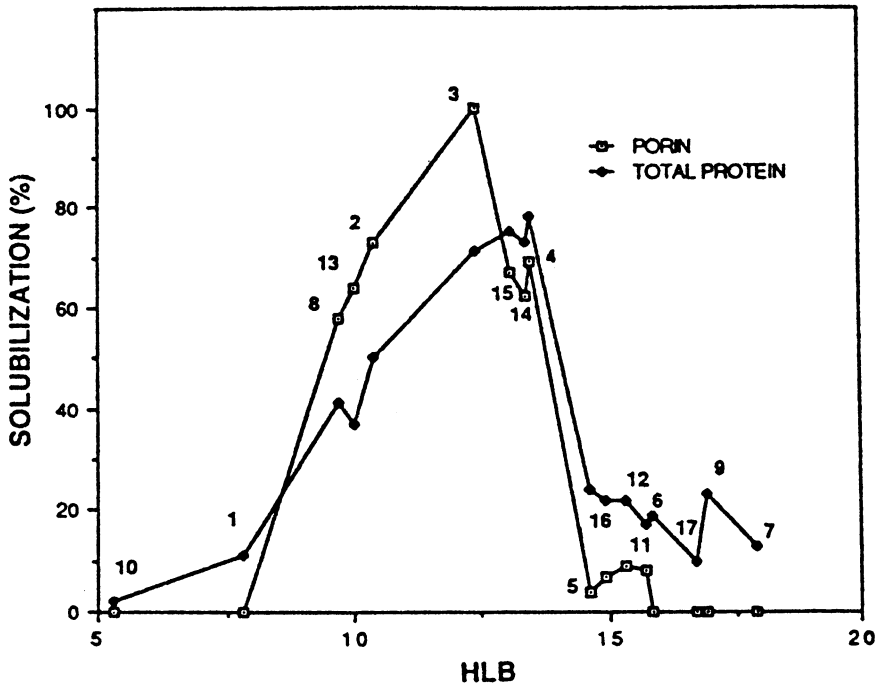

Fig. 1. Solubilisation of total mitochondrial membrane protein and porin with surfactants covering a range of HLB. The surfactant concentrations were 2% (w/v). The numbers refer to the surfactants listed in Table 4. Reproduced from De Pinto et al. (1989), with permission.

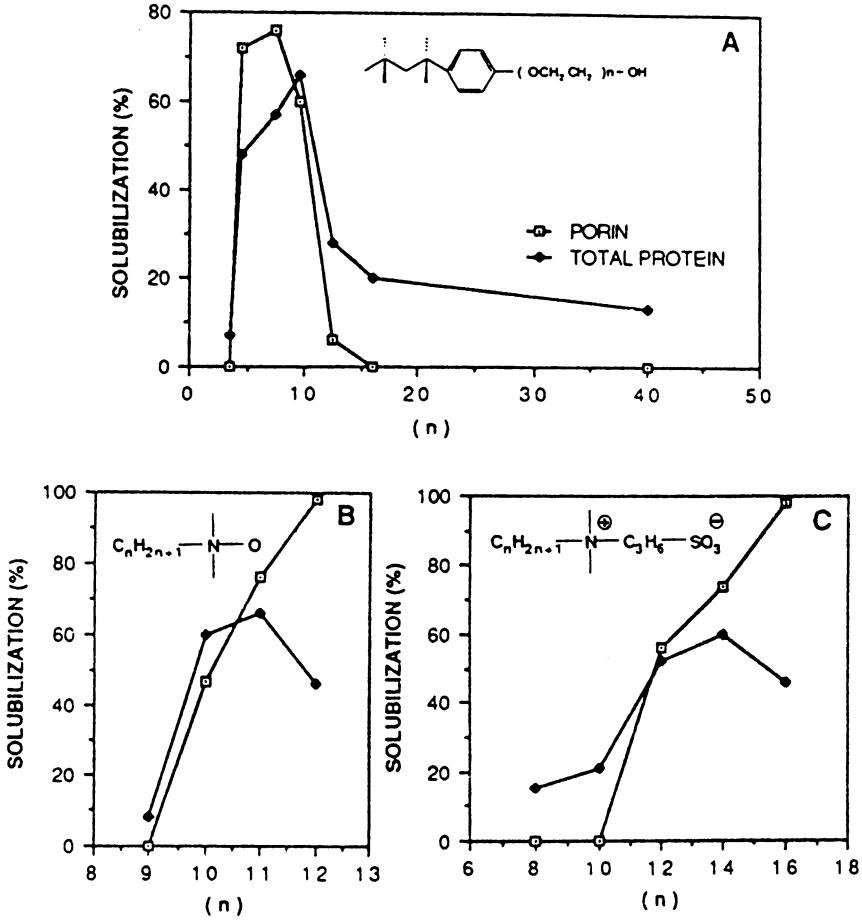

Fig. 2. Solubilisation of total mitochondrial membrane protein and porin with structural changes in three classes of surfactant (concentrations 1%, w/v). (A) Triton X series where *n* is the number of oxyethylene units (see Table 4). (B) Alkyldimethylamino *N*-oxide series where *n* is the number of carbon aroms in the alkyl chain. (C) Zwittergent series (*n* as in B). Reproduced from De Pinto et al. (1989), with permission.

porin was achieved with the C16 zwittergent (Z316).

These results demonstrate that porin solubilisation, compared to total mitochondrial protein, was more efficient with the more hydrophobic members of each series. With most of the surfactants studied the protein eluted as protein–surfactant micelles in the void volume of hydroxyapatite/celite columns.

4.3. Sarcoplasmic reticulum Ca^{2+}-ATPase

Sarcoplasmic reticulum Ca^{2+}-ATPase is a membrane protein which is vulnerable to deactivation when removed by surfactant extraction from the membrane bilayer. It has however been possible to prepare active preparations by use of $C_{12}E_8$, myristoylphosphoglycerocholine, Tween 80, dodecyl-β-D-maltoside (Lund et al., 1989 Kraghhansen et al., 1993) and *N*,*N*-dimethylalkylamine *N*-oxides (Andriamainty, et al., 1997). The enzyme in the presence of Ca^{2+} ions (the so-called E_1 state) is more stable than in the presence of EGTA [ethylene-bis(oxyethylenenitrilo)tetraacetic acid] (the so-called E_2 state). The inactivation rate constants in a wide range of surfactants have been measured in the E_1 state (Lund et al., 1989) and some of the data are shown in Table 5. Also

Table 5
Inactivation of lipid-depleted Ca^{2+}-ATPase (pH 7.5, 20°C) by surfactants in the E_1 state and during turnover (addition of 5 mM Mg ATP)[a]

