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Examination of the Protein Composition of the Cell Envelope of *Escherichia coli* by Polyacrylamide Gel Electrophoresis

CARL A. SCHNAITMAN

Department of Microbiology, University of Virginia School of Medicine, Charlottesville, Virginia 22901

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An envelope preparation containing the cell wall and cytoplasmic membrane of *Escherichia coli* was obtained by breaking the cells with a French pressure cell and sedimentating the envelope fraction by ultracentrifugation. This fraction was prepared for polyacrylamide gel electrophoresis by dissolving the protein in an acidified *N,N'*-dimethylformamide, removing lipids by gel filtration in the same organic solvent and removing the solvent by dialysis against aqueous urea solutions. More than 80% of the total protein of the envelope fraction was recovered in soluble form. Electrophoresis on sodium dodecyl sulfate-containing gels yielded from 20 to 30 well-resolved bands of protein. One major protein band was observed on the gels. This protein had a molecular weight of 44,000 and accounted for as much as 40% of the total protein of the envelope fraction. A double-labeling technique was used to examine the protein composition of the envelope fraction from cells grown under different sets of conditions which result in large changes in the levels of membrane-bound oxidative enzymes. These changes in growth conditions resulted in only minor alterations in the protein profiles observed on the gels, suggesting that this organism is able to adapt to changes in growth environment with only minor modifications of the major proteins of the cell envelope.

The concept that membranes contain structural or organizational proteins and catalytic proteins (5, 6) is difficult to examine in a rigorous fashion. *Escherichia coli* provides a biological system which, for several reasons, is well suited for testing this hypothesis. The extensive development of genetic systems in this organism permits the use of mutants and genetic techniques to determine the function of major membrane proteins of unknown or unmeasurable catalytic activity. The availability of systems for studying conditional lethal mutants permits the study of alterations in membrane components which are essential for the life of the organism. That the organism is adaptable to a wide variety of growth conditions and carbon sources permits experimental flexibility which cannot be attained with higher organisms.

The successful use of this organism as a tool for studying membrane proteins requires the solution of two problems. First, an adequate system must be devised for the separation and identification of membrane proteins. This is the subject of the present report which describes the application of an improved method for disc electrophoresis of membrane proteins to the examination of the protein composition of the *E. coli* envelope. Second, the envelope of *E. coli* contains in addition to the cytoplasmic membrane a cell wall of complex and presumably proteinaceous structure. Hence, methods are required for the separation of these two components. The companion paper (19) describes the resolution of the cell wall and cytoplasmic membrane and the localization of proteins in these two subcellular organelles.

MATERIALS AND METHODS

Cultures and growth conditions. The bacterial strain used was *E. coli* $O111_{B4}$ obtained from E. Heath, Department of Physiological Chemistry, The Johns Hopkins School of Medicine. This strain was used because the chemical composition of the cell envelope lipopolysaccharide has been thoroughly examined (7). Cultures were grown at 37 C on a rotary shaker in a medium containing 2 g of NH_4Cl, 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.026 g of Na_2SO_4, 0.021 g of $MgCl_2$, and a trace of $FeCl_3$ per liter. The final *p*H of the medium was 7.0, and the carbon source consisted of either 0.5% glucose, 1% glycerol, or 1% succinic acid adjusted to *p*H 7.0 with NaOH. Carbon sources and isotopes, as indicated below, were added after autoclaving the salts. Late log-phase cultures were obtained by inoculating 500 ml of medium in a 2,000-liter flask with 25 to 50 ml of an overnight culture

grown on the same medium and incubating for 3 hr. Anaerobic cultures were grown at 37 C in sealed flasks with a mixture of 95% N_2 and 5% CO_2 bubbled through the medium.

Reagents and chemicals. Sodium dodecyl sulfate (SDS) was obtained from Eastman Organic Chemicals, Rochester, N.Y. Spectrographic-grade *N,N'* dimethyl formamide manufactured by Matheson-Coleman and Bell was obtained from Preiser Scientific, Bethesda, Md. All reagents for polyacrylamide gel electrophoresis were obtained from Canal Industrial Corporation, Rockville, Md. NCS solubilizer was obtained from Amersham/Searle Corp., Des Plaines, Ill. Uniformly-labeled ^{14}C-L-leucine (316 mCi/mmole), ^{14}C-L-tyrosine (454 mCi/mmole), L-leucine-*4,5-^{3}H* (40 Ci/mmole), and L-tyrosine-*3,5-^{3}H* (51 Ci/mmole) were obtained from Schwarz BioResearch, Orangeburg, N.Y. Other chemicals were reagent-grade or better.

