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SOLUBILIZATION OF PLASMA MEMBRANES IN ANIONIC, NON-IONIC AND ZWITTERIONIC SURFACTANTS FOR ISO-DALT ANALYSIS: A CRITICAL EVALUATION

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SUMMARY**

A critical study has been made of the solubilizing properties of anionic, non-ionic and zwitterionic surfactants to be used in the first dimension of two-dimensional isoelectric focusing–sodium dodecyl sulphate (IF–SDS) maps. Excess of SDS is a powerful solubilizing agent, but prevents proteins from entering the IF gel. Nonidet P-40 (NP-40)–urea mixtures are mediocre solubilizing agents, but are compatible with the IF dimension. Zwitterionic detergents (zwittergents) appear to exhibit a well balanced solubilizing power and are able to generate sharp two-dimensional maps, with round spots and minimal vertical and horizontal streaking. SB3-12 and SB3-14 appear to have the best solubilizing properties; shorter surfactants (SB3-8) exhibit a poor solubilization efficiency, while longer detergents (SB3-16) bind too strongly to hydrophobic regions in proteins. The random performance of non-ionic detergents has been attributed to their ability to form mixed micelles with the carrier ampholytes used in the IF step: depending on the relative ratio of NP-40 to Ampholines, different types of micelles would be formed, which, when reaching appropriate stoichiometries and charge densities, would mimic the behaviour of natural zwittergents. Acceptable two-dimensional maps can be obtained when the sample is lysed in limiting amounts of SDS (SDS:protein = 1:3), suggesting that excess of free SDS in solution is deleterious to the IF process.

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** Abbreviations: Bis = N,N'-methylenebisacrylamide; SB3-8, SB3-10, SB3-12, SB3-14, SB3-16 = zwitterionic (sulphobetaine) detergents with tails of 8, 10, 12, 14 or 16 carbon atoms, respectively; NP-40 = Nonidet P-40 = *p-tert.*-octylphenylpolyoxyethylene; SDS = sodium dodecyl (lauryl) sulphate; IF = isoelectric focusing; PAGE = polyacrylamide gel electrophoresis.

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INTRODUCTION

Most surfactants employed to date in the investigation of membrane proteins are either anionic (*e.g.*, SDS) or non-ionic of the polyoxyethylene type [*e.g.*, Nonidet P-40 (NP-40) or the essentially identical Triton X-100][1,2]. Both classes have drawbacks owing to their variable binding affinity to proteins. With SDS, while most if not all of the integral membrane proteins are solubilized, the resulting SDS–protein complexes exhibit a total loss of biological activity and are not readily amenable to analysis by surface-charge electrophoretic methods, such as isoelectric focusing (IF)[3]. The non-ionic detergents also appear to bind membrane proteins in a highly co-operative manner at levels near their critical micelle concentrations (CMC)[4]. However, unlike SDS, they appear to have a relatively low solubilization efficiency, although in general they do not interfere with the biological activity of the solubilized specimen.

A third class, the zwitterionic surfactants, was first introduced by Allen and Humphries[5] for the IF fractionation of a milk fat globule and red blood cell membranes. The properties of these detergents have been thoroughly investigated by Gonenne and Ernst[6], Ernst[7] and Hjelmeland and co-workers[8,9]. The advantage of these surfactants is that they are homogeneous organic compounds with known physicochemical properties, are non-denaturing (in the sense that they do not perturb the secondary or tertiary structure of proteins), are effectively neutral (*i.e.*, they display zero net charge in the pH 3–10 range), are fully soluble from 0 to 100°C and show only minor responses to changes in pH, ionic strength and temperature[9].

The selection of the right type of detergent is important in the analysis of membrane polypeptides by two-dimensional techniques (2-D PAGE) and for the generation of high-resolution IF–SDS maps, with reproducible spot positions and relative densities from run to run[10]. O'Farrell[11] found that SDS could be used initially to solubilize samples for 2-D PAGE, if NP-40 were included in the IF gel; proteins appeared to migrate according to native charge, while free SDS formed mixed micelles with NP-40 which would collect at the anodic gel extremity. The procedure was not reproducible, however, and had to be carefully standardized by Ames and Nikaido[12]. Use of urea–NP-40 alone had been found to result in incomplete solubilization of erythrocyte membranes[13,14], although improved solubilization could be obtained under alkaline conditions (pH 10.3) by addition of potassium carbonate[15]. As for the zwitterionic detergents (zwittergents), which had appeared so promising, there are reports that their solubilizing properties are even less effective than those of the standard urea–NP-40 mixture[16,17].

Our group is actively involved in the study of myogenesis *in vitro*; during this process, mononucleated myoblasts divide actively, followed by a cell–cell recognition step were gap junctions appear between the cell, which become metabolically coupled[18]. After a lag period, the mononucleated myoblasts fuse to form large multinucleated myotubes. In this sequence of events, the role of plasma membrane components is crucial. Changes in the biosynthesis as well as post-translational changes due to glycosylation and phosphorylation of the different plasma membrane proteins have been described in detail recently[19,20]. These studies led us to postulate that changes in glycosylation might be at the basis of the cell–cell recognition step before fusion takes place[19]. To unravel this biological process, it is fundamental to be able

to follow possible transformations of plasma membrane proteins by a high-resolution and highly reproducible 2-D PAGE technique. As we have had unsatisfactory results with standard SDS and urea–NP-40 solubilization techniques, we have re-investigated methodologies employing zwittergents and have standardized a zwitterionic-based IF–SDS map.

EXPERIMENTAL

Chemicals

All chemicals were of analytical-reagent grade, unless indicated otherwise. Ultra-pure urea was purchased from Schwarz Mann (Spring Valley, NY, U.S.A.). Acrylamide, N,N′-bisacrylamide (Bis), ammonium persulphate and N,N,N′,N′-tetramethylethylenediamine (TEMED) were of electrophoresis grade from Bio-Rad Labs. (Richmond, CA, U.S.A.). Nonidet P-40 (NP-40) was from Fluka (Buchs, Switzerland), Ampholines from LKB (Bromma, Sweden); zwitterionic detergents (zwittergents) from Calbiochem Behring (La Jolla, CA, U.S.A.); sodium dodecyl sulphate (SDS) of special biochemical grade from BDH (Poole, U.K.), phenylmethylsulphonyl fluoride (PMSF), trypsin inhibitor, Tris base and Tris–HCl from Sigma (St. Louis, MO, U.S.A.), [^{35}S]methionine (more than 1000 Ci mmol^{-1}) from Amersham International (Amersham, U.K.), NS2T and X-omatic regular intensifying screens, made by Kodak (Rochester, NY, U.S.A.) from Euromedica (Paris, France), 2-mercaptoethanol from Koch-Light (Colnbrook, U.K.), acetic acid, orthophosphoric acid, glycerol, sodium hydroxide and potassium chloride from Merck (Darmstadt, F.R.G.), methanol from Prolabo (Paris, France), agarose from Pharmacia (Uppsala, Sweden), Dulbecco's modified Eagle medium (DMEM); foetal calf serum (FCS) from Gibco and methylated ^{14}C-labelled molecular weight markers (myosin, phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase and lactoglobulin A) (3–30 μCi/mg) from N.E.N. Oreieich (F.R.G.).