Surfactant	k_{E1} (min^{-1})	k_{TO} (mm^{-1})	V_o (μmol/mg/min)
C_8E_4	0.41	4.2	0.25
$C_{12}E_8$	0.0020	0.08	5.6
$C_{12}E_{<10>}$	0.0023	0.11	4.1
$C_{18}E_{<10>}$(Brij 76)	0.125	1.4	0.9
Octylglucoside	0.16	1.1	0.27
Decylmaltoside	0.0015	0.12	0.70
Dodecylmaltoside	0.0016	0.07	1.6
Deoxycholate	0.155	–	–
CHAPS	0.0055	0.02	0.1
Dodecyldimethylamine oxide	1.3	3.5	0.3
$C_{12}E_{<4>}$ (Brij 52)	0.0021	–	–
$C_{12}SE_{<20>}$ (Tween-80)	0.0019	–	<0.1
Diocytphosphatidylcholine (di C_8 PC)	0.21	98	–

[a] Data from Lund et al. (1989. k_{E1}, inactivation rate constant measured in the E_1 state; k_{TO}, inactivation rate constant; V_o, the initial velocity during turnover.

shown are the inactivation rate constants and initial reaction velocity during turnover in the presence of Mg-ATP. Although the inactivation rate constants vary widely amongst the different types of surfactants they are considerably higher during turnover (k_{TO}) than in the E_1 state (k_{E1}). Excluding steroid-based surfactants there is an inverse correlation between k_{TO} and V_o. It is suggested that the higher degree of lability during turnover is related to the different stabilities of the various intermediates in the catalytic cycle.

The effects of hydrophobic chain length on the inactivation constant k_{E1} is very considerable, short-chain surfactants such as C_8E_4, octylglucoside (OBG) and dioctylphosphatidylcholine have inactivation rate constants 80–200 times greater than found for $C_{12}E_8$. Deoxycholate also inactivates with a rate constant 78 times that of $C_{12}E_8$. In general, polyoxyethylene glycol and glucoside detergents are the head groups of choice for extraction with retention of activity. It should be noted that some surfactants, particularly the long-chain members of the Tween family, show a rapid and a slow phase due to the formation of monomeric and oligomeric Ca^{2+}-ATPase–surfactant complexes respectively.

Several different modes of surfactant binding to Ca^{2+}-ATPase can be envisaged as illustrated in Fig. 3. The hydrophobic domain of the enzyme can be envisaged to insert into a lamellar surfactant phase or surfactant binding can be of the micellar or monolayer type. The binding of the series of non-ionics, dodecyldimethylamineoxide, dodecylmaltoside, $C_{12}E_8$ and Triton X-100 decreases in the order given and is inversely related to the cross-sectional areas of the surfactants in monolayers at an air–water interface and in micelles (Møller and Lemaire, 1993). From the size of the hydrophobic domain of the enzyme and the area per molecule of the surfactants it is concluded that monolayer binding to the hydrophobic domain is the most appropriate model.

4.4. ATPase-active P-glycoprotein

The multidrug transporter P-glycoprotein is a member of the class of traffic ATPases of membrane-associated proteins. It can be isolated from the plasma membranes of a multidrug-resistant cell line of Chinese hamster ovary cells using CHAPS as the surfactant (Doige et al., 1993 Sharom et al., 1995) and has a molecular mass of the order of 175 000. In 0.4 mM CHAPS it has an ATPase activity of the order of 0.3–0.4 μmol/min/mg. Studies have been made on the effects of a range of surfactants on the ATPase activity of

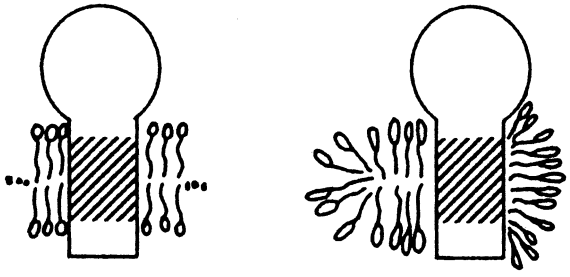

Fig. 3. Schematic representation of different modes of binding of surfactants to delipidated Ca^{2+}-ATPase: (A) insertion into lamellar surfactant phase; (B, left-hand side) micellar type binding; (C, right-hand side) monolayer type binding. The hatched areas of the Ca^{2+}-ATPase represent the hydrophobic domains. Reproduced from Lund et al. (1989), with permission.

partially purified P-glycoprotein solubilised in 0.4 mM CHAPS which have demonstrated some interesting activation effects and also phospholipid protection against thermal inactivation.

Fig. 4 shows the effects of a range of surfactants on the ATPase activity relative to the activity in 0.4 mM CHAPS. CHAPS, octylglucoside and deoxycholate all deactivated at high concentrations. Addition of low levels of octylglucoside or deoxycholate, however, stimulate activity. This pattern of behaviour is also seen on the addition of Triton X-100 but at much lower concentrations (Fig. 4B). These data suggest that CHAPS is the surfactant of choice for isolation of the P-glycoprotein. It was found that after CHAPS solubilisation at a concentration of 8 mM the preparation could be stored with no loss of activity for several months at −70°C, but activity was lost on storage at 4°C. Dialysis to remove more than 99% of the CHAPS resulted in a loss of 10–20% activity. The retention of activity after removal of CHAPS suggested that the P-glycoprotein had tightly bound annular lipid. To test this hypothesis the thermal stability of the glycoprotein was assessed in the presence of a range of membrane lipids to determine the levels of protection given by different lipids. Fig. 5 shows the results of experiments in which P-glycoprotein in 0.4 mM CHAPS was incubated for 1 h at 23°C with various phospholipids. In the absence of added lipids the ATP activity falls to approximately 25% of its initial value after 1 h at 23°C. The results in Fig. 5 clearly show that certain lipids, notably asolectin [30% phosphatidylcholine–30% phosphatidylethanolamine (PE)–31% phosphatidylinositol], DPPE and phosphatidylserine (PS) protect P-glycoprotein against thermal denaturation and DPPE restores activity after thermal denaturation. There is a strong correlation between lipid fluidity and the ability to restore activity. Lipids with low chain melting temperatures (T_m) were more effective than those with high T_m. Complete delipidation inactivates ATPase activity but it can be restored by fluid lipid mixtures (Sharom, 1995).