Isolation of the crude envelope fraction. Cultures were harvested by centrifugation at 0 C and suspended to a volume of 100 ml in 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.8) containing 1 mM ethylenediaminetetraacetic acid (EDTA). This suspension was placed in a Sorvall Omnimixer blender and treated for 1 min at a speed control setting of 7.5. This procedure removes flagella, pili, and capsular material. This procedure did not result in any cell breakage either, as determined by electron microscopy of fixed, sectioned preparations or as determined by release of the cytoplasmic enzyme glucose-6-phosphate dehydrogenase. The cells were again centrifuged at 0 C and suspended to 25 ml in the same buffer solution. Approximately 1 mg each of pancreatic ribonuclease and deoxyribonuclease were stirred into the suspension, and the cells were broken by passage twice through an Aminco French pressure cell operated in a motor-driven press at the maximum working pressure. The broken cell suspension was then made 2 mM in Mg^{2+} by the addition of $MgCl_2$ and centrifuged at 5,000 rev/min in a Sorvall SS-34 rotor for 5 min to remove intact cells and debris. The supernatant fluid was then centrifuged in a Spinco 50Ti rotor for 45 min at 50,000 rev/min. The pellet containing the particulate fraction was then suspended to 5 ml in the Tris-EDTA buffer described above and treated with 0.2 mg of the detergent Lubrol WX per mg of protein. The use of Lubrol WX for facilitating the removal of soluble proteins from membrane preparations has been described elsewhere (18). After treatment with Lubrol WX, the suspension was again centrifuged at 50,000 rev/min for 45 min in the Spinco 50Ti rotor. In some of the earlier preparations, the pellet was then suspended in 15% (w/w) sucrose, layered over 55% (w/w) sucrose, and centrifuged for 2 hr in the Spinco SW 25.1 rotor at 25,000 rev/min. The interface band was removed, diluted with buffer as above to reduce the sucrose concentration, and centrifuged as above. This procedure removed any remaining intact cells. However, since the remaining intact cells amounted to less than 5% of the total protein, the procedure was discontinued because it resulted in some loss of envelope material.

Solubilization and electrophoresis of envelope proteins. The procedure for solubilization and removal of lipid is essentially as previously reported (16, 17), but is described here in greater detail. The solvent system for solubilizing the envelope proteins consisted of nine parts of cold *N,N'*-dimethylformamide and one part of 0.1 M HCl (DMF + HCl; 23). This solvent is unstable, becoming more basic upon standing. Decomposition was minimized by keeping all solutions at 0 C or below, and freshly prepared solvent was used for all procedures. Freshly prepared DMF + HCl had a *p*H of 2.3, as measured with a conventional *p*H electrode. The amine content of commercial batches of DMF varies considerably; thus, with some batches of solvent, redistillation or increasing the HCl concentration to 0.12 M were necessary to lower the *p*H to 2.3. Even slight increases in the *p*H of the DMF + HCl causes a reduction in protein solubility.

The final pellet of the envelope fraction was suspended in a small amount of distilled water, and 0.3 ml of this suspension (containing not more than 10 mg of protein) was placed in a glass homogenizer. DMF + HCl (6.0 ml) was added, and the protein was dispersed by a few gentle strokes of the homogenizer. This solution was allowed to stand for 15 to 30 min at 0 C and was then centrifuged at 17,000 rev/min in the Sorvall SS-34 rotor for 15 min to remove any precipitated carbohydrate or protein. The supernatant fraction was then chromatographed at 4 C on a column (1.5 by 50 cm) of Sephadex LH-20 which had previously been washed with at least two column volumes of fresh DMF + HCl. The protein was eluted from the column with DMF + HCl, and the location of the protein peak was determined by observing the optical density at 280 nm. The protein content of the peak was estimated by assuming optical density of 0.900 for a solution in a 1-cm cuvette containing 1 mg of protein/ml. The fractions containing the protein were pooled and dialyzed overnight at 4 C against 80 volumes of 8 M urea in 35% acetic acid followed by dialysis at room temperature for 6 to 8 hr against 80 volumes of 8 M urea in 1% sodium acetate. The contents of the bag were then removed and made 0.1% in SDS by the addition of 10% SDS solution. The sample was placed in a fresh dialysis bag and dialyzed overnight at room temperature under an atmosphere of N_2 against 80 volumes of a solution containing 8 M urea, 0.1% SDS, and 0.1% 2-mercaptoethanol in 0.1 M sodium phosphate buffer (*p*H 7.2). In most cases, the samples were then concentrated to about 2 to 3 mg of protein per ml by vacuum dialysis in collodion bags or by dialysis against dry Sephadex or powdered cane sugar.