Cell culture

Culture of cells of the L_6 line was performed according to Crerar *et al.*[21].

Labelling of the cells

Confluent L_6 cells ($1.5 \cdot 10^4$ cells/cm^2) (5–10% of nuclei in myotubes) were labelled at 37°C in 5 ml of DMEM per 100 mm diameter plate (not containing methionine), 10% FCS and 20 μCi/ml of [^{35}S]methionine. After 22 h the radioactive media were removed and the cultures were washed several times with 10 ml of ice-cold phosphate-buffered saline (PBS). The cells were scraped into PBS and resuspended in ice-cold Tris buffer containing 0.1 *M* Tris–HCl (pH 7.5), 0.25 *M* KCl, 1 mg/ml of soybean trypsin inhibitor and 1 m*M* PMSF.

Plasma membrane purification

This was performed as described previously[19].

Sample preparation

Labelled plasma membranes were solubilized by three different methods.

Method 1. Membranes were solubilized in an aqueous solution containing SDS

at an SDS:proteins ratio of 10:1 or 1:3 according to Ames and Nikaido[12]. The mixture was boiled at 100°C for 3 min and solid urea was added to a final concentration of 9 *M* and NP-40 to a final concentration of 2%. One volume of lysis buffer[11] was added and the sample was either loaded immediately on an isoelectric focusing gel or stored frozen at −80°C.

Method 2. Membranes were solubilized in 2% NP-40 in water by boiling for 3 min, then solid urea to a final concentration of 9 *M* and one volume of lysis buffer were added. The sample was either loaded on an isoelectric focusing gel or stored frozen at −80°C.

Method 3. Purified membranes were homogenized in a solution containing 2% or 4% zwittergent (SB3-8, SB3-10, SB3-12, SB3-14 or SB3-16) and boiled for 3 min. After cooling the samples to room temperature, one volume of a solution containing 8 *M* urea and 4% of NP-40 was added. The samples were either run immediately or stored frozen at −80°C.

Isoelectric focusing gels

Isoelectric focusing gels were 11 cm long. They were either prepared exactly as described by O'Farrell[11] when methods 1 and 2 were used for samples solubilization (see preceding section) or by a modification of this technique when the membranes had been solubilized by zwittergents. In this instance, the gels used were 4% T and 5% C* containing 7 *M* urea, 0.1% of zwittergent, 2% of ampholines, 0.01% of ammonium persulphate and 0.07% of TEMED. All solutions were carefully filtered before use and the mixture was thoroughly degassed before adding the TEMED.

In all instances, the gels were overlayed with a 5 *M* urea solution and left to polymerize for 2 h at room temperature. The urea was then removed and replaced with lysis buffer[11] containing 5 *M* urea and allowed to stand for a further 2 h. The samples were loaded on the gels, overlayed with a solution of 3 *M* urea and run from the cathode to the anode. The gels were run at 350 V for 17 h and then at 500 V for a further 1 h, which makes a total of 6450 Vh. The gels were subsequently removed from the tubes and stored at −80°C. The anode and cathode solutions were 0.01 *M* H_3PO_4 and 0.02 *M* NaOH, respectively.

Determination of the pH gradient of the gels

This was performed as described by Righetti and Drysdale[22].

SDS polyacrylamide gel electrophoresis

The 8.5 cm long 5–15% polyacrylamide gradient gels were prepared according to Laemmli[23] and a stacking gel containing 4% acrylamide was cast on top of them. The pH of the gels was adjusted to 8.2 according to Johnson[24] in order to allow a better resolution of low-molecular-weight proteins. The isoelectric focusing gels were

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$$* \; T = \frac{\text{Acrylamide (g)} + \text{N,N'-methylenebisacrylamide (g)}}{\text{Volume (ml)}} \cdot 100$$

$$C = \frac{\text{N,N'-methylenebisacrylamide (g)}}{\text{Acrylamide (g)} + \text{N,N'-methylenebisacrylamide (g)}} \cdot 100$$

defrosted at room temperature and allowed to equilibrate in SDS sample buffer[11] at 4°C for 30 min[24]. Gel slabs were run at 10 mA per slab until the bromophenol blue entered the stacking gel and then at 5 mA until the bromophenol blue front arrived at the bottom of the gel.

RESULTS

Membrane solubilization in excess SDS

Labelled plasma membranes were solubilized in excess of SDS (SDS:protein = 10:1), the mixture was boiled at 100°C for 3 min and then solid urea was added to a final concentration of 9 *M*. After adding one volume of lysis buffer, the sample was separated in a typical O'Farrell IF gel, containing 2% NP-40 and 8 *M* urea[11]. The IF gel rod was then subjected to SDS electrophoresis in a Laemmli discontinuous pore gradient SDS gel slab[18]. The resulting 2-D map is shown in Fig. 1. Although most of the membrane polypeptides entered the IF gel, when the IF gel rod is satur-