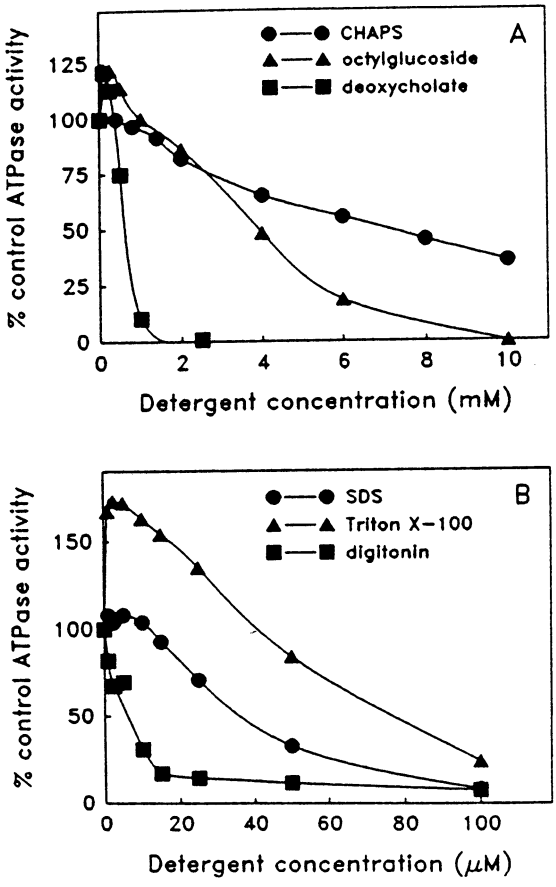

Fig. 4. Effect of various surfactants on P-glycoprotein ATPase activity. The P-glycoprotein (1–2 μg per 100 μl) was solubilised in 0.4 mM CHAPS and had an initial activity of 0.3–0.4 μmol/min/mg. Reproduced from Doige et al. (1993) with permission from Elsevier Science BV.

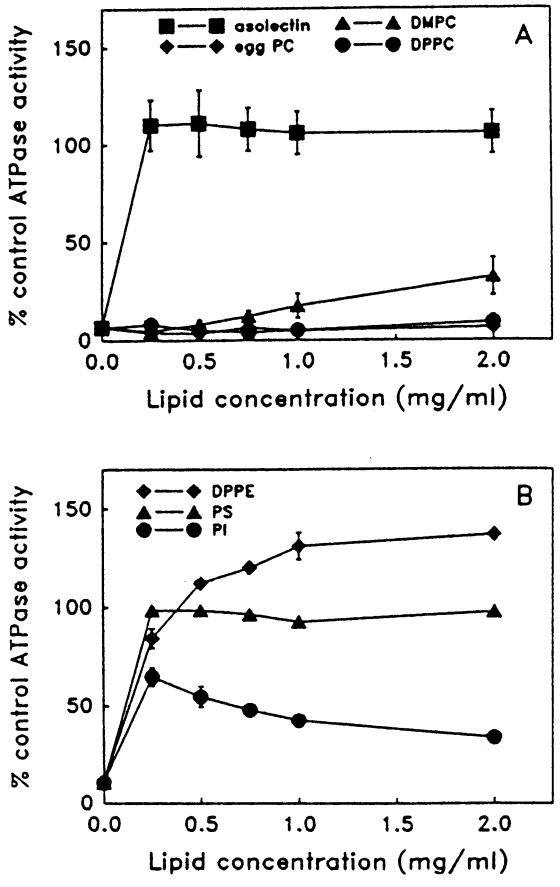

Fig. 5. Protection against thermal inactivation of the P-glycoprotein ATPase activity by phospholipids. The P-glycoprotein (1–2 μg per 100 μl) in 0.4 mM CHAPS was incubated for 1 h at 23°C with the lipids shown. In the absence of lipids the activity falls to approximately 25% of its initial value (0.3–0.4) μmol/min/mg). Reproduced from Doige et al. (1993) with permission from Elsevier Science BV.

P-glycoprotein has also been found to use alkylphenol exothylate surfactants as substrates. Thus P-glycoprotein can protect organisms from the toxic and oestrogenic effects of, for example, nonylphenol ethoxylate, but not from the biodegradation product nonylphenol (Loo and Clarke, 1988).

4.5. Bacteriorhodopsin

Bacteriorhodopsin is a light-driven proton pump which constitutes 75% of the mass of the purple membrane of *Halobacterium halobium*, the remaining 25% being lipid. It has a molecular mass of 25 000 and consists of seven transmembrane helices. The chromophore is a retinal molecule covalently linked to a lysine residue (lys-216) by a Schiff base. In the dark the retinal is a mixture of the 13-*cis* and all-*trans* isomers which on irradiation with light coverts to the all-*trans* isomer. The light energy is converted into a transmembrane proton gradient which is used to synthesise ATP. In the membrane, bacteriorhodopsin occurs as trimers although proton pumping is brought about by the monomeric species.

The solubilisation of the purple membrane by surfactants, unlike many membrane solubilisation processes which occur in minutes, takes hours to days to reach equilibrium. The reason for the slow solubilisation is suggested to relate to the tight crystalline organisation of the membrane which prevents insertion of surfactants into the bilayer rather than any specific effects attributable to the bacteriorhodopsin molecule itself (Viguera et al., 1994). Triton X-100 has frequently been used for solubilisation (Viguera et al., 1994 Meyer et al., 1992 Kovács et al., 1995), although studies with a range of surfactants have also been reported (Massotte and Aghion, 1991 Ikematsu et al., 1995). The emphasis of much of the work reported is on the effects of surfactants on the photochemical properties of bacteriorhodopsin. The absorbance spectra of both the light-adapted and dark-adapted molecules have maxima in the region of 550–570 nm with a maximum difference (ΔA) absorption at 590 nm. If a sample is irradiated and ΔA followed as a function of time in the dark the rate of dark adaptation can be quantified as the time (τ) for ΔA to fall to e^{-1} of its initial value (Massotte and Aghion, 1991). In the absence of surfactant τ is 41 min, which compares with values of 21 min (SDS), 30 min (deoxycholate) and 40 min (dodecylmaltoside). Two surfactants, Triton X-100 and Nonidet P40 (octylphenolethylene oxide condensate), however, give variable but much longer values of τ and it is in those surfactants that the bacteriorhodopsin dissociates from the trimeric to the monomeric state. In the monomeric state the rate of dark adaptation is very slow. The absorbance maxi mum (λ_{max}) of the dark-adapted *Halobacterium halobuim* membrane is at 565 nm and that of the

light-adapted membrane at 573 nm—i.e. the absorption spectra of the dark-adapted is blue-shifted compared to the light-adapted. The turbidity of the dark-adapted membrane (absorbance at 700 nm), the absorbance at 560 nm and the wavelength of maximum absorption can be used to follow the solubilisation of the membrane on addition of increasing amounts of Triton X-100 (Meyer et al., 1992). Fig. 6 shows that there are a number of steps in the solubilisation