Gel electrophoresis was carried out as previously described (16) in the SDS-containing gel system described by Maizel (10). With some recent batches of acrylamide monomers, it has been necessary to reduce the amount of ammonium persulfate catalyst to prevent uneven banding resulting from too rapid polymerization. Best results have been obtained when the ammonium persulfate catalyst is adjusted for each batch of reagents so that polymerization occurs in 30 to 60 min at room temperature.

Examination of acrylamide gels. Gels were fixed

and stained with Coomassie Blue, as previously described (16), for direct observation of protein bands. Gels containing labeled protein were preserved by fixing and staining as above or by freezing and storage at −20 C. Radioactivity was determined by a modification of the method of Basch (1). Gels were sliced into sections 1.25 mm thick, and each slice was placed in a scintillation vial containing 0.5 ml of NCS solubilizer. The vials were sealed and placed in a water bath at 60 C for 2 hr. The samples were then cooled, and 10 ml of toluene-based counting fluid was added to each vial. The vials were allowed to stand at 4 C overnight before counting. This results in a swelling of the gel slices so that they are permeated by the counting fluid, and both ^{3}H and ^{14}C are recovered completely with minimal quenching.

Enzyme and biochemical assays. Protein was determined by the procedure of Lowry et al. (9), by using bovine serum albumin as a standard. The radioactivity of protein from cells grown on labeled leucine and tyrosine was determined by adding samples up to 50 μliters to 1 ml of 5% calf serum containing 0.5 mM unlabeled leucine and tyrosine. The sample was precipitated by the addition of 1 ml of cold 10% trichloroacetic acid. After centrifugation, the precipitate was dissolved in 1.0 ml of NCS solubilizer and transferred to a counting vial containing 10 ml of toluene-based counting fluid. This procedure resulted in complete precipitation of radioactive protein from samples dissolved in DMF + HCl or in 8 M urea solutions in which precipitation was not possible in the absence of carrier protein.

Succinic dehydrogenase (EC 1.3.99.1), formic dehydrogenase (EC 1.2.2.1), and lactic dehydrogenase (EC 1.1.2.4) were assayed polarographically with a Clark electrode by measuring oxygen uptake in the presence of the dye phenazine methosulfate. Succinic dehydrogenase was assayed by preincubating the enzyme for 10 min in an assay system containing 0.05 M phosphate buffer (*p*H 7.6), 25 mM succinate, 10 mM EDTA, and 2 mM KCN (18). Since KCN does not completely inhibit respiration, air was bubbled through the preincubation mixture to maintain O_2 saturation. The reaction was initiated by the addition of 0.05 ml of 40 mM phenazine methosulfate to 1.95 ml of the preincubation mixture. Formic dehydrogenase was assayed by preincubating the enzyme for 1 min in 1.85 ml of an assay medium containing 0.25 M phosphate buffer (*p*H 6.5), 10 mM EDTA, and 2 mM KCN. The reaction was started by the addition of 0.1 ml of 0.5 M sodium formate and 0.05 ml of 40 mM phenazine methosulfate. Lactic dehydrogenase was estimated in an assay medium containing 0.05 M phosphate buffer (*p*H 7.6), 10 mM EDTA, 2 mM KCN, and 90 mM sodium DL-lactate. The reaction was started by addition of phenazine methosulfate as above. Glucose-6-phosphate dehydrogenase was assayed exactly as described by Noltmann et al. (13). All assays were conducted at room temperature (23 C). Total phospholipid was estimated as lipid-extractable phosphate exactly as previously described (18).

RESULTS

Properties of the envelope fraction. The envelope fraction used in these studies was free of cytoplasmic contamination as judged by the absence of the enzyme glucose-6-phosphate dehydrogenase. Ninety-five per cent of the glucose-6-phosphate dehydrogenase of intact cells was recovered from the supernatant of the first Spinco centrifugation after breakage in the French pressure cell. The envelope preparations which were freed of any remaining intact whole cells by centrifugation onto a cushion of 55% (w/w) sucrose contained no detectable glucose-6-phosphate dehydrogenase when assayed under conditions which would have detected 1% of the activity present in a corresponding amount of whole cells. This preparation has been examined extensively by electron microscopy of fixed, sectioned preparations and consists entirely of membrane vesicles and fragments of cell wall similar in appearance to that reported for intact cells (2, 11). No ribosomal particles were observed by electron microscopy. Detailed observations on the morphology of this preparation are presented in the companion paper (19). The phospholipid content of this preparation was 0.30 to 0.35 μmoles of lipid-extractable phosphate per mg of protein, as compared to a value for intact cells of 0.075 μmoles per mg of protein.

