SOLUBILIZATION OF *ESCHERICHIA COLI* MEMBRANE PROTEINS BY APROTIC SOLVENTS

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1. Introduction

Relatively few tightly membrane-bound proteins have so far been highly purified in functional form [1,2]. This seems to be due in large part to problems of inactivation and aggregation caused by detergents which are commonly employed for solubilization. An alternative methodology has been developed in the case of the extremely hydrophobic C_{55}-isoprenoid alcohol kinase which has been highly purified in organic solvent mixtures based on butanol-1 [3,4]. However, less than 2% of the total membrane protein was solubilized by butanol-1 in the initial solubilization step performed at pH 4.2. A virtually complete solubilization by butanol-1 or other organic solvents studied so far is possible only at strongly acidic pH values [4,5].

It is now reported that certain aprotic solvents bring about an extensive solubilization of *E. coli* membrane proteins under mild conditions (pH 6–8). Some of the results have been briefly communicated [6,7].

2. Experimental

2.1. *Membrane preparations*

2.1.1. Total membrane preparation

Escherichia coli, strain ML 308-225, was grown in minimal medium M 63 [8] supplemented by 0.35% (w/v) potassium succinate and a total membrane fraction was prepared by the method of Osborn and Munson [9]. After washing four times with 0.25 M sucrose, 3.3 mM Tris–HCl, 1 mM EDTA, pH 7.8, containing 5 mM $MgCl_2$, the final membrane pellet (114 mg total protein from 10 g cells) was suspended in 20 ml of the same buffer and stored frozen at −22°C.

Abbreviation: SDS, sodium dodecylsulphate

2.1.2. Membrane vesicle preparation

Vesicles were prepared from succinate-grown cells of *E. coli* ML 308-225 [10] by the procedure of Kaback [11]. The final membrane fraction was suspended to 13 mg/ml protein in 100 mM potassium phosphate, pH 6.6, and stored in liquid nitrogen.

2.2. *Solubilization procedure*

Suspensions of total membranes (20 ml, 114 mg protein) or of membrane vesicles (3 ml, 39 mg protein) were divided into seven equal portions. Membrane pellets were isolated by centrifugation (30 min, 100 000 × *g*, 2°C) and were then suspended in 5 ml of the following buffers: 500 mM sodium acetate, pH 4.0 or 5.0; 500 mM sodium citrate, pH 6.0; 500 mM Tris–HCl, pH 7.0, 8.0 or 9.0; 0.25 M sucrose, 3.3 mM Tris–HCl, 1 mM EDTA, pH 7.8. Mg^{2+} was added to each suspension to a final concentration of 5 mM and the membrane fractions were re-isolated as described above. The membrane samples were washed once more by the same procedure and were then resuspended in 500 μl of the same buffer. Portions of 50 μl of the various suspensions were kept frozen at −22°C.

For solubilization, 450 μl organic solvent, or buffer as a control, was added to the thawed membrane sample, followed by incubation (30 min, 25°C, Eppendorf shaker No. 3300). Undissolved material

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was removed by centrifugation (120 min, 100 000 × *g*, 15°C) and was then solubilized by heating (5 min, 95°C) in 100 μl SDS-containing sample buffer [12]. The supernatant contained the solubilized proteins which were analyzed for protein content and by SDS–slab-gel electrophoresis. For the latter method, 100 μl aliquots were used. Organic solvent was removed in vacuo, 30 μl sample buffer [12] was added and the samples were heated (5 min, 95°C).

2.3. *Protein determination*

The sample solution (1 vol.) and an aqueous solution of SDS (2% w/v) (1 vol.) were mixed and heated (5 min, 95°C). Aliquots were then analyzed by a modified Lowry procedure [4], using bovine serum albumin (Serva) as a standard.

2.4. *Gel electrophoresis*

SDS–slab-gel electrophoresis was carried out using an apparatus similar to that described by Reid and Bielesky [13]. The gel compositions and buffers used were as described by Laemmli [12] except that the acrylamide concentration of the stacking gel was increased to 5% (w/v). The running gel contained 10% acrylamide. Staining of the protein bands in the gels was performed using Coomassie Blue at 60°C [14].

3. Results and discussion

3.1. *Solubilization of membrane polypeptides by N-methylpyrrolidone*

To illustrate the solubilization effect of aprotic solvent, the results obtained using *N*-methylpyrrolidone are shown in fig.1. Treatment of the total membrane fraction at pH 5–6 (gel Nos 2,3) resulted in about 18% solubilization (Lowry test). Butanol-1 solubilized only 2% protein under identical conditions (gel No. 7).