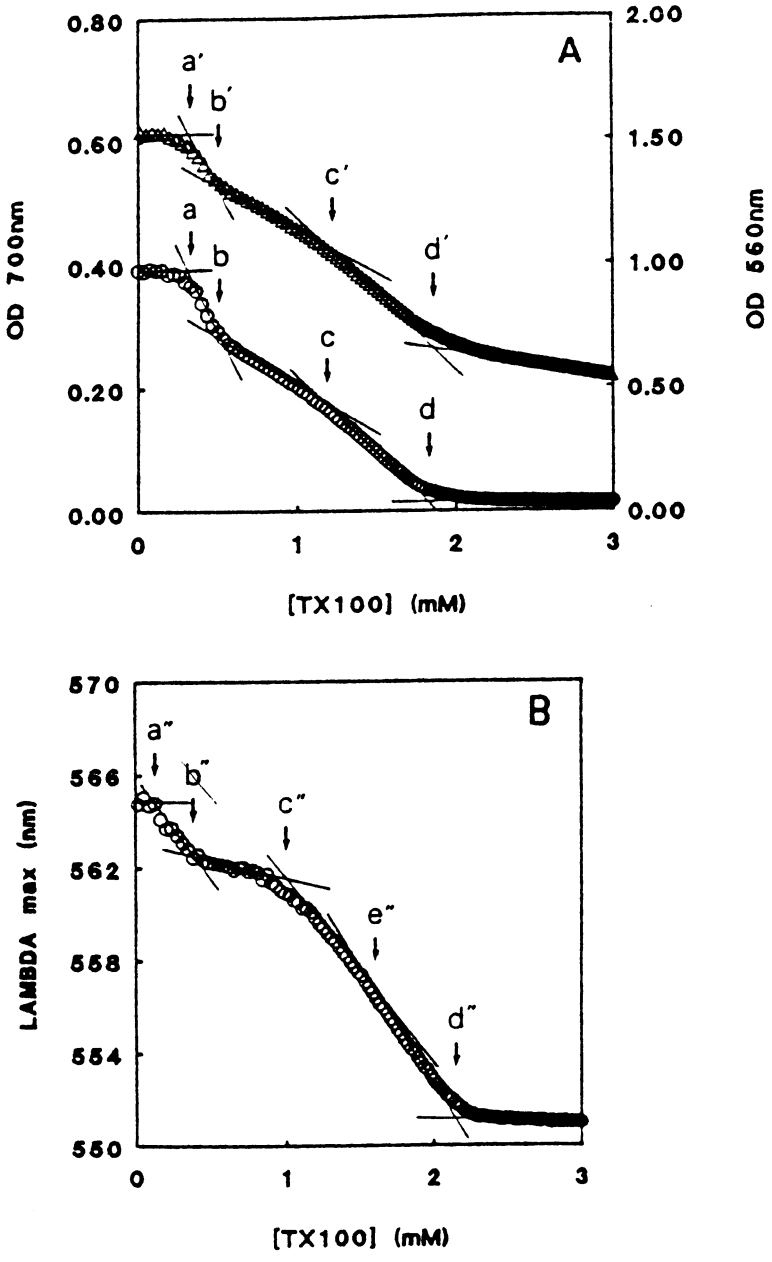

Fig. 6. Solubilization of dark-adapted purple membranes by Triton X-100. (A) Absorbance at 700 nm (o) and 560 nm (Δ). (B) λ_{max}. The bacteriorhodopsin concentration was 0.9 mg/ml. The break points indicated by letters are discussed in the text. Reproduced from Meyer et al. (1992) with permission from Elsevier Science BV.

process which are reflected by breaks in the absorbance and λ_{max} curves. In the absorbance profiles there are four break points (a–d) at both wavelengths which appear at similar Triton X-100 concentrations except for a slight difference between d and d′. The λ_{max} variations are similar but there is a small additional break point e″ and there are significant shifts to both lower (a″, b″ and c″) and higher (d″) Triton X-100 concentrations. The absorbance profiles reflect changes in the size of aggregates in the solubilised membrane dispersion whereas the λ_{max} profiles relate to the microenvironment of bacteriorhodopsin. The break points a, a′ and a″ are perhaps the start of the solubilisation process and d, d′ and d″ the finish, but the points b(b″) and c(c″) cannot be assigned to any specific events. It is suggested that they could relate to the destruction of the membrane bilayer and the formation of monomeric bacteriorhodopsin, respectively. The Triton X-100 (as TX-100) concentrations for all the break points are found to be linearly related to both concentration of bacteriorhodopsin [BR] and the sum of [BR] plus the lipid concentration [L] and are described by the equation:

$$[\text{TX-100}]_{total} = [\text{TX-100}]_{free} + R_{eff}([\text{BR}] + [\text{L}]) \quad (3)$$

where $[\text{TX-100}]_{free}$ is the free monomeric surfactant concentration and R_{eff} the effective surfactant concentration in the membrane aggregates. At the end of the solubilisation process (d″), it is estimated that the solution contains mixed micelles in which there are about 64 molecules of Triton X-100 per bacteriorhodopsin molecule so that each bacteriorhodopsin helix would be surrounded by at least nine surfactant molecules.

The reconstitution of bacteriorhodopsin in liposomes has been brought about by the use of CHAPS, CHAPSO and octylthioglucoside (Denkov et al., 1998) to produce active proteoliposomes of homogeneous size which are very well suited for transport studies (Cladera et al., 1997). Neutron scattering studies have also been reported on monomeric bacteriorhodopsin in reconstituted unilamellar liposomes (Hunt et al., 1997). During the process of liposome reconstitution of bacteriorhodopsin after surfactant removal from lipid–dodecylmaltoside micelles a new gel-like phase was observed and the rate of surfactant removal strongly influenced the final liposome morphology (Lambert et al., 1998).

4.6. Rhodopsin

Rhodopsin is the light-sensitive visual pigment of the rod photoreceptor cells in the retina. It consists of the integral membrane protein opsin with the covalently-linked chromophore 11-*cis*-retinal (total molecular mass 38 000). Like bacteriorhodopsin it is a member of the family of integral membrane proteins which have seven transmembrane helical domains. It is insoluble in aqueous media and requires surfactants for solubilisation. A relatively wide range of surfactants were used by De Grip et al. (1992) in an attempt to produce crystals from bovine rhodopsin suitable for diffraction studies. The long-term thermal stability of rhodopsin in many surfactants is not high and denaturation occurs in 10 min at temperatures between 40 and 60°C. The most suitable surfactants for long-term stability are octylglucoside, octylmannoside, decyl- and dodecylmaltosides, $C_{12}E_8$, $C_{12}E_{10}$ and CHAPSO (De Grip et al., 1992). Triton X-114 has also been used in a phase-partitioning study (Justice et al., 1995).

Rhodopsin contains mannose residues which have been exploited to isolate it from octylglucoside-solubilised extracts by affinity chromatography (Litman, 1992) using a concanavalin A–Sepharose column. The purity of rhodopsin, as determined from the ratio of the absorbance at 280 nm to the absorbance at 500 nm, was critically dependent on the surfactant used to solubilise the rod outer segment disc membranes; octylglucose was found to give rhodopsin of greater purity than material solubilised by cetyltrimethylammonium bromide. Concanavalin A affinity chromatography has been used to isolate rhodopsin from CHAPSO extracts, followed by exchange of the CHAPSO for lauryldimethylamine oxide (LDAO) in which the rhodopsin remains stable for at least 50 h (Schafmeister et al., 1993). The latter study described the design and use of a 24 amino acid peptide surfactant (called a peptitergent), for solubilisation of integral membrane proteins which had a helical se-

quence with a hydrophobic face and a hydrophilic face, although the long term solubility of rhodopsin in the peptitergent was less than in LDAO. An infrared spectroscopic method for the detection of residual surfactant in phospholipid liposomes reconstituted from dodecylmaltoside-solubilised rhodopsin (Pistorius et al., 1994) and the removal of surfactant from surfactant–lipid rhodopsin micelles to form proteoliposomes, by use of inclusion compounds of the cyclodextrin type, have also been described (De Grip et al., 1998).

