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Comparison of the Polypeptide Composition of *Escherichia coli* Outer Membranes Prepared by Two Methods

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Escherichia coli outer membranes were prepared by centrifugation to equilibrium in sucrose gradients and then treated with Sarkosyl in the presence of ethylenediaminetetraacetate. The polypeptide profiles of the two outer membrane preparations were compared by two-dimensional polyacrylamide gel electrophoresis. The patterns obtained were not identical, and Sarkosyl removed several minor proteins from the outer membrane.

The cell envelope of *Escherichia coli* and other related species contains two membranes: the inner (or cytoplasmic) membrane and the outer membrane. Several methods exist for the separation of the outer membrane (9), one of which relies upon its resistance to solubilization by the detergent Sarkosyl (4). Because of the relative simplicity of the method, Sarkosyl has been widely used for the preparation of outer membranes. Although major outer membrane proteins remain unextracted by Sarkosyl (4), it is unknown whether all outer membrane proteins behave in the same manner (9). Thus the assumption, made by several authors (5, 8, 14), that minor envelope proteins found in the Sarkosyl-soluble fraction derive from the inner membrane may be invalid. This question has therefore been examined by two-dimensional polyacrylamide gel electrophoresis (10), which is capable of resolving minor components in complex mixtures of proteins.

Outer membranes were prepared from *E. coli* K-12 strain JC3272 (3) by centrifugation to equilibrium in sucrose gradients by the method of Smit et al. (13). Their purity with respect to contamination by inner membranes was assessed by estimation of NADH dehydrogenase (EC 1.6.99.3) and succinic dehydrogenase (EC 1.3.99.1). Both enzymes are located in the inner membrane (11). Comparison of enzyme specific activities in the crude cell lysates with those in the outer membrane fraction indicated negligible contamination (<5%) of outer membranes by inner membranes (Table 1). Treatment of these outer membranes with Sarkosyl (0.5%, vol/vol) (4) failed to alter the specific activity of the enzymes (Table 1) but caused removal of about 1.2% of the total proteins.

The polypeptide contents of the two outer membrane preparations were compared by two-dimensional gel electrophoresis (Fig. 1). Features of these profiles are illustrated diagrammatically in Fig. 2. Although many of the polypeptides (namely, proteins Ia, Ib, and II*) were common to both preparations, it was clear that the profiles were not identical. In particular, treatment with Sarkosyl removed a number of high-molecular-weight polypeptides (see areas within dashed boxes in Fig. 2A and B). Treatment with Sarkosyl led to the appearance of several spots (group A, Fig. 2B) not found in the control preparation. These proteins appeared as a series of spots at the same position in the isoelectric focusing dimension and with different positions in the second dimension. Since they were located below two major proteins (Ia and Ib), the minor spots are probably artifacts (see reference 10). Treatment with Sarkosyl led to the appearance of some spots (namely, 1 to 3, Fig. 2A and B) not found or barely detectable in the untreated preparation. This may result from

TABLE 1. *NADH dehydrogenase and succinic dehydrogenase in cell fractions*[a]

Enzyme	Crude lysate	Outer membrane	Outer membrane after extraction with Sarkosyl
NADH dehydrogenase	0.32	0.011	0.013
Succinic dehydrogenase	0.55	0.009	0.012

[a] The crude lysate obtained by French press disruption of whole bacteria was fractionated on sucrose gradients (13) to produce outer membranes. These were suspended (100 μg of protein per ml) in 3 mM EDTA (pH 7.2) and treated with 0.5% (vol/vol) Sarkosyl as described (4). Sarkosyl-treated outer membranes were recovered by centrifugation (78,000 × *g*, 2 h, 4°C) and washed twice in water. Enzymes were assayed following published procedures (1, 3), and activities are expressed as micromoles per minute per milligram of protein. Values are the means of duplicate determinations for each fraction.