Recovery of envelope proteins during preparation for gel electrophoresis. The data in Table 1 illustrate the recovery of acid-precipitable protein during the steps of solubilization and gel filtration before gel electrophoresis. The recovery of proteins from the gels after electrophoresis has

TABLE 1. *Recovery of acid-precipitable membrane protein during preparation for gel electrophoresis*[a]

Fraction	Total counts/min	Recovery
		%
Crude envelope fraction..	3.78×10^6	(100)
Soluble in DMF + HCl..	3.10×10^6	84
Recovered from LH-20 column chromatography.....	3.03×10^6	82
Recovered after final dialysis step.....	2.93×10^6	80

[a] Recovery of protein is based on the final crude envelope fraction prepared as described in the text. The culture used was grown to late log phase from an overnight inoculum, and the final culture contained 0.2 μCi/ml each of ^{3}H-leucine and ^{3}H-tyrosine.

also been examined by comparing the total radioactivity in the gel slices to the amount layered on the gel; in several consecutive experiments, all of the radioactivity was recovered from the gel. When samples in the urea-phosphate buffer-SDS solution are concentrated by vacuum dialysis, approximately 30% of the protein is not recovered from the dialysis membrane. However, the gel pattern obtained with concentrated samples is identical both qualitatively and quantitatively to that obtained with unconcentrated samples, indicating that this loss is not restricted to any particular species or molecular weight of protein. Concentration has been found to be of value in obtaining higher resolution of bands on the gels. Although satisfactory bands can be obtained with sample volumes as large as 250 μliters per gel, the sharpest resolution of closely spaced bands is obtained when the sample volume is 50 μliters or less per gel.

Recovery as indicated in Table 1 has been routinely obtained with fresh preparations of *E. coli* envelopes; however, as noted previously for mammaliam membrane proteins (16), recovery drops sharply when preparations are aged for 24 hr or more at 4 C or frozen and thawed. This loss of recoverable protein is usually accompanied by the appearance of substantial amounts of protein at the top of the gels, suggesting protein denaturation.

Gel electrophoresis of envelopes from glucose-grown cells. Figure 1 illustrates the appearance of acrylamide gels of the envelope fraction, as observed by scanning of stained gels and by slicing the gels and measuring the radioactive label. Figures 1A and 1B illustrate gels which were fixed overnight and stained with Coomassie Blue. The correlation between the stained bands and the distribution of the radioactivity is generally good. However, a significant improvement in resolution is obtained (Fig. 1C) when the gels are frozen immediately after termination of the electrophoresis and then sliced immediately after thawing, as opposed to preservation by fixation in sulfosalicylic acid. This is probably due to the fact that considerable diffusion of the protein bands can take place during the time required for the penetration of the fixative, a phenomenon supported by the observation that during fixation the low-molecular-weight proteins towards the bottom of the gels show much more diffusion than the bands near the top of the gel. It is also worth noting that with this method the level of protein between the resolved bands drops to a baseline of almost zero. The resolution of this technique is good, and is limited primarily by the gel-slicing techniques.