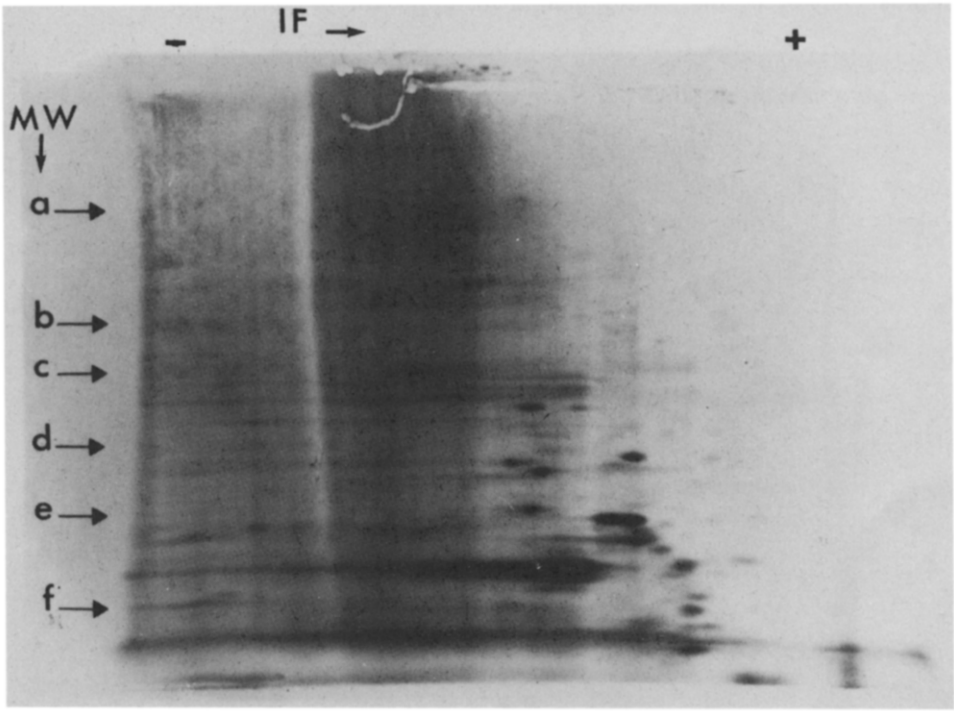

Fig. 1. Confluent L6 cells were labelled *in vitro* with [^{35}S]methionine (20 μCi/ml) for 22 h. Plasma membranes were purified and solubilized in SDS at an SDS to proteins ratio of 10:1 as described in method 1; $1.6 \cdot 10^6$ cpm were loaded on the isoelectric focusing gel prepared exactly as described by O'Farrell[11]. The first-dimension gel was frozen for 24 h and then loaded on an SDS–polyacrylamide gradient gel (5–15% of acrylamide) and run overnight. The gel was subsequently fixed, dried and exposed for 1 week. Methylated ^{14}C-labeled molecular weight markers were included in the SDS dimension. Their migration is indicated by arrows. (a) Myosin, M_r 200 kD; (b) phosphorylase B, M_r 92.5 kD; (c) bovine serum albumin, M_r 69 kD; (d)ovalbumin, M_r 46 kD; (e) carbonic anhydrase, M_r 30 kD;(f) lactoglobulin A, M_r 18.37 kD.

ated with SDS denaturing buffer and run in the SDS dimension, the peptide chains appear as continuous streaks on the left-hand side of the SDS gel slab. Only a few species with M_r lower then 69 kD were detected in the neutral to mildly basic pH region and appear to be effectively focused. These results were a constant finding whenever an excess of SDS was used in the solubilization procedure. This system was therefore abandoned.

Membrane solubilization in urea–Nonidet

Plasma membranes were solubilized by method 2 by boiling in 2% NP-40 for 3 min. On cooling, solid urea was added to a final concentration of 9 *M* and then the sample was diluted 1:1 with lysis buffer. The IF run was in a gel containing also 2% NP-40 and 8 *M* urea. The resulting 2-D map is shown in Fig. 2; there is a slight improvement over the SDS-solubilized sample of Fig. 1, but the overall performance of this method is still poor. Mostly low p*I*, low M_r species are able to enter the gel, the remaining polypeptides precipitating at the cathodic gel surface (seen in the 2-D map as a long string to the left). Anodic sample application was even more disastrous, as practically everything precipitated at the application point (not shown).

Membrane solubilization in zwittergents (SB3-8 to SB3-16)

Myoblast membranes were then solubilized in 2% or 4% zwittergent (method

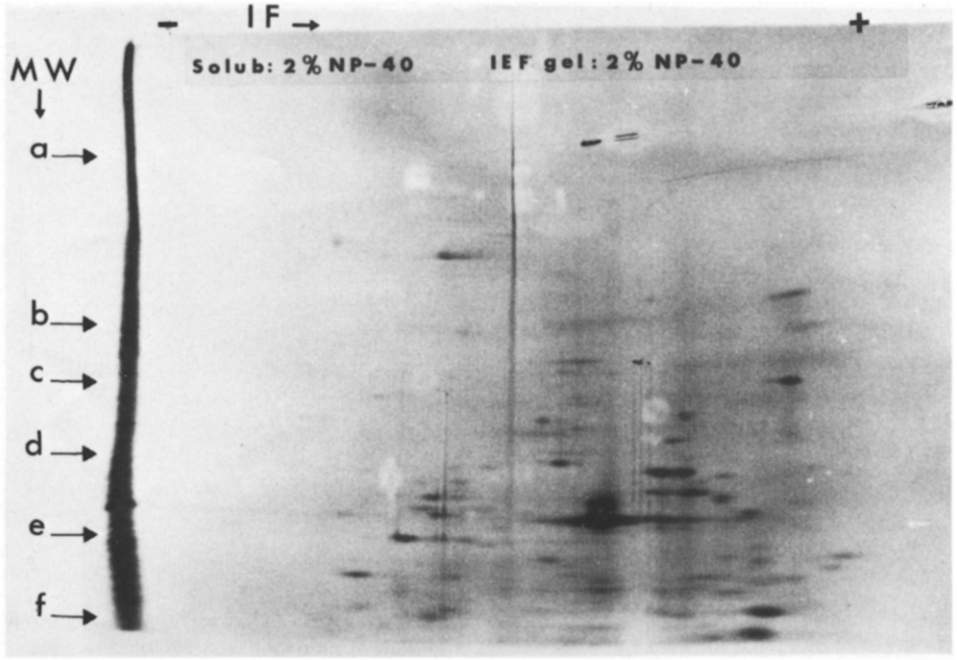

Fig. 2. Confluent L6 cells were labelled *in vitro* with [^{35}S]methionine (20 μCi/ml) for 22 h. Plasma membranes were purified and solubilized in NP-40 at a final concentration of 2% as described in method 2; $1.6 \cdot 10^6$ cpm were loaded on the isoelectric focusing gel. The pH gradient was linear between pH 4.00 and 7.2. The first-dimension gel was frozen for 24 h and then loaded on an SDS–polyacrylamide gradient gel (5 15% of acrylamide) and run overnight. The gel was subsequently fixed, dried and exposed for 1 week. Molecular weight markers (a–f) were included in the SDS run (see legend to Fig. 1).