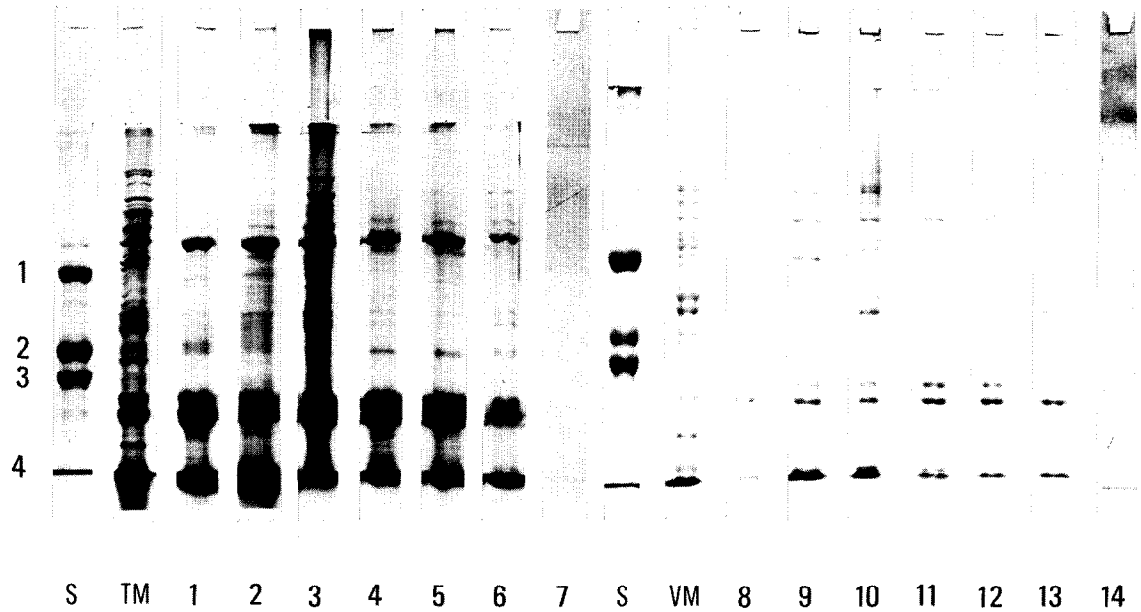

Fig.1. Solubilization by *N*-methylpyrrolidone. Gels labeled 'S' show the following standard polypeptides of mol. wt [14]: 68 000, 43 000, 40 000 and 25 700, for bovine serum albumin (1), ovalbumin (2), aldolase (3) and chymotrypsinogen (4), respectively. The gel labeled 'TM' was obtained with the total membrane preparation. Gel Nos 1–6 show the polypeptides solubilized from the total membrane preparation with *N*-methylpyrrolidone at pH values 4, 5, 6, 7, 8 and 9, respectively. Gel No. 7 shows a butanol-1 extract from a similar experiment (pH 6). The gel labeled 'VM' was obtained with the vesicle membrane preparation. Gels Nos 8–13 show the polypeptides solubilized from the vesicle preparation with *N*-methylpyrrolidone at pH values 4, 5, 6, 7, 8 and 9, respectively. Gel No. 14 shows a butanol-1 extract from a similar experiment (pH 5). The indicated pH values refer to the pH values of the various buffers used in the solubilization procedure (see Experimental).