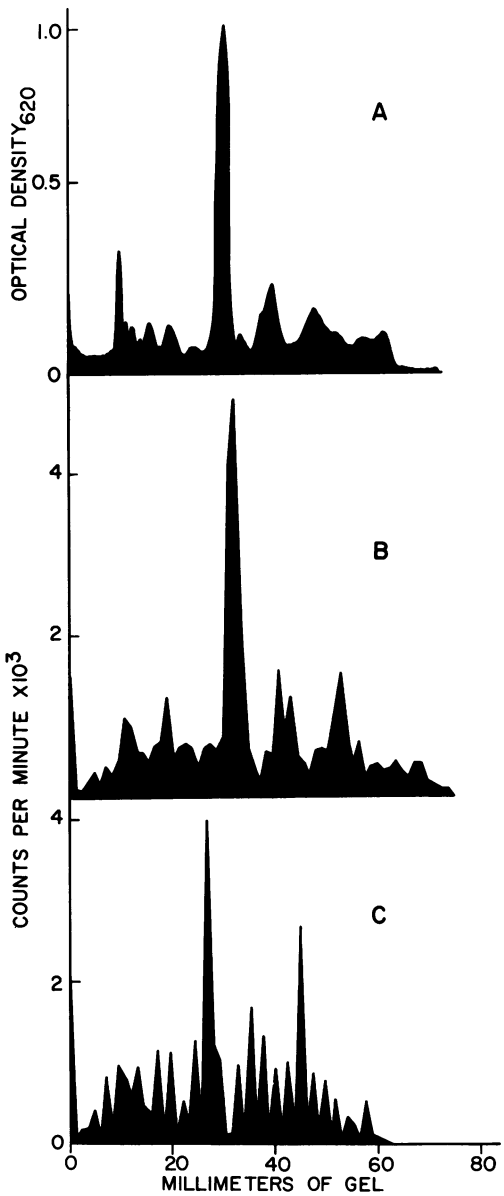

FIG. 1. *Acrylamide gel profiles of envelope protein. (A) Optical density tracing at 620 nm. (B) Radioactivity profile of a gel which was fixed and stained with Coomassie Blue before slicing. (C) Radioactivity profile of a gel which was preserved by freezing. The culture was grown to late log phase with glucose as carbon source, and both the inoculum and the final culture contained 0.2 μCi/ml each of ^{3}H-leucine and tyrosine.*

A conspicuous feature of the gel pattern of *E. coli* envelopes is the single major peak observed in Fig. 1. This peak accounts for from 25 to 40% of the protein in the *E. coli* envelope

and has been observed in similar amounts in three other strains of *E. coli* which have been examined to date. The molecular weight of this protein has been estimated to be 44,000 by the procedure of Shapiro et al. (20), by using bovine serum albumin monomer and dimer, ovalbumin, trypsin, and cytochrome *c* as standards. In gels which are somewhat overloaded to resolve minor components (such as those shown in Fig. 2), this peak appears to split into two closely spaced peaks. However, when the amount of protein is reduced by two- to fourfold, this protein is again observed as a single symmetrical peak, even when the gels are run up to 10 hr. Thus, it appears that the peak represents a single protein or a mixture of proteins so similar that they cannot be resolved by this technique. It is significant to note that similar splitting of an apparently homogeneous protein into two closely spaced bands has been observed by Renaud et al. (14) in a study of microtuble protein, a protein which shares with membrane proteins the property of insolubility in most aqueous solvents. It is possible that this is indicative of some form of interaction between individual proteins with a strong tendency towards aggregation.

E. coli B, *E. coli* C, and *E. coli* K-10 have also been examined by this technique. The protein patterns observed with these strains were virtually identical to those obtained with *E. coli* strain O111 used in this study. The protein composition of the envelope fraction has also been examined by phenol-acetic acid technique of Takayama et al. (21) and by the modification of the technique described for *Mycoplasma* membranes by Rottem and Razin (15). This procedure yielded poor results, with large amounts of protein required to produce stainable bands and excessive amounts of protein remaining near the top of the gels. Direct solubilization of the envelope protein in 1% SDS in 8 M urea has also been used to prepare proteins for electrophoresis, but this provided much poorer resolution than the procedure employed in this study.

Essentially all of the proteins resolved on the gels have a molecular weight of less than 120,000, suggesting that they are fully dissociated into monomeric polypeptide chains. Substitution of 10^{-3} M dithiothreitol for the 2-mercaptoethanol had no effect on the gel patterns. Inclusion of urea up to 6 M in the gels had no effect other than a slight loss of resolution of individual bands. In most experiments, the amount of protein remaining at the top of the gels was from 3 to 6% of the total protein (radioactivity) recovered from the gels.