3) by boiling for 3 min. On cooling, the sample was diluted 1:1 with a solution of 8 *M* urea and 4% NP-40. The IF gel contained 7 *M* urea and, in general, 0.1% zwittergent. As can be seen clearly in Fig. 3, the inclusion of these detergents in the IF gels resulted in a shift of the pH gradient towards the alkaline side in all regions; the slope of the curve was maintained, which indicates a uniform displacement in the pH gradient. As could be deduced from theoretical considerations as the length of the aliphatic chain increased, there was an increase in the extent of the shift towards the

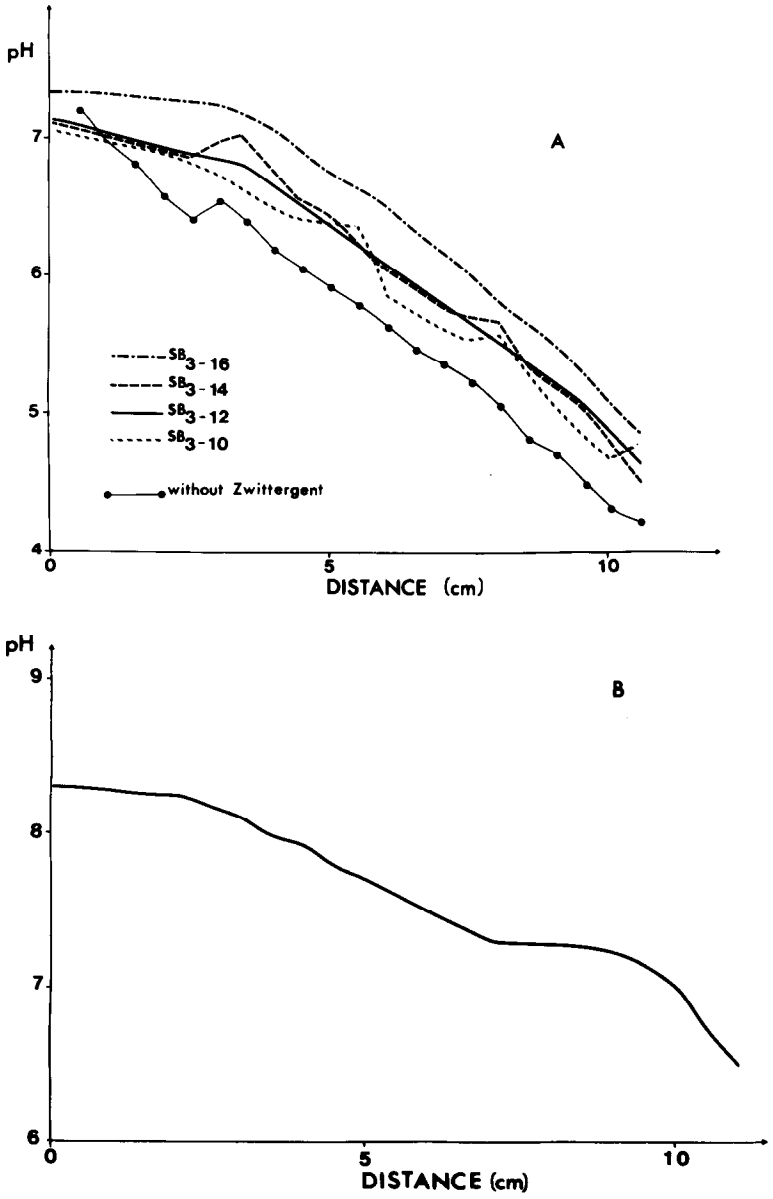

Fig. 3.

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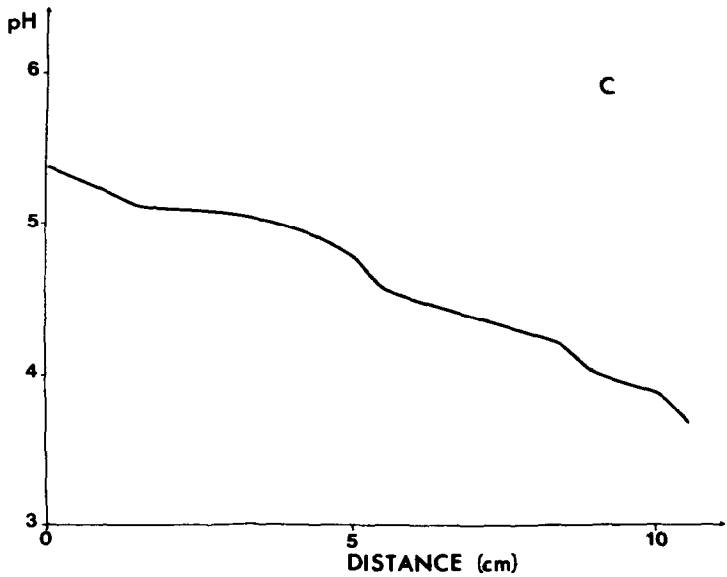

Fig. 3. pH gradient for isoelectric focusing gels. (A) SB3-10, SB3-12, SB3-14 and SB3-16 were included or not, containing 2% of ampholines (1.6% of pH range 5–8 and 0.4% of pH range 3.5–10). (B) Containing ampholines of basic pH (0.8% of pH range 7–9, 0.8% of pH range 9–11 and 0.4% of pH range 3.5–10) and 0.1% of SB16. (C) Containing ampholines of acidic pH (0.8% of pH range 2.5–4; 0.8% of pH range 4–6 and 0.4% of pH range 3.5–10) and 0.1% of SB16. The zero point on the abscissas is the bottom of the well, *i.e.*, the origin of the electrophoresis.

basic side. The resulting 2-D map, in the case of SB3-10, is shown in Fig. 4: compared with solubilization with anionic and neutral detergents, there is a marked increase in solubilizing power. Most of the membrane polypeptides appear to be able to enter the IF gel, and the 2-D map now shows a well balanced distribution of low and high p*I* components, as well as low and high M_r species. Very few components precipitate at the cathodic gel extreme, as shown by the faint string on the left-hand side of Fig. 4. Although the improvement in the 2-D map is considerable, the polypeptide spots in the 2-D plane are not fully resolved; many of them, especially in the high M_r region, appear as elongated or fused ellipsoids, some of the filaments reaching up to the cathodic gel surface, suggesting a mechanism of continuous precipitation–solubilization cycles for the whole duration of the IF step. In addition to horizontal streaking (IF dimension), there is also a considerable vertical streaking, especially in the centre of the map, suggesting solubilization difficulties in the SDS step. In order to see if better solubilizing properties could be achieved, we tried a higher homologous series, with progressively longer hydrocarbon. As shown in Fig. 5, SB3-12 seems to achieve an almost ideal balance in solubilizing power, vertical and horizontal streaking being almost negligible, with a further reduction in the amount of material precipitated at the origin in the IF step. The 2-D maps obtained with SB3-12 or SB3-14 are practically indistinguishable (Fig. 6), suggesting that the two species of zwittergent can be used interchangeably. Raising the level of detergent to 4% in the solubilization step produced essentially identical maps; also, reducing the amount of surfactant in the IF gel from 0.1 to 0.01% did not alter the pattern of the 2-D map