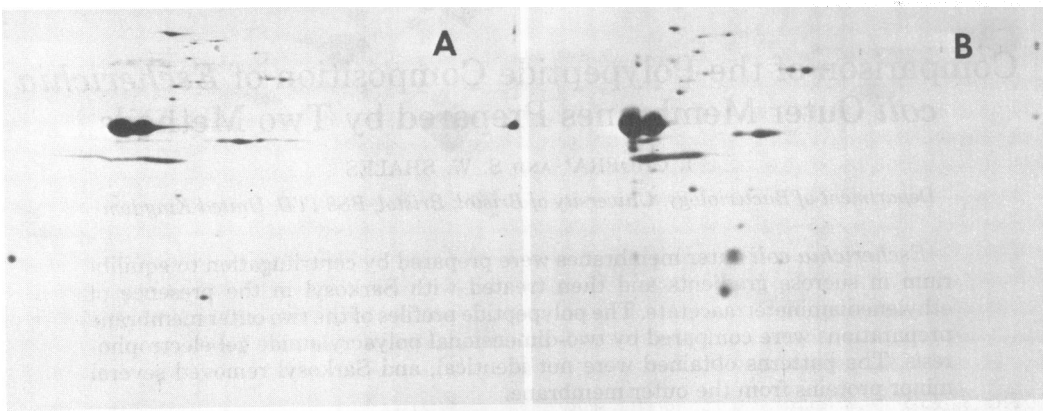

FIG. 1. *Two-dimensional electrophoretic separation of outer membrane proteins. (A) Outer membranes separated by centrifugation in sucrose gradients (13) and (B) after treatment with Sarkosyl (4). Two-dimensional electrophoresis was performed essentially as described previously (6) with the following modifications. Protein solubilized in the sample preparation buffer for isoelectric focusing was quantified (15) to permit identical protein loading (125 μg). After separation in the first dimension, the cylindrical isoelectric focusing gels were fixed and stained as described previously (7). Before the second dimension separation these gels were equilibrated for 1.5 h as described previously (7).*

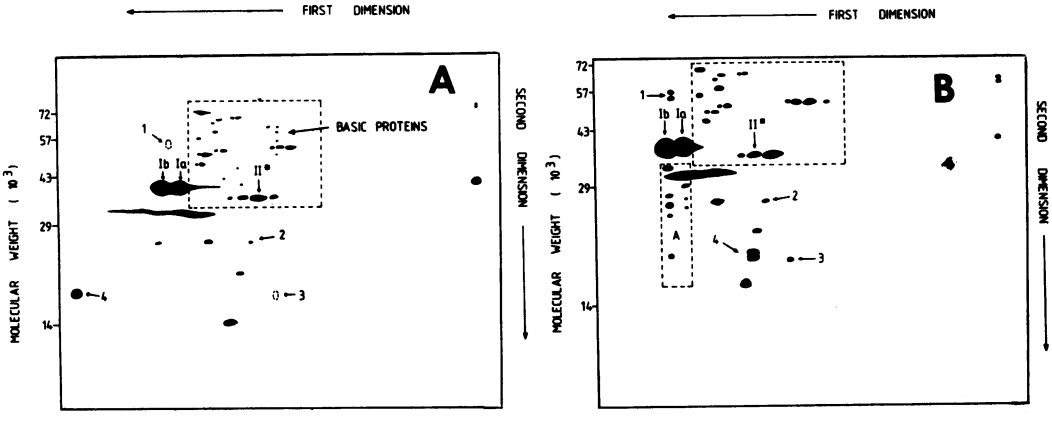

FIG. 2. *Diagrammatic representation of the gels shown in Fig. 1. (A) Outer membranes separated by centrifugation in sucrose gradients (13) and (B) after treatment with Sarkosyl (4). The positions of outer membrane proteins Ia, Ib, and II* are indicated. These proteins were identified by the use of mutants which lacked the individual proteins (2). The pH's in the regions of proteins Ia/Ib and II* were approximately 5.0 and 6.4, respectively (2). The molecular weight range was estimated by use of a cross-linked standard (monomer to pentamer of a 14,300 polypeptide; British Drug Houses product number 44223 2U). The group of spots designated A in (B) are probably artifacts (see text). Spots 1 to 4 and those designated basic proteins are discussed in the text.*