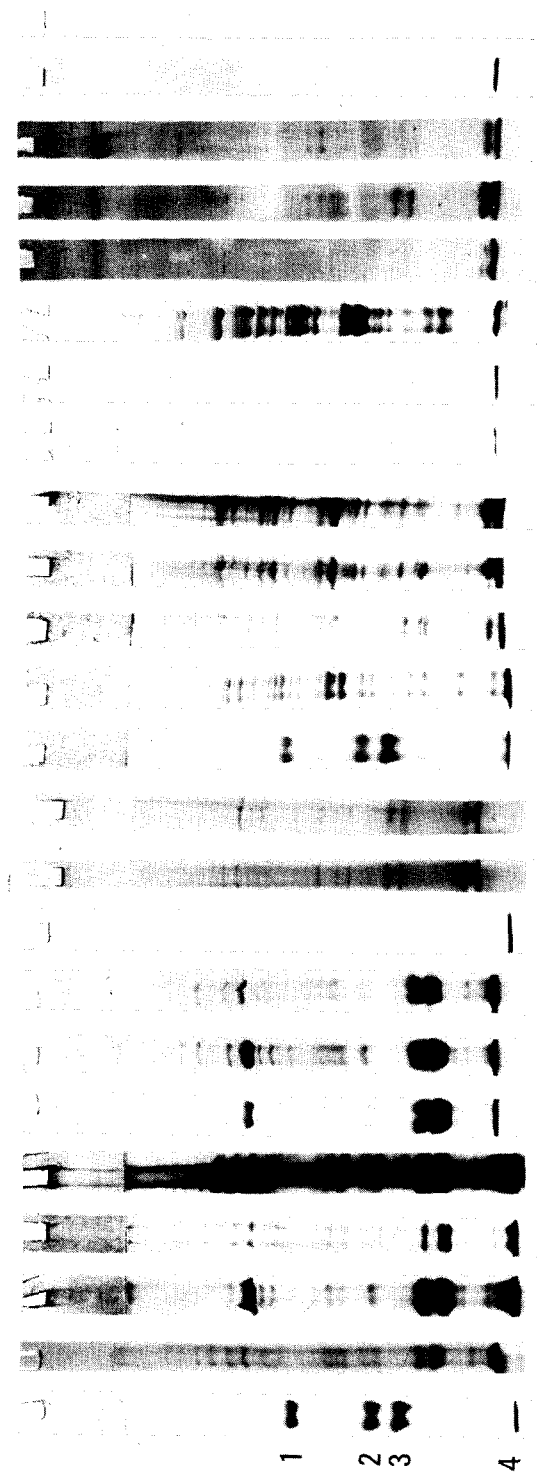

Fig.2. Influence of extraction conditions on polypeptide gel patterns. The designations 'S', 'TM' and 'VM' were used as in fig.1. Gel Nos 1–20 were taken from a number of separate experiments. The positions and staining intensities of the polypeptide bands are therefore not always directly comparable. Gel Nos 1–9 were obtained with the total membrane fraction. The solvents and pH conditions used for solubilization were as follows: Gel Nos 1–3, hexamethylphosphoric triamide at pH values 4, 6 and 9, respectively. Gel Nos 4–6, dimethylsulfoxide at pH values 4, 6 and 9, respectively. Gel No. 7, *N*-methylpyrrolidone at pH 7.8, in the presence of 100 mM $Ca(OAc)_2$. Gel No. 8, hexamethylphosphoric triamide at pH 7.8, in the presence of 1 M NH_4OAc. Gel No. 9, same experiment as No. 8, with omission of NH_4OAc. Gel Nos 10–20 were obtained with the membrane vesicle fraction. The following solvents and pH conditions were used for extraction: Gel Nos 10–12, hexamethylphosphoric triamide at pH values 4, 6 and 9, respectively. Gel Nos 13–15, dimethylsulfoxide at pH values 4, 7 and 9, respectively. Gel Nos 16–18, tetramethylurea at pH 5, 7 and 9, respectively. Gel No. 19, Surfynol 61 at pH 7. Gel No. 20, dimethylformamide at pH 7.

At pH 4 and pH 7–9, solubilization was less effective (9% of total protein; gel Nos 1,4–6). With the membrane vesicle fraction, which is enriched in inner membrane material [11], the percentage of protein solubilized at pH 5–7 was again about 20% (gel Nos 9–11). Butanol-1 eluted about 7% of the total protein (gel No. 14).

3.2. *Solubilization by other aprotic solvents*

Compared to *N*-methylpyrrolidone, dimethylsulfoxide solubilized slightly less protein (15% at pH 5) of the total membrane fraction, and distinctly less from the vesicle preparation (6% at pH 5), although in the latter case solubilization increased to 17% at pH 9. SDS–gels for these experiments are shown in fig.2 (gel Nos 4–6 and 13–15). About 15% of the total vesicle protein was solubilized by tetramethylurea between pH 4 and pH 9 (fig.2, gel Nos 16–18), while dimethylformamide was much less effective under the same conditions (gel No. 20). 3,5-Dimethyl-1-hexin-3-ol (Surfynol 61, Serva Co., Heidelberg), a protic solvent with detergent properties, was also rather ineffective (fig.2, gel No. 19). The most effective solvent among those investigated was hexamethylphosphoric triamide which solubilized 40–60% protein from the total membrane fraction between pH 4 and 9 (fig.2, gel Nos 1–3) and even more protein (60–80%) from the vesicle preparation (gel Nos 10–12). Hexamethylphosphoric triamide was difficult to remove in vacuo and the residual amount of solvent interfered somewhat with the electrophoretic separation. It can be seen, however, that quite a number of polypeptides were solubilized.

3.3. *Influence of salt*

Treatment of the total membrane fraction with hexamethylphosphoric triamide, in the additional presence of 100 mM LiCl or NH_4OAc, resulted in the solubilization of 80–90% of the total protein (see fig.2, gel Nos 8,9). The same percentage of total protein was solubilized from membrane vesicles under these conditions. These were the highest yields of solubilized protein attained. In the presence of 100 mM $Ca(OAc)_2$, however, no protein was solubilized from the total membrane preparation and no polypeptides were detected by gel electrophoresis (fig.2, gel No. 7).

3.4. *Selectivity*

A comparison of gel patterns from solubilization experiments where pH values and/or the solvent used were varied (figs 1,2) indicated that a certain selectivity existed with regard to the solubilized polypeptides. For example, the prominent outer membrane polypeptides of molecular weights between 30 000 and 40 000 appeared to be preferentially eluted from the total membrane preparation. Such observations of selectivity argue against the possibility that treatment with aprotic solvent merely resulted in fragmentation to membrane particles not sedimenting in the ultracentrifuge.

3.5. *Functional activity*

When almond β-glucosidase or trypsin were dissolved in 90% *N*-methylpyrrolidone or hexamethylphosphoric triamide and kept for 2 h at 25°C, full enzyme activities were still present upon dilution into the respective assay buffers (data not shown). Other examples for the survival of enzyme activities in organic solvent have been reviewed [15,16]. A reconstitution of the *E. coli* lactose permease system has been achieved by the solubilization technique described in the present paper [10]. Preliminary gel chromatographic data (controlled pore glass CPG-10 (118 Å), hydroxypropylated Sephadex G-50, Sepharose CL-6B) indicate that phospholipids are present in monomeric form in the aprotic solvent extracts and fractionation of the polypeptides should become possible.

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