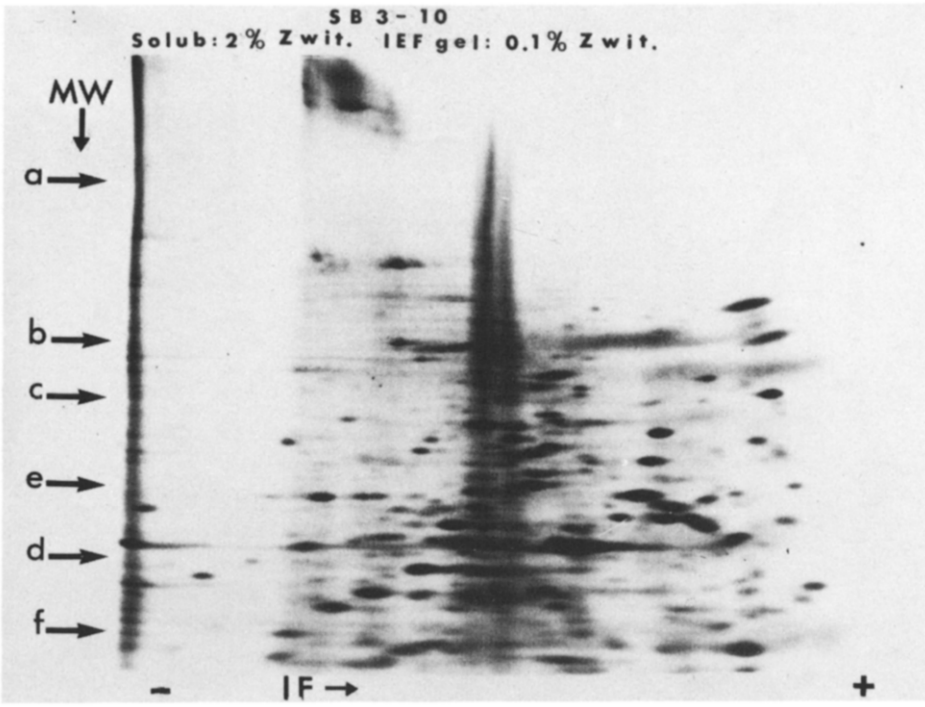

Fig. 4. Confluent L6 cells were metabolically labelled with [^{35}S]methionine (20 μCi/ml) for 22 h. Plasma membranes were purified and solubilized in SB3-10 exactly as described in method 3; 2 · 10^6 cpm were loaded on an isoelectric focusing gel (4% T and 5% C) containing 7 *M* urea, 0.1% zwittergent, 2% of ampholines (1.6% of pH 5–8 and 0.4% of pH 3.5–10), 0.01% ammonium persulphate and 0.07% of TEMED. The pH gradient was linear between pH 4.00 and 7.2. The first-dimension gel was frozen for 24 h and then loaded on an SDS–polyacrylamide gradient gel (5–15% of acrylamide) and run overnight. The gels were subsequently fixed, dried and autoradiographed using Kodak NS2T films. The exposure time was 1 week. Molecular weight markers (a–f) were included in the SDS run (see legend to Fig. 1).

(not shown). However, when the longer zwittergent (SB3-16) was tried, poorer maps were obtained (Fig. 7); horizontal streaking again became pronounced and there appeared to be a marked loss of polypeptides in the low M_r low and neutral p*I* region. This was accompanied by an increase of material precipitated at the cathodic gel end (Fig. 7, left-hand side).

It should be emphasized that, at present, 2-D maps are able to resolve polypeptide chains only in the pH 4–7 range. When alkaline pH ranges were tried, most of the material never entered the gel (Fig. 8A); only a few, low M_r, components migrated in the IF gel, but as a continuous filament spanning the distance from the cathode to the anode. The black, solid line on the left clearly indicates massive precipitation of the applied sample. Better, but still very poor, results were obtained in the acidic pH range (pH 3–5) (Fig. 8B); only low M_r, high p*I* components enter the IF gel, again mostly as continuous filaments connected to the precipitate at the sample application site. Although the results presented here refer to SB3-16, identical data were obtained with all the other surfactants tested (SB3-8 to SB3-14) (data not shown).

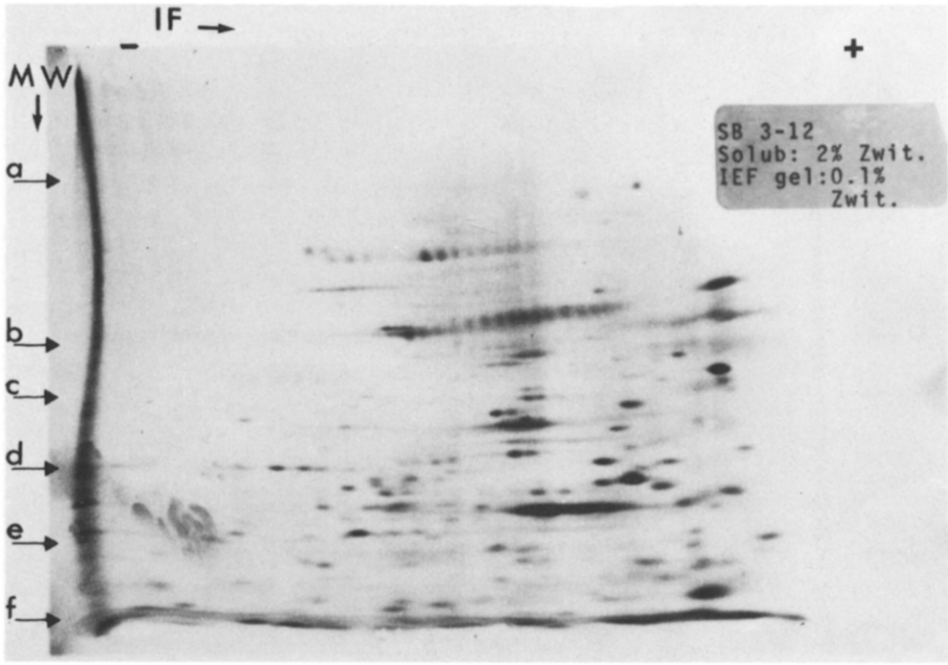

Fig. 5. L6 confluent cells were labelled, plasma membranes were purified and solubilized in SB3-12 as described in the legend to Fig. 4; $1.6 \cdot 10^6$ cpm were loaded on an isoelectric focusing gel as described under Experimental, which was run overnight (for details see legend to Fig. 4). The gel was frozen, loaded on an SDS–polyacrylamide gel (5–15% of acrylamide), run overnight, dried and autoradiographed as described in the legend to Fig. 4. The exposure time was 1 week. Molecular weight markers (a–f) were included in the SDS run (see legend to Fig. 1).