5. General conclusions

- A very wide range of surfactant types is now available for use in membrane solubilisation and within a given surfactant type structural variations encompassing a range of hydrophilic/lipophilic balance (HLB) are also available.
- The cost and purity of the surfactants to be used can be a consideration. Other factors being equal it is always preferable to choose surfactants which are readily available in pure form and in bulk at relatively low cost.
- The problem presented to researchers in the solubilisation and extraction of a particular membrane protein in an active form is establishing the criteria to be used in making a choice of surfactant. None denaturing surfactants are usually required, but care must be exercised to ensure that a particular surfactant is none denaturing over the entire range of concentration it is likely to be used.
- A rigorous approach to the extraction problem requires the use of a range of surfactant types in order to establish which type gives the most satisfactory results in terms of yield and retention of functionality. The rigorous approach has at present been limited to a few specific membrane proteins.
- Some knowledge of the properties of the protein/glycoprotein to be isolated is helpful, especially if the molecule resembles membrane proteins/glycoproteins for which isolation methods have already been established.
- It should always be borne in mind that each and every membrane protein/glycoprotein is unique and although general principles of solubilisation can be applied to a given membrane component the researcher should always be aware of the possibility of specific idiosyncrasies of protein/glycoprotein interactions which can influence the outcome of a particular choice of surfactant.

References

Alonso, A., Urbaneja, M.A., Goñi, F.M., Caromona, F.G., Canovas, F.G., Gomez-Fernandez, J.C., 1987. Kinetic studies on the interaction of phosphatidylcholine liposomes with Triton X-100. Biochim. Biophys. Acta 902, 237–246.

Altaf, A., Ankers, T.V., Kaderbhai, N., Mercer, E.I., Kaderbhai, M.A., 1997. Acid lipase of castor bean lipid bodies: isolation and characterisation. J. Plant Biochem. 6, 13–18.

Andriamainty, F., Filipek, J., Devinsky, F., Balgavy, P., 1997. Effect of *N*,*N*-dimethylalkylamine *N*-oxidases on the activity of purified sarcoplasmic reticulum (Ca–Mg)ATPase. Pharmazie 52, 240–242.

Anstee, D.J., 1990. Blood-group-active surface molecules of the human red-blood-cell. Vox Sang. 58, 1–20.

Baldwin, S.A., Baldwin, J.M., Lienhard, G.E., 1982. Monosaccharide transporter of the human erythrocyte—characterization of an improved preparation. Biochemistry 21, 3836–3842.

Barthélémy, P., Maurizis, J.C., Lacombe, J.M., Pucci, B., 1998. A new class of sulfoxide surfactants derived from Tris. Synthesis and preliminary assessments of their properties. Bioinorg. Med. Chem. Lett. 8, 1559–1562.

Bauer, K.H., Reinhart, T., Stenz, R., 1997. Dextran fatty acid esters as polymeric surfactants with low hemolytic activity. Macromol. Symp. 120, 39–45.

Beyhl, F.E., 1986. Interactions of detergents with microsomal enzymes: Effects of some ionic and nonionic detergents on glucose-6-phosphatase. IRCS Med. Sci. 14, 417–418.

Bodier, C., 1981. Phase separation of integral membrane proteins in Triton X-114 solution. J. Biol. Chem. 256, 1604–1607.

Brunner, J., Hauser, H., Semenza, G., 1978. Single bilayer lipid–protein vesicles formed from phosphatidylcholine and small intestinal sucrose-isomaltase. J. Biol. Chem. 253, 7538–7546.

Casey, J.R., Reithmeier, R.A.F., 1993. Detergent interaction with Band 3, a model polytopic membrane protein. Biochemistry 32, 1172–1179.

Cash, C.D., Hechler, V., Mersel, M., Maitre, M., 1996. Kinetic characterisation and solubilization of γ-hydroxybutyrate receptors from rat brain. Neurosci. Lett. 209, 25–28.

Ciruela, F., Blanco, J., Canela, E.I., Lluis, C., Franco, R., Mallol, J., 1994. Solubilization and molecular characterization of the nitrobenzylthioinosine binding-sites from pig-kidney brush-border membranes. Biochim. Biophys. Acta 1191, 94–102.

Cladera, J., Rigaud, J.L., Villaverde, J., Dunach, M., 1997. Liposome solubilization and membrane protein reconstitution using Chaps and Chapso. Eur. J. Biochem. 243, 798–804.

De Grip, W.J., van Oostrum, J., de Caluwe′, G.L.J., 1992. Studies towards the crystallization of the rod visual pigment rhodopsin. Crystal Growth 122, 357–384.

De Grip, W.J., Van Oostrum, J., BoveeGuerts, P.H.M., 1998. Selective detergent-extraction from mixed detergent/lipid/protein micelles, using cyclodextrin inclusion compounds: a novel generic approach for the preparation of proteoliposomes. Biochem. J. 3330, 667–674.

Denkov, N.D., Yoshimura, H., Kouyama, T., Walz, J., Nagayama, K., 1998. Electron cryomicroscopy of bacteriorhodopsin vesicles: mechanism of vesicle formation. Biophys. J. 74, 1409–1420.

De Pinto, V., Benz, R., Palmieri, F., 1989. Interaction of non-classical detergents with the mitochondrial porin. A new purification and characterization of the pore-forming unit. Eur. J. Biochem. 183, 179–187.

Doige, C.A., Yu, X.H., Sharom, F.J., 1993. The effects of lipids and detergents on ATPase-active P-glycoprotein. Biochim. Biophys. Acta 1146, 65–72.

El-Sayert, M.Y., Roberts, M.F., 1985. Charged detergents enhance the activity of phospholipase C (*Bacillus cereus*). Biochim. Biophys. Acta 831, 133–141.

Englund, A.K., Lundahl, P., 1993. Isoelectric-focusing in immobilized pH gradients of the glucose-transporter from human red-cell membranes. Electrophoresis 14, 1307–1311.

Florine, K.I., Feigenson, G.W., 1987. Protein redistribution in model membranes-clearing M13 coat protein from calcium-induced gel-phase regions in phosphatidylserine phosphatidylcholine multilamellar vesicles. Biochemistry 26, 2978–2983.

Fuchs, C., Vine, N., Hills, M.J., 1996. Purification and characterisation of the acid lipase from the endosperm of castor-oil seeds. J. Plant Physiol. 149, 23–29.