increased solubility of the proteins in the sample preparation buffer after prior exposure to Sarkosyl. The isoelectric point of a low-molecular-weight protein (spot 4, approximate molecular weight 16,000) (Fig. 2) appeared to be affected by exposure to Sarkosyl.

Treatment of outer membranes with Sarkosyl failed to remove residual inner membrane proteins as judged by the levels of NADH dehydrogenase and succinic dehydrogenase (Table 1). However, the apparent loss of minor proteins after treatment of the outer membrane with Sarkosyl could still be interpreted as indicating that it is contaminated with inner membrane or periplasmic proteins and that these proteins are solubilized by Sarkosyl. Polypeptides from the inner membrane (13) and periplasmic space (12) were therefore separated by two-dimensional electrophoresis to determine whether the proteins solubilized by Sarkosyl could be detected in these fractions. Enlarged areas of gels comparable with those in Figs. 1 and 2 are shown in

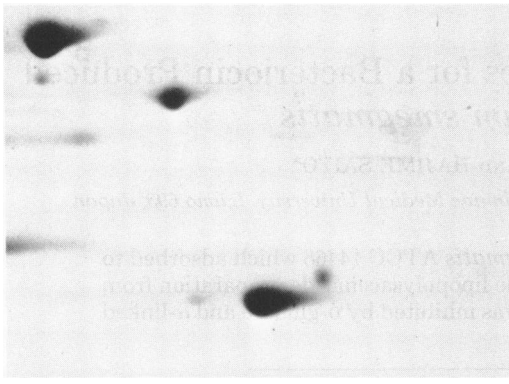

FIG. 3. *Two-dimensional electrophoretic separation of inner membrane proteins. Inner membranes separated by centrifugation in sucrose gradients (13) were washed twice in water and then analyzed by two-dimensional electrophoresis as described in the legend to Fig. 1. Only a high-molecular-weight region of the gel is illustrated, comparable with the boxed areas in Fig. 2.*

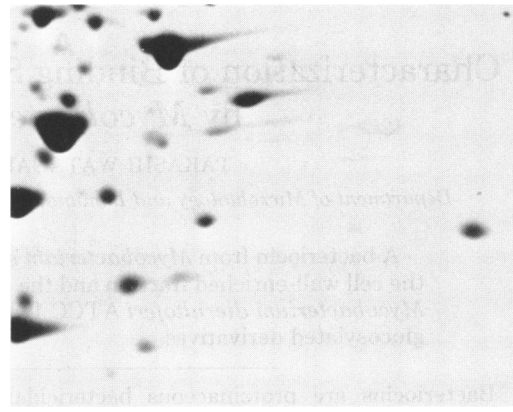

FIG. 4. *Two-dimensional electrophoretic separation of periplasmic proteins. Periplasmic proteins, prepared as by Sato et al. (12), were concentrated by lyophilization and then analyzed by two-dimensional electrophoresis as described in Fig. 1. Only a high-molecular-weight region of the gel is illustrated, comparable with the boxed area in Fig. 2 and that in Fig. 3.*

Fig. 3 (inner membrane) and Fig. 4 (periplasmic fraction). Although some of the polypeptides removed by Sarkosyl from the outer membrane could indeed be contaminants, a group of basic proteins (see Fig. 2A) that were solubilized by the detergent appear to be genuine outer membrane proteins.

The results presented here show that treatment with Sarkosyl can lead to loss of proteins from the outer membrane. Caution should therefore be exercised before concluding that a protein found in the Sarkosyl-soluble extract is necessarily derived from the cytoplasmic membrane.

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