Membrane solubilization in limiting amounts of SDS

We were intrigued by the poor performance of the SDS solubilized samples (Fig. 1), as there seems to be a general agreement that SDS is by far the best solubilizing agent[3,11,12]. We therefore tried a new solubilization procedure, using limiting amounts of SDS. When myoblast membranes were dissolved at an SDS to protein ratio of 1:3 and then run under standard conditions, the resulting 2-D map was spectacular (Fig. 9). The map thus obtained is similar to that generated in SB3-10 (see Fig. 3), not only for its positive (good distribution and spreading of the spots in the p*I*–M_r plane) but also for its negative aspects (pronounced vertical and horizontal streaking). It still appears, however, that a significant amount of radioactivity fails to enter the gel, as shown by the thick and black filament on the left of Fig. 4.

DISCUSSION

Zwittergents versus neutral detergents

The limited reports that have appeared so far on the use of zwittergents for membrane protein solubilization have been conflicting. Whereas on the one hand Gonenne and Ernst[6] and Hjelmeland and co-workers[8,9] reported a superior solubilizing power of zwittergents over non-ionic detergents (Triton X-100 and NP-40), on

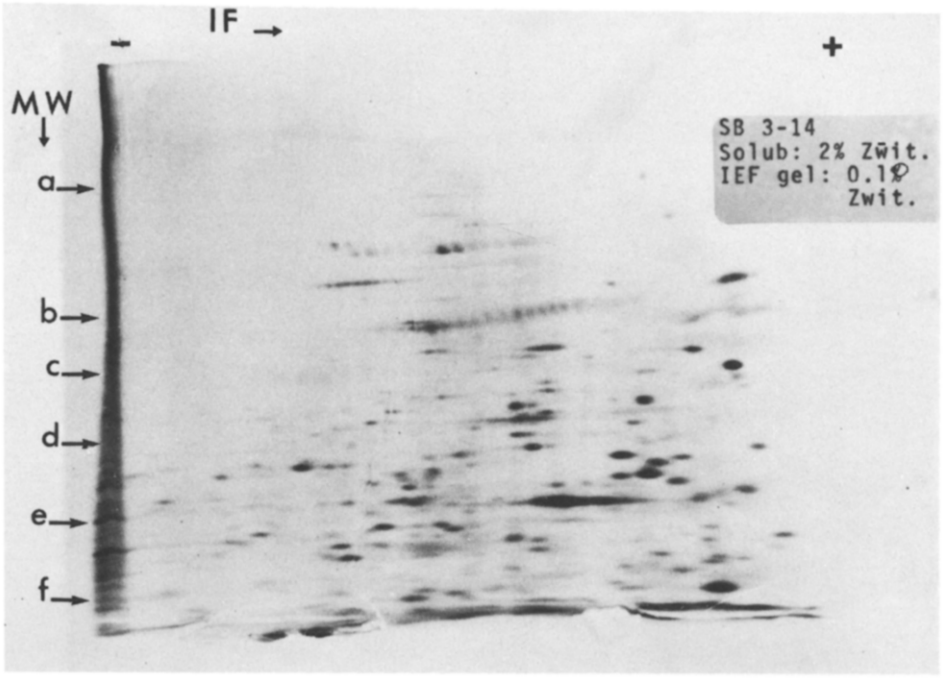

Fig. 6. L6 cells were labelled, plasma membranes were purified and solubilized in SB3-14 as described in the legend to Fig. 4; $1.6 \cdot 10^6$ cpm were loaded on a isoelectric focusing gel as described under Experimental, which was run overnight (for details see legend to Fig. 4). The gel was then frozen, loaded on an SDS–polyacrylamide gel (5–15% of acrylamide), run overnight, dried and autoradiographed as described in the legend to Fig. 4. The exposure time was 1 week. Molecular weight markers (a–f) were included in the SDS run (see legend to Fig. 1).

the other hand Burghes *et al.*[16] and Booz and Travis[17] reached the opposite conclusions. With CHAPS {3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulphonate} it was actually found that it could disaggregate cytochrome P-450 to a species having M_r in the neighbourhood of 100,000 daltons, while the same protein in Triton N-101 had an M_r of 300,000, indicating the presence of higher aggregates[9]. In our hands zwittergents, especially SB3-12 and SB3-14, appear to have a better solubilizing power not only than neutral but also than anionic detergents on myoblast membranes. In a way, this does not seem surprising: the general zwitterionic amphilic character of SBn's is similar to that of the diacylphospholipids phosphatidylcholine (lecithin) and phosphatidylethanolamine, which are the major constituents of cellular membranes. It is no wonder that they are the best substitutes for the lipids in the native membrane, and this could also explain their ability to retain the enzymatic activity of membrane constituents[6]. In fact, the solubilizing properties of neutral detergents *per se* have not been proved conclusively. It should be remembered that, in most instances, neutral detergents have been used in connection with carrier ampholytes for the IF analysis of membrane components. Ampholine species have been demonstrated to form mixed micelles with NP-40, especially with the higher p*I* components, which would have a higher hydrophobicity than low p*I* amphoteres[25]. It is