Garcia, M.L., Viitanen, P., Foster, D.L., Kaback, H.R., 1983. Mechanism of lactose translocation in proteoliposomes reconstituted with Lac carrier protein purified from *Escherichia coli*. 1. Effect of pH and imposed membrane-potential on efflux, exchange and counterflow. Biochemistry 22, 2524–2531.

Gorga, J.C., Lienhard, G.E., 1984. One transporter per vesicle-determination of the basis of the insulin effect on glucose-transport. Fed. Proc. 43, 2237–2241.

Hammond, J.R., 1994. Functional reconstitution of pharmacologically distant subtypes of nucleoside transporters in liposomal membranes. J. Pharm. Exp. Ther. 271, 906–917.

Hankamer, B., Nield, J., Zheleva, D., Boekema, E., Jansson, S., Barber, J., 1997. Isolation and biochemical characterisation of monomeric and dimeric photosystem II complexes from spinach and their relevance to the organisation of photosystem II in vivo. Eur. J. Biochem. 243, 422–429.

Helenius, A., Sarvas, M., Simons, K., 1981. Asymmetric and symmetric membrane reconstitution by detergent elimination studies with Semliki-Forest virus spike glycoprotein and penicillinase from the membrane of *Bacillus lichteniformis*. Eur. J. Biochem. 116, 27–35.

Helenius, A., Simons, K., 1975. Solubilization of membranes by detergents. Biochim. Biophys. Acta 415, 29–79.

Hicks, G.R., Rice, M.S., Lomax, T.L., 1993. Characterization of auxum-binding proteins from Zucchini plasma-membrane. Planta 189, 83–90.

Hunt, J.F., McCrea, P.D., Zaccai, G., Engelman, D.M., 1997. Assessment of the aggregation state of integral membrane proteins in reconstituted phospholipid vesicles using small angle neutron scattering. J. Mol. Biol. 273, 1004–1019.

Ikematsu, M., Sugiyama, Y., Iseki, M., Muneyuki, E., Mizukami, A., 1995. Direct reconstitution of bacteriorhodopsin into planar phospholipid bilayers-detergent effect. Biophys. Chem. 54, 155–164.

Jackson, M.L., Litman, B.J., 1982. Rhodopsin-phospholipid reconstitution from octyl glucoside solubilized samples by a detergent dilution techniques. Biochemistry 21, 5601–5608.

Jennings, M.L., 1984. Oligomeric structure and the anion transport function of human-erythrocyte Band-3 protein. J. Membr. Biol. 80, 105–117.

Jones, M.N., 1992. Surfactant interactions with biomembranes and proteins. Chemical Society Reviews 21, 127–136.

Jones, M.N., Chapman, D., 1995a. Micelles, Monolayers and Biomembranes. Wiley-Liss, New York, pp. 143–179.

Jones, M.N., Chapman, D., 1995b. Micelles, Monolayers and Biomembranes. Wiley-Liss, New York, pp. 199–219.

Jones, M.N., Chapman, D., 1995c. Micelles, Monolayers and Biomembranes. Wiley-Liss, New York, pp. 1–23.

Jones, M.N., Chapman, D., 1995d. Micelles, Monolayers and Biomembranes. Wiley-Liss, New York, pp. 203–208.

Jones, M.N., Manley, P., Wilkinson, A.E., 1982a. The dissociation of glucose oxidase by sodium *n*-dodecylsulphate. Biochem. J. 203, 285–291.

Jones, M.N., Manley, P., Midgley, P.J.W., Wilkinson, A.E., 1982b. The dissociation of bovine and bacterial catalase by sodium *n*-dodecylsulphate. Biopolymers 21, 1435–1451.

Jones, O.T., Earnest, J.P., McNamee, M.G., 1987a. In: Findlay, J.B.C., Evans, W.H. (Eds.), Biological Membranes: A Practical Approach. IRL Press, Oxford, pp. 139–177.

Jones, M.N., Finn, A., Mosavi-Movahedi, A., Waller, B.J., 1987b. The activation of *Aspergillus niger* catalase by sodium *n*-dodecylsulphate. Biochim Biophys. Acta 913, 395–398.

Jones, M.N., 1996. In: Magdassi, S. (Ed.), Surface Activity of Proteins: Chemical and Physiochemical Modifications. Marcel Dekker, New York, pp. 237–284.

Justice, J.M., Murtagh, J.J., Moss, J., Vaughan, M., 1995. Hydrophobicity and subunit interactions of rod outer segment protein investigated using Triton X-114 phase partitioning. J. Biol. Chem. 270, 17970–17976.

Kapitza, H.-G., Jacobson, K.A., 1986. In: Ragan, C.I., Cherry, R.J. (Eds.), Techniques for the Analysis of Membrane Proteins. Chapman and Hall, London, pp. 345–375.

Khamlichi, S., Loirat, M.J., Blanchard, D., LeMarc, M., Boully, P., Cartron, J.P., Bertrand, O., 1994. Influence of the size of the polar head of nonionic detergents of membrane-proteins immunoaffinity purification. J. Biochem. Biophys. Methods 29, 123–134.

Kirkpatrick, F.H., Gordesky, S.E., Marinetti, G.V., 1974. Differential solubilization of proteins, phospholipids and cholesterol of erythrocyte membranes by detergents. Biochim. Biophys. Acta 345, 154–161.

Klausner, R.D., Bridges, K., Tsunoo, H., Blumenthal, R., Weinstein, J.N., Ashwell, G., 1980. Reconstitution of the hepatic asialoglycoprotein receptor with phospholipid vesicles. Proc. Natl. Acad. Sci. USA 77, 5087–5091.

Koetters, P.J., Chou, H.F., Jonas, A.J., 1995. Reconstitution of lysosomal sulfate transport in proteoliposomes. Biochim. Biophys. Acta 1244, 311–316.

Kovács, I., Hollós-Nagy, K., Váró, G., 1995. Dark adaptation and spectral changes in Triton-X-100-treated bacteriorhodopsin. J. Photochem. Photobiol. B Biol. 27, 21–25.

Kraghhansen, U., Lemaire, M., Noel, J.P., Gulikkrzywicki, T., Møller, J.V., 1993. Transitional steps in the solubilization of protein-containing membranes and liposomes by nonionic detergents. Biochemistry 32, 1648–1656.

Lambert, O., Levy, D., Ranck, J.L., Leblanc, G., Rigaud, J.L., 1998. A new 'gel-like' phase in dodecyl maltoside-lipid mixtures: implications in solubilization and reconstitution studies. Biophys. J. 74, 918–930.

Levitzki, A., 1985. Reconstitution of membrane-receptor systems. Biochim. Biophys. Acta 822, 127–153.

Lévy, D., Bluzat, A., Seigneuret, M., Rigaud, J-L., 1990. A systematic study of liposome and proteoliposome reconstitution involving Bio-bead-mediated Triton X-100 removal. Biochim. Biophys. Acta 1025, 179–190.