Effect of growth conditions on the E. coli envelope protein composition. *E. coli* provides an ideal system for studying the relationship between membrane composition and enzymatic formation, since it is capable of growth on a variety of substrates and under both aerobic and anaerobic conditions. Pronounced changes in membrane-bound enzymes occur under these variations of growth conditions. One well-documented example is the repression of oxidative enzymes by growth on glucose and its catabolites (4, 12, 22). The data shown in Table 2 illustrate the changes in the levels of the membrane-bound enzymes, succinic, lactic, and formic dehydrogenase, under the conditions employed in this study. The level of these enzymes varied by more than threefold, with succinate as the carbon source as opposed to cells grown on glucose. With glycerol as the carbon source, intermediate levels of these enzymes were observed. Similar but more dramatic changes in levels of membrane bound succinic dehydrogenase, reduced nicotinamide adenine dinucleotide oxidase, and cytochrome b_1 were observed by Gray et al. (3) in a study of the enzyme levels in aerobic and anaerobic glucose-grown *E. coli*. To determine whether these changes in enzyme levels were accompanied by changes in membrane proteins as observed in gels of the envelope fraction, the envelopes from cells grown under different conditions were compared by a double labeling technique. Cultures grown under two different conditions were labeled with ^{3}H-leucine and ^{14}C-tyrosine, respectively (legend of Fig. 2). The cultures were then washed and mixed together, and the envelope fraction was isolated as described above. This procedure was necessary to avoid as much as possible any artifacts arising from gel slicing or from minor differences in isolation procedure. To increase the resolution of minor components, somewhat larger amounts of protein were layered on the gels (75 to 100 μg of protein per gel), and the electrophoresis time was increased to 8 hr. The results of three different combinations of growth conditions are summarized in Fig. 2. The protein patterns in all three experiments were similar, when allowance is made for gel-slicing artifacts. The changes observed in each of the individual proteins resolved on the gels are rather small in relation to the observed changes in enzymatic activity. This suggests that the enzymes themselves are such minor components of the membrane that they cannot be resolved on the gels or that they are removed from the membrane by the isolation procedure. The greatest change appeared to be between glucose and succinate-grown cultures. In almost all cases, the changes were quantitative rather than qualitative in nature and represented less than a twofold change in individual peaks. One exception to this