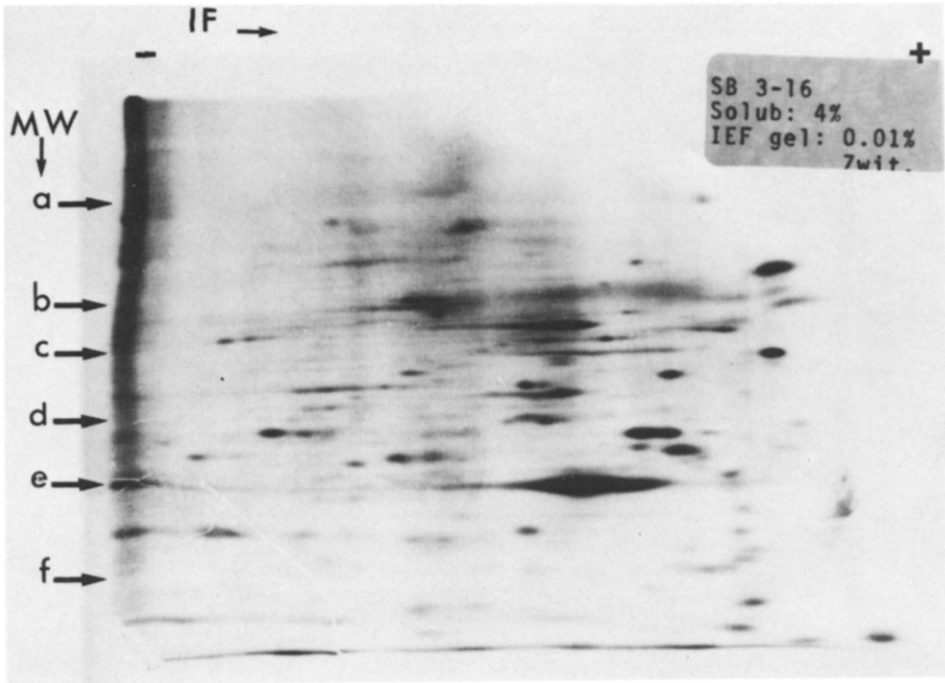

Fig. 7. L6 confluent cells were labelled, plasma membranes were purified and solubilized in SB3-16 as described in legend to Fig. 4; $1.6 \cdot 10^6$ cpm were loaded on an isoelectric focusing gel as described under Experimental, which was run overnight (for details see legend to Fig. 4). The gel was loaded on an SDS polyacrylamide gel (5–15% of acrylamide), run overnight, dried and autoradiographed as described in the legend to Fig. 4. The exposure time was 1 week. Molecular weight markers (a–f) were included in the SDS run (see legend to Fig. 1).

tempting to suggest that perhaps part of the solubilizing power of neutral detergents could be due to the "zwitterionic" properties of the mixed micelles of NP-40–Ampholine. This could also explain the random behaviour of neutral detergents. For instance, with synaptic membranes from rat forebrain, a lysis in 2% NP-40 and 2% Ampholine (pH 5–7) afforded only 50% solubilization of the total sample radioactivity, while 80% solubilization efficiency was achieved by simply increasing to 6% the amount of Ampholine in the lysis buffer and in the IF gel[26,27]. This behaviour is consistent with our hypothesis of "zwitterionic" mixed micelles, as high levels of Ampholines could have shifted the equilibrium towards the formation of "zwitterionic micelles" resembling natural zwittergents in charge density. It is therefore not surprising that there is such a scatter in literature reports on the solubilizing power of neutral surfactants[3]: different amounts and types of carrier ampholytes used would alter the properties and charge density of the resulting mixed micelles. The different amounts of urea used (in general from 5 to 9 *M*) and different temperatures in different experimental protocols would also suggest the formation of mixed micelles having random stoichiometries and variable solubilizing power from one laboratory to another. What is lacking here is a control experiment in which membranes are solubilized and electrophoretically separated in the complete absence of carrier ampholytes (work is in progress along these lines).

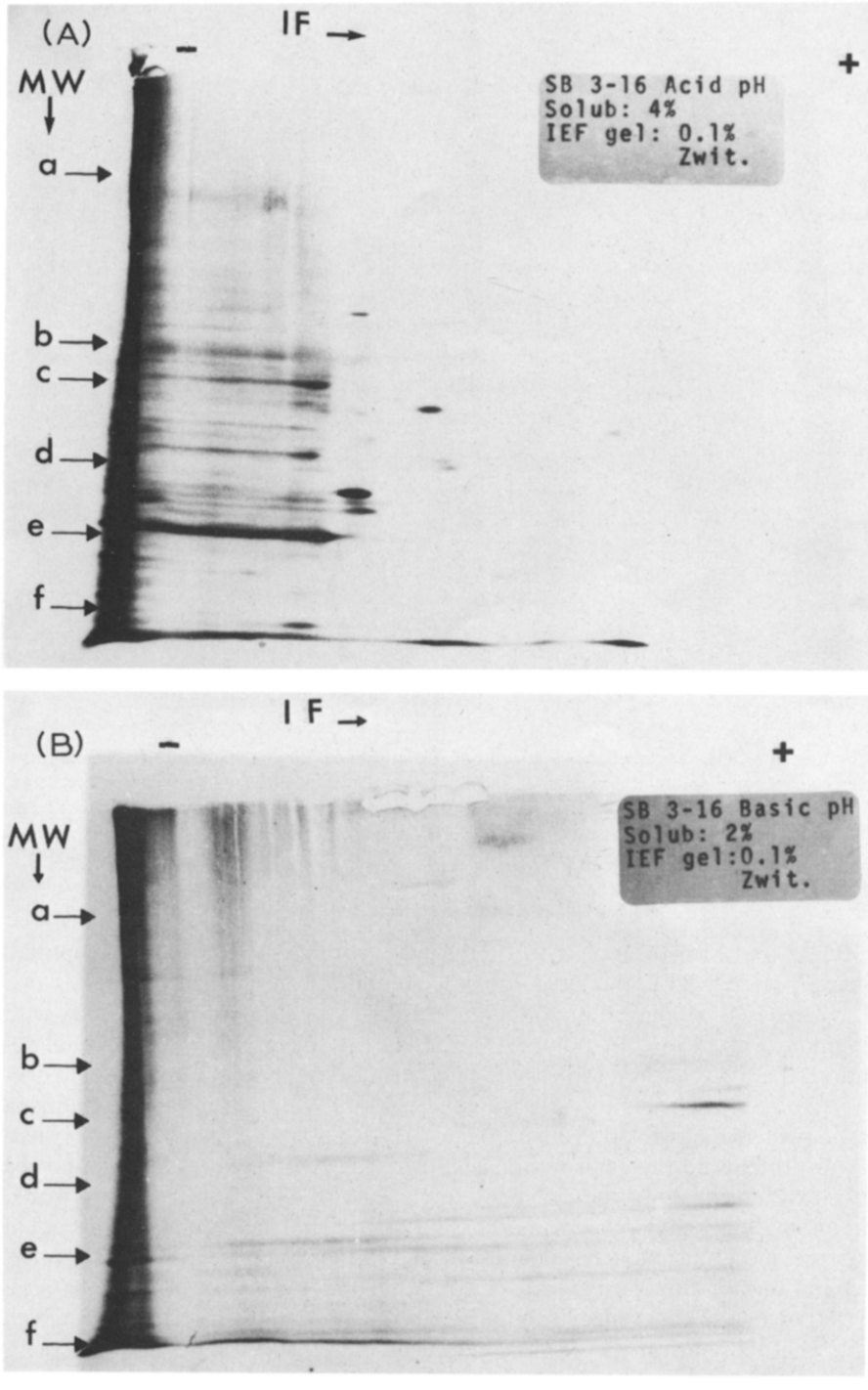

Fig. 8. L6 confluent cells were labelled and plasma membranes were obtained as described in the legend to Fig. 4. They were subsequently solubilized in SB3-16 as described under Experimental; $1.6 \cdot 10^6$ cpm were loaded on an isoelectric focusing gel done using either: (A) Ampholines of acidic pH (0.8% of pH 2.5–4, 0.8% of pH 4–6 and 0.4% of pH 3.5–10); the pH gradient was linear between pH 3.8 and 5.4; or (B) Ampholines of basic pH (0.8% of pH 7–9, 0.8% of pH 9–11 and 0.4% of pH 3.5–10); the pH gradient was linear between pH 4.7 and 8.3. The remainder of the procedure was as described in the legend to Fig. 4. The exposure time in both instances was 1 week. Molecular weight markers (a–f) were included in the SDS run (see legend to Fig. 1).