Lévy, D., Gulik, A., Bluzat, A., Rigaud, J-L., 1992. Reconstitution of the sarcoplasmic reticulum Ca^{2+}-ATPase: mechanisms of membrane protein insertion into liposomes during reconstitution procedures involving the use of detergents. Biochim. Biophys. Acta 1107, 283–298.

Lichtenberg, D., 1985. Characterization of the solubilization of lipid bilayers by surfactants. Biochim. Biophys. Acta 821, 470–478.

Lichtenberg, D., Robson, R.J., Dennis, E.A., 1983. Solubilization of phospholipids by detergents: structural and kinetic aspects. Biochim. Biophys. Acta 737, 285–304.

Litman, B.J., 1992. Purification of rhodopsin by concanavalin A affinity chromatography. Methods Enzymol. 81, 150–153.

Long, A.R., Hall, J.L., Williams, L.E., 1997. Chromatographic resolution, purification and characterisation of H^+-PPase and H^+-ATPase from *Ricinus* cotyledon. J. Plant Physiol. 151, 16–24.

Loo, T.W., Clarke, D.M., 1988. Nonylphenol exothylates, but not nonylphenol, are substrates of the human multidrug resistance P-glycoprotein. Biochem. Biophys. Res. Commun. 247, 478–480.

Lopez Corcuera, B., Alcantara, R., Vazquez, J., Aragon, C., 1993. Hydrodynamic properties and immunological identification of the sodium-coupled and chloride-coupled glycine transporter. J. Biol. Chem. 268, 2239–2243.

Low, P.S., 1986. Structure and function of the cytoplasmic domain of Band-3-center of erythrocyte-membrane peripheral protein interactions. Biochim. Biophys. Acta 864, 145–167.

Lund, S., Orlowski, S., de Foresta, B., Champeil, P., Le Maire, M., Møller, J.V., 1989. Detergent structure and associated lipid as determinants in the stabilization of solubilized Ca^{2+}-ATPase from sarcoplasmic reticulum. J. Biol. Chem. 264, 4907–4915.

Lundahl, P., Watanabe, Y., Takayi, T., 1992. High-performance hydroxyapatite chromatography of integral membrane-proteins and water-soluble proteins in complex with sodium dodecylsulfate. J. Chromatogr. 604, 95–102.

Marchesi, V.T., Andrews, E.P., 1971. Glycoproteins: isolation from cell membranes with lithium diiodosalicylate. Nature 174, 1247–1248.

Massotte, D., Aghion, J., 1991. Bacteriorhodopsin 'detergent monomers' blue shift and velocity of light-dark adaptation. Biochem. Biophys. Res. Commun. 181, 1301–1305.

Mazer, N.A., Benedek, G.B., Carey, M.C., 1980. Quasi elastic light-scattering of aqueous biliary lipid systems. Mixed micelle formation in bile salt–lecithin solutions. Biochemistry 19, 601–619.

McDonnel, A., Donner, D.B., 1981. Affinity of the hepatic insulin-receptor is influenced by membrane phospholipids. J. Biol. Chem. 256, 1051–1057.

Meyer, O., Ollivon, M., Paternostre, M.T., 1992. Solubilization steps of dark-adapted purple membrane by Triton X-100. A spectroscopic study. FEBS Lett. 305, 249–253.

Meyuhas, D., Lichtenberg, D., 1995. The effect of albumin on the state of aggregation and phase transformations in phosphatidylcholine–sodium cholate mixtures. Biochim. Biophys. Acta 1234, 203–213.

Møller, J.V., Lemaire, M., 1993. Detergent binding as a measure of hydrophobic surface-area of integral membrane-proteins. J. Biol. Chem. 268, 18659–18672.

More, J.E., Jones, M.N., 1983. The efect of membrane composition and alcohols on the insulin-sensitive reconstituted monosaccharide-transport system of rat adipocyte plasma-membranes. Biochem. J. 216, 113–120.

Nakanishi, M., Tetsuka, T., Kagawa, Y., Moriyama, A., Sasaki, M., Hirata, H., 1993. Solubilization and reconstitution of high-affinity and low-affinity Na^+-dependent neutral L-α-amino acid transporters from rabbit small intestine. Biochim. Biophys. Acta 1151, 193–200.

Nelson, C.A., 1971. The binding of detergents to proteins Part 1: The maximum amount of dodecylsulphate bound to proteins and the resistance to binding of several proteins. J. Biol. Chem. 246, 3895–3901.

Panagiotidis, C.H., Reyes, M., Sievertsen, A., Boos, W., Shuman, H.A., 1993. Characterisation of the structural re-

quirements for assembly and nucleotide-binding of an ATP-binding cassette transporter. The maltose transporter-system of *Eschericha coli*. J. Biol. Chem. 268, 23685–23696.

Pande, S.V., Murthy, M.S.R., 1994. A modified micro-Bradford procedure for elimination of interference from sodium dodecylsulphate, other detergents and lipids. Anal. Biochem. 220, 424–426.

Parker, W., Song, P-S., 1992. Protein structures in SDS micelle–protein complexes. Biophys. J. 61, 1435–1439.

Partearroyo, M.A., Pilling, S.J., Jones, M.N., 1991. The lysis of isolated fish (*Oncorhynchus mykiss*) gill epithelial cells by surfactants. Comp. Biochem. Physiol. 100C, 381–388.

Paternostre, M., Viard, M., Meyer, O., Ghanam, M., Ollivon, M., Blumenthal, R., 1997. Solubilization and reconstitution of vesicular stomatitus virus envelope using octylglucoside. Biophys. J. 72, 1683–1694.

Pederson, S.E., Ross, E.M., 1982. Functional reconstitution of β-adrenergic receptors and the stimulatory GTP-binding protein of adenylate-cyclase. Proc. Natl. Acad. Sci. USA 79, 7229–7232.

Pick, U., 1981. Liposomes with a large trapping capacity prepared by freezing and thawing of sonicated phospholipid mixtures. Arch. Biochem. Biophys. 212, 186–194.

Pistorius, A.M.A., Schuurmans Stekhover, F.M.A.H., Bovee-Geurts, P.H.M., de Grip, W.J., 1994. Quantitative analysis of residual detergent in proteoliposomes by Fourier transform infrared spectroscopy. Anal. Biochem. 221, 48–52.

Pouliot, J.F., Beliveau, R., 1994. Extraction of brain capillary membrane-proteins using Triton X-114. Biochem. Mol. Biol. Int. 34, 1075–1084.

Reviakine, I., Stoylova, S., Holzenburg, A., 1996. Surfactosomes-A novel approach to the reconstitution and 2-D crystallization of membrane-proteins. FEBS Lett. 380, 296–300.

Richard, P., Rigaud, J-L., Gräber, P., 1990. Reconstitution of CFOF1 into liposomes using a new reconstitution procedure. Eur. J. Biochem. 193, 921–925.