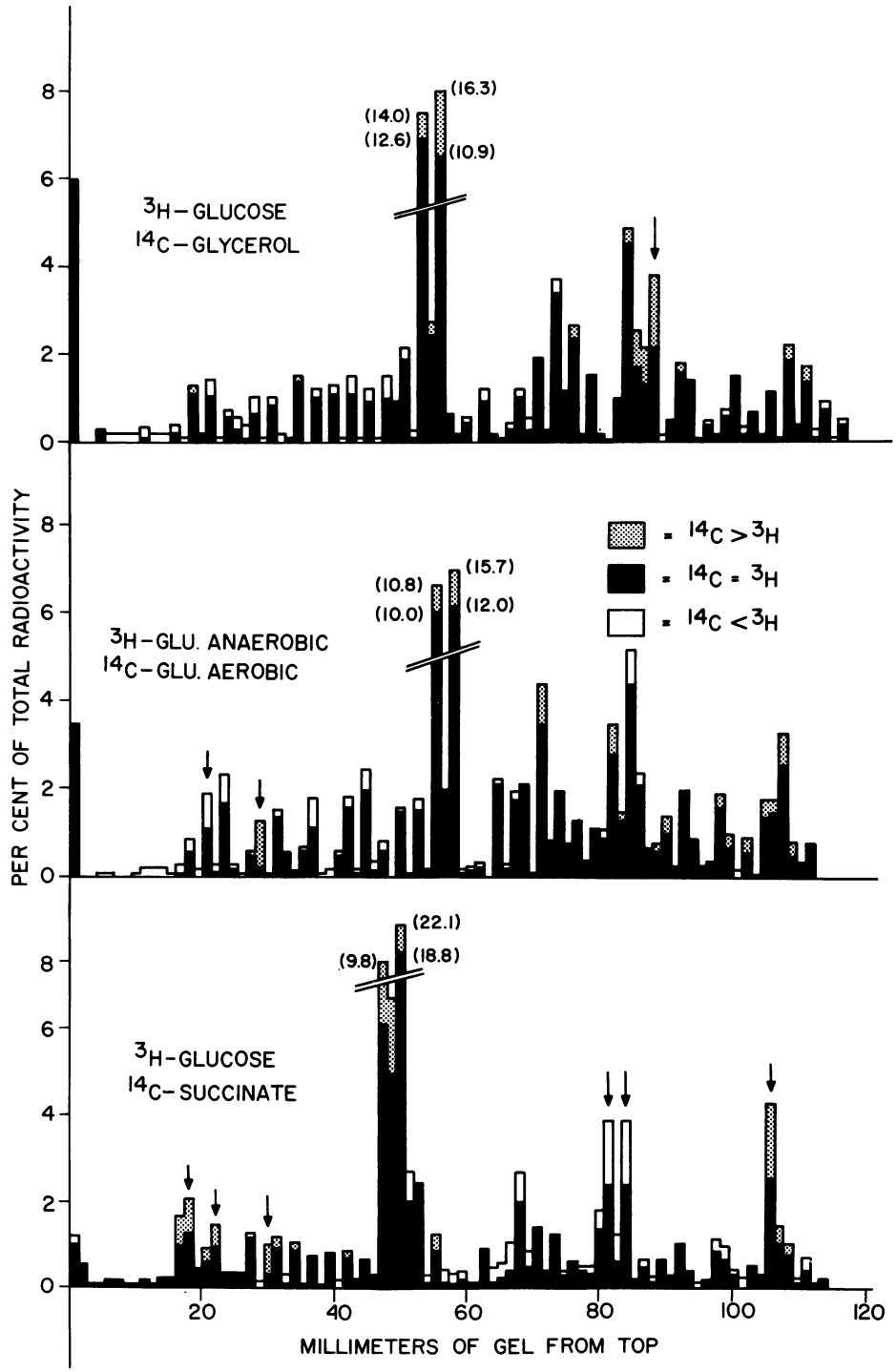

FIG. 2. *Comparison of the acrylamide gel profiles of envelopes from cultures grown under different conditions. In each comparison, one culture was grown in the presence of 0.05 μCi/ml each of ^{14}C-leucine and tyrosine, whereas the other was grown in the presence of 0.1 μCi/ml each of ^{3}H-leucine and tyrosine. The cultures were mixed before isolation of the envelope fraction. The numbers in the brackets indicate the per cent of total counts in the major peaks. Peaks showing more than 30% variation between the respective growth conditions are indicated by arrows.*

TABLE 2. *Effect of carbon source on levels of membrane-bound dehydrogenases in sonic particles*

Carbon source	Succinic dehydrogenase[a]	Lactic dehydrogenase[a]
Glucose	230	87
Glycerol	556	189
Succinate	880	293

[a] Expressed as nanomoles of substrate consumed per minute per milligram protein. Cultures were grown to late log phase on minimal salts plus the respective carbon sources. Sonic particles were obtained as previously described (17).

is the second peak indicated by an arrow in the comparison of aerobic and anaerobic cultures, in which a protein present in aerobic cultures appears missing in anaerobic cultures.

DISCUSSION

The procedure used in this study for resolving the envelope proteins of *E. coli* appears to be an improvement over other gel systems which have been employed with bacterial membrane or envelope preparations (8, 15). The patterns obtained are quite reproducible and indicate the presence of between 20 and 30 protein bands. Resolution of labeled envelope proteins is limited only by the slicing techniques which are used for preparing the samples for counting. A good recovery of proteins is obtained, and little protein remains at the top of the gels. The reasons for the increased resolution of membrane proteins with this system are not known; this may be due either to the more effective dissolution of the membrane proteins by the organic solvent solubilization or to the removal of lipids which are present in aqueous systems in a micellar form and which may effect the stacking of proteins at the top of the gel. The effectiveness of organic solvents for the solubilization of membrane proteins under mild conditions has been demonstrated in recent studies by Zahler (24), who was able to reconsitute both the morphological and spectroscopic properties of membrane proteins dissolved in acidic organic solvents by readdition of lipid and removal of the solvent.

Two models might be proposed for the membrane of a facultative bacterium such as *E. coli* to explain the ability of this organism to change substantially the enzymatic makeup of the membrane as a response to different conditions of nutrition and oxygen tension. The first model would predict that the major constituents of the membrane, which may or may not be enzymatic in nature, would show marked changes in relative amounts. This would provide a variable "template" onto which enzymatic proteins would be attached, and the changes in enzymatic composition would merely reflect these changes in structural or organizational membrane proteins. An alternative model would predict that the structural components of the membrane would remain essentially unchanged, and that enzymatic proteins concerned with specific functions could be inserted into this basic framework as needed by the organism. Within the limitations of the techniques used in this study, it appears that the latter hypothesis is more likely. Changes from aerobic to anaerobic culture and from fermentable to nonfermentable carbon sources produced relatively minor changes in the major proteins resolved on the gels as compared to the large differences in membrane-bound enzymes. This second hypothesis would be more economical for the cell. This may in fact be the definition of a facultative organism which is able to effect a substantial change in metabolic pathways for substrate utilization with relatively minor alterations in cellular composition.

It is interesting to note that the envelope preparations examined in this study appear to contain one protein species which may account for as much as 40% of the total protein of the preparation. It is unlikely that this protein is a component of the cytoplasmic membrane in view of the complexity of function of the bacterial membrane. It would seem more reasonable that this protein represents one of the major components of the outer layers of *E. coli* envelope, since this structure has a much simpler function. The localization of this protein and its relation to the *E. coli* envelope is the subject of the next paper in this series.