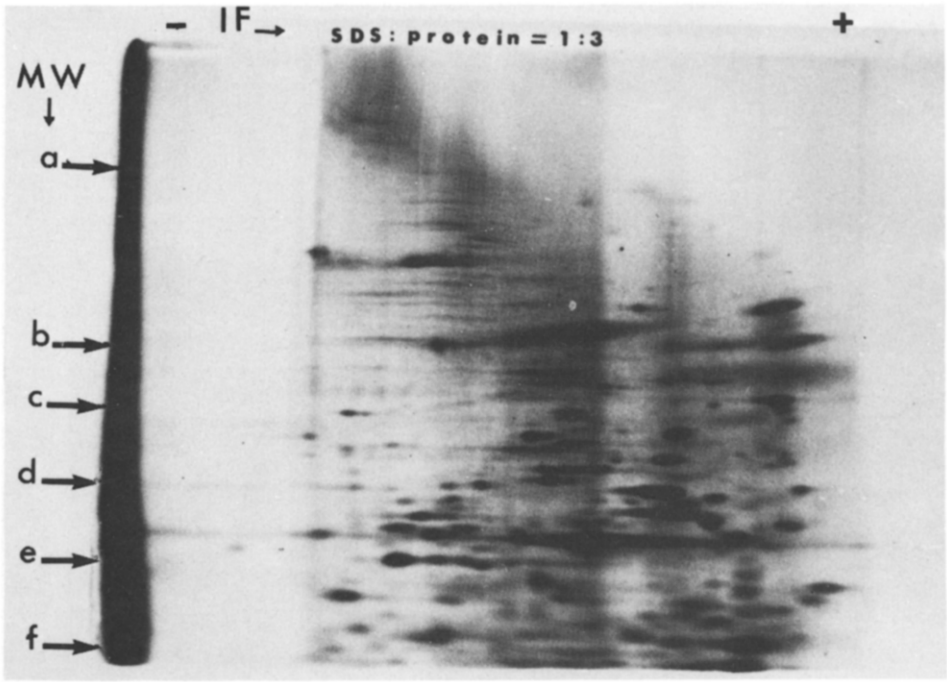

Fig. 9. Confluent L6 cells were labelled *in vitro* with [^{35}S]methionine (20 μCi/ml) for 22 h. Plasma membranes were purified and solubilized in SDS at an SDS to protein ratio of 1:3 as described in method 1; $1.6 \cdot 10^6$ cpm were loaded on an isoelectric focusing gel as in ref. 11, which was run overnight. The gel was loaded on an SDS–polyacrylamide gel (5–15% of acrylamide), which was run overnight, fixed, dried and autoradiographed. The exposure time was 1 week. Molecular weight markers (a–f) were included in the SDS run (see legend to Fig. 1).

SB3-12 and SB3-14 appear to be the two zwittergents that achieve optimal solubilizing power; SB3-8 exhibited too low solubilization efficiency and was discarded, whereas SB3-16 gave horizontal and vertical streaking in the 2-D map, with a concomitant loss of low M_r polypeptides. Hence it appears that, for optimal solubilization, the hydrocarbon tail should have just the right length and right hydrophobicity. SB3-8 is probably too short to bind efficiently to hydrophobic domains in membrane proteins; at the opposite extreme, SB3-16 is too long and binds too tenaciously to proteins so that, during the SDS run, the zwittergent moiety cannot be efficiently exchanged along the polypeptide chain with SDS micelles. This would result in anomalous behaviour during the SDS electrophoresis step. That this could be the correct mechanism has been demonstrated in the case of SDS: some commercial preparations of this surfactant are contaminated by higher homologues, especially tetradecyl- and hexadecylsulphates. The latter bound to proteins with such an avidity that its removal was extremely difficult[28,29].

Zwittergents versus anionic detergents

It is generally agreed that nothing can surpass SDS solubilization[3]. We fully agree, but what we are observing is what happens after the proteins are in solution,

namely their electrophoretic movement in the IF gel. When using the SDS levels typical of SDS denaturing buffer (SDS protein ratio of at least 10:1)[30], the proteins obviously failed to migrate correctly (Fig. 1). It was only when reading the review of Dunn and Burghes[3] that we realized that we should have used a much lower ratio (SDS:protein = 1:3). This was obviously a printing error, as Ames and Nikaido[12] would never have used such an unfavourable SDS to protein ratio. Luckily, we did not notice this printing error and performed the wrong experiments, which led us to the excellent separation in Fig. 9. The interpretation may be that when there is an excess of free SDS in solution, either because of problems of too high conductivity in the sample zone, or because of a possible interaction of free SDS micelles with carrier ampholytes, the membrane proteins aggregate. Consequently, the pH gradient is perturbed and artifactual (microheterogeneity) banding patterns are observed. For the opposite reason, when the amount of SDS is limiting, and little or no free SDS is available in solution, a substantial proportion of membrane polypeptides are able to enter the IF gel and to migrate to their p*I* position. Thus SDS can be a valid substitute for NP-40 or zwittergents in the lysis buffer provided, however, that it is used in limiting amounts, well below the total protein level. This would have two beneficial effects: (a) there would be no unbound SDS free in solution and (b) as the protein would be only partially SDS saturated, its removal from the polypeptide moiety in exchange for NP-40 or zwittergents during the IF step would be greatly facilitated.

NOTE ADDED IN PROOF

After this manuscript had been completed, another paper dealing with zwittergent solubilization of plasma and mitochondrial membranes was brought to our attention[31]. Even though the results were obtained by a completely different approach (turbidimetry rather than the 2-D techniques used in our work), their overall conclusions were strikingly similar to our own, with zwittergent 3-14 exhibiting the highest solubilizing power, superior to taurodeoxycholate, Triton X-100, octylglucoside and even CHAPS. Moreover, a model was proposed that we had suspected all along, namely that different detergents could have different solubilizing properties on different types of membranes. Zwittergents are very effective on both plasma and mitochondrial membranes, whereas neutral detergents are effective only on the latter and exhibit a very low disaggregating power on plasma membranes.

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