Rigaud, J-L., Paternostre, M.T., Bluzat, A., 1988. Mechanisms of membrane-protein insertion into liposomes during reconstitution procedures involving the use of detergents. 2. Incorporation of the light-driven protein pump bacteriorhodopsin. Biochemistry 27, 2677–2688.

Roelofsen, B., Denkamp, J., 1994. Plasma-membrane phospholipid asymmetry and its maintenance—the human erythrocyte model. Curr. Top. Membr. 40, 7–46.

Ruiz, J., Goñi, F.M., Alonso, A., 1988. Surfactant-induced release of liposomal contents: a survey of methods and results. Biochim. Biophys. Acta 937, 127–134.

Saito, S., Tsuchiya, T., 1984. Characteristics of *n*-octyl β-D-thioglucopyranoside, a new non-ionic detergent useful for membrane biochemistry. Biochem. J. 222, 829–832.

Sankaran, D., Lau, L.C.L., Ng, M.L., 1997. Interaction of kunjin virus with octyl-D-glucose extracted Vero cell plasma membrane. J. Virol. Methods 63, 167–173.

Sayid, M., Isaac, R.E., Harrow, I.D., 1997. Purification and properties of a membrane aminopeptidase from *Ascaris suum* muscle that degrades AFI and AF2. Mol. Biochem. Parasitol. 89, 225–234.

Schafmeister, C.E., Miercke, L.J.W., Stroud, R.M., 1993. Structure at 2.5Å of a designed peptide that maintains solubility of membrane proteins. Science 262, 734–738.

Schuck, P., Legrum, B., Passow, H., Schubert, D., 1995. The influence of 2 anion transporter inhibitors, 4,4′-diisothiocyanatodihydrostilbene-2,2′-disulfonate and 4,4′-dibenzoylstilbene-2,2′-disulfonate, on the self-association of erythrocyte Band-3 protein. Eur. J. Biochem. 230, 806–812.

Scotto, A.W., Zakin, D., 1986. Reconstitution of membrane-proteins-catalysis by cholesterol of insertion of integral membrane proteins into preferred lipid bilayers. Biochemistry 25, 1555–1561.

Sefton, B.M., Buss, J.E., 1987. The covalent modification of eukaryotic proteins with lipid. J. Cell Biol. 104, 1449–1453.

Segrest, J.P., Wilkinson, T.M., Sheng, L., 1979. Isolation of glycophorin with deoxycholate. Biochim. Biophys. Acta 554, 533–537.

Seyfang, A., Duszenko, M., 1993. Functional reconstitution of the *Trypanosoma-brucei* plasma-membrane D-glucose transporter. Eur. J. Biochem. 214, 593–597.

Sharom, F.J., Yu, X.H., Chu, J.W.K., Doige, C.A., 1995. Characterization of the ATPase activity of P-glycoprotein from multidrug-resistant Chinese-hamster ovary cells. Biochem. J. 308, 381–390.

Sharom, F.J., 1995. Characterisation and functional reconstitution of the multidrug transporter. J. Bioenerg. Biomembr. 27, 15–22.

Shiao, Y-J., Chen, J-C., Wang, C-T., 1989. The solubilization and morphological change of human-platelets in various detergents. Biochim. Biophys. Acta 980, 56–68.

Singer, S.J., Nicholson, G.L., 1972. The fluid mosaic model of the structure of cell membranes. Cell membranes are viewed as two-dimensional solutions of orientated globular proteins and lipids. Science 175, 720–731.

Steck, T.L., 1974. The organization of proteins in the human red blood cell membrane. J. Cell Biol. 62, 1–19.

Straubinger, R.M., Düzgünes, N., Papahadjopoulis, D., 1985. pH-sensitive liposomes mediate cytoplasmic delivery of encapsulated macromolecules. FEBS Lett. 179, 148–154.

Stump, D.D., Zhou, S.L., Berk, P.D., 1993. Comparison of plasma-membrane FABP and mitochondrial isoform of aspartate-aminotransferase from rat-liver. Am. J. Physiol. 265G, 894–902.

Tanner, M.J., Martin, P.G., High, S., 1988. The complete amino acid sequence of the human erythrocyte membrane anion-transport protein deduced from cDNA sequence. Biochem. J. 256, 703–712.

Thomas, D.D., 1986. In: Ragan, C.I., Cherry, R.J. (Eds.), Techniques for the Analysis of Membrane Proteins. Chapman and Hall, London, pp. 377–431.

Thorne, C.J.R., 1978. Techniques in Protein and Enzyme Biochemistry, vol. 104. Elsevier-North Holland Biomedical Press, Amsterdam, p. 1.

Tiller, G.E., Mueller, T.J., Dockler, M.E., Strive, W.G., 1984. Hydrogenation of Triton X-100 eliminates its fluorescence and ultraviolet-light adsorption while preserving its detergent properties. Anal. Biochem. 141, 262–266.

Tocanne, J.-F., Dupon-Cezanne, L., Lopez, A., 1994. Lateral diffusion of lipids in model and natural membranes. Prog. Lipid Res. 33, 203–237.

Urbaneja, M.A., Nieva, J.L., Goñi, F.M., Alonso, A., 1987. The influence of membrane composition on the solubilizing effects of Triton X-100. Biochim. Biophys. Acta 904, 337–345.

Vachon, V., Delisle, M.C., Giroux, S., Laprade, R., Beliveau, R., 1995. Factors affecting the stability of the renal sodium-phosphate symporter during its solubilization and reconstitution. Int. J. Biochem. Cell Biol. 27, 311–318.

VanVeldhoven, P.P., Asselberghs, S., Eyssen, H.J., Mannaertis, G.P., 1996. Stabilization and partial purification of Triton X-100 solubilized trihydroxycoprostanyl CoA synthase. Biochem. Mol. Biol. Int. 40, 447–457.

Viguera, A.R., Gonzalezmanas, J.M., Taneva, S., Goñi, F.M., 1994. Early and delayed stages in the solubilization of purple membrane by a polyoxyethylenic surfactant. Biochim. Biophys. Acta 1196, 76–80.

Weber, K., Osborn, M., 1975. In: Neurath, H., Hill, R.L., Boeden, C.-L. (Eds.), The Proteins, vol. 1, 3rd edn. Academic Press, San Diego, pp. 179–223.

Wheeler, T.J., Hauck, M.A., 1985. Reconstitution of the glucose transporter from bovine heart. Biochim. Biophys. Acta 818, 171–182.

Wong, P., 1993. The state of association of Band-3 of the human erythrocyte membrane-evidence of a hexamer. Biochim. Biophys. Acta 1151, 21–27.

Zardeneta, G., Horowitz, P.M., 1994. Protein refolding at high concentrations using detergent/phospholipid mixtures. Anal. Biochem. 218, 392–398.

Zimmerman, M.L, Daleke, D.L., 1993. Regulation of a candidate amino phospholipid-transporting ATPase by lipid. Biochemistry 32, 12257–12263.