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LITERATURE CITED

1. Basch, R. S. 1968. An improved method for counting tritium and carbon-14 in acrylamide gels. Anal. Biochem. 26:184–188.
2. DePetris, S. 1967. Ultrastructure of the cell wall of *Escherichia coli* and the chemical nature of its constituent layers. J. Ultrastruct. Res. 19:45–83.
3. Gray, C. T., J. W. T. Wimpenny, D. E. Hughes, and M. R. Mossman. 1966. Regulation of metabolism in facultative bacteria. I. Structural and functional changes in *Escherichia coli* associated with shifts between the aerobic and anaerobic states. Biochim. Biophys. Acta 117:22–32.
4. Gray, C. T., J. W. T. Wimpenny, and M. R. Mossman. 1966. Regulation of metabolism in facultative bacteria. II. Effects of aerobiosis, anaerobiosis and nutrition on the formation

of Krebs cycle enzymes in *Escherichia coli*. Biochim. Biophys. Acta 117:33–41.

5. Green, D. E., N. E. Haard, G. Lenaz, and H. I. Silman. 1969. On the noncatalytic proteins of membrane systems. Proc. Nat. Acad. Sci. U.S.A. 60:277–284.
6. Green, D. E., H. D. Tisdale, R. S. Criddle, P. Chen, and R. M. Bock. 1961. The isolation and properties of the structural protein of mitochondria. Biochem. Biophys. Res. Commun. 5:109–119.
7. Heath, E. C., R. M. Mayer, R. D. Edstrom, and C. A. Beaudreau. 1966. Structure and biosynthesis of the cell wall lipopolysaccharide of *Escherichia coli*. Ann. N.Y. Acad. Sci. 133:315–333.
8. Jones, T. H., and E. P. Kennedy. 1969. Characterization of the membrane protein component of the lactose transport system of *Escherichia coli*. J. Biol. Chem. 244:5981–5987.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
10. Maizel, J. V., Jr. 1966. Acrylamide gel electrophoresis by mechanical fractionation: radioactive adenovirus proteins. Science 151:988–990.
11. Murray, R. G. E. 1968. Bacterial cell wall anatomy in relation to the formation of spheroplasts and protoplasts. p. 1–18. *In* L. B. Guze (ed.), Microbial protoplasts, spheroplasts, and L-forms. The Williams & Wilkins Co., Baltimore, Md.
12. Neiderhardt, F. C., and B. Magasanik. 1956. Inhibitory effect of glucose on enzyme formation. Nature 178:801–802.
13. Noltmann, E. A., C. J. Grubler, and S. A. Kuby. 1961. Glucose 6-phosphate dehydrogenase. I. Isolation of the crystalline enzyme from yeast. J. Biol. Chem. 236:1225–1230.

Renaud, R. L., A. J. Rowe, and I. R. Gibbons. 1968. Some properties of the protein forming the outer fibers of cilia. Cell Biol. 36:79–90.

15. Rottem, S., and S. Razin. 1967. Electrophoretic patterns of membrane proteins of mycoplasma. J. Bacteriol. 94:359–364.
16. Schnaitman, C. 1969. Comparison of rat liver mitochondrial and microsomal membrane proteins. Proc. Nat. Acad. Sci. U.S.A. 63:412–419.
17. Schnaitman, C. 1969. Alteration of membrane proteins in a chlorate-resistant mutant of *Escherichia coli*. Biochem. Biophys. Res. Commun. 37:1–5.
18. Schnaitman, C., and J. W. Greenawalt. 1968. Enzymatic properties of the inner and outer mitochondrial membranes of rat liver mitochondria. J. Cell Biol. 38:158–175.
19. Schnaitman, C. A. 1970. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. 104:890–901.
20. Shapiro, A. L., E. Vinuela, and J. V. Maizel, Jr. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. 28:815–820.
21. Takayama, K. D., H. MacLennan, A. Tzagoloff, and C. D. Stoner. 1964. Studies on the electron transfer system. LXVII. Polyacrylamide gel electrophoresis of the mitochondrial electron transfer complexes. Arch. Biochem. Biophys. 114:223–230.
22. Umbarger, H. E. 1954. The influence of the environment on acetate metabolism in *Escherichia coli*. J. Bacteriol. 68:140–145.
23. Zahler, P. 1968. Blood group antigens in relation to chemical and structural properties of the red cell membrane. Vox Sang. 15:81–101.
24. Zahler, P. 1969. The structure of the erythrocyte membrane. Experientia 25:449–456.