DEMONSTRATION OF MISSING MEMBRANE PROTEINS IN A COLICIN-TOLERANT MUTANT OF E. COLI K12.

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A mutant which was tolerant to the killing action of colicin E1 because it contains an extensive deletion in the tol C locus of E. coli was characterized in some detail. Physiological growth studies showed that this mutant was sensitive to a wide range of antibiotics and detergents, indicating an alteration of the cell envelope. By using first polyacrylamide gel electrophoresis and secondly a technique using radioactive double label and a sucrose-SDS gradient, it was possible to demonstrate the loss of a membrane protein in this tol C deletion mutant.

Our understanding of the relationship between the structural organization and function of cell membranes is still very limited. However, research in this area should be greatly facilitated by the isolation and detailed investigation of potential membrane mutants. Recent studies have shown that bacterial mutants tolerant to the killing action of colicins (protein antibiotics liberated by certain bacterial strains) have altered membrane characteristics (1,2,3,4,5,7 and 8). These colicin-tolerant bacteria are still able to adsorb the colicin but are no longer killed by them. The mutation to tolerance modifies the bacterial membrane in such a way as to give protection against the killing action of the adsorbed colicin. Our immediate objective has

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been to determine the molecular basis for colicin-tolerance and relate the genetic and physiological properties of these mutants to the actual changes that occur in the membrane organization when the gene products from the colicin-tolerant locus tol C (tolerant only to colicin El) are absent. This paper describes the demonstration of missing components from the membrane of a deletion mutant of the tol C gene of E. coli K12.

MATERIALS AND METHODS

Bacterial strains X36 (6) and the colicin tolerant mutant tol C 101 derived from X36 by E. Whitney were kindly provided by Drs. E. Whitney and S. Silver (8). The tol C mutant 101 was shown by phage P1 transduction analysis to carry an extensive deletion of the tol C locus of E. coli (8). The sensitivity of this tol C mutant to various antibiotics was determined by placing Dispens-O-Dics (Difco) of different antibiotics on a lawn of the strain to be tested (5).

Membrane proteins were prepared for polyacrylamide gel electrophoresis by growing cells overnight in nutrient broth with vigorous aeration at 37°C. Cells were harvested by low speed centrifugation and washed with 0.1 M Tris-HCl buffer, pH 8.1, three times before disruption in an Aminco French pressure cell at 15,000 p.s.i. The membrane proteins were then isolated and solubilized as described elsewhere (9,11).

Electrophoresis using sodium dodecyl sulfate (SDS)-acrylamide gel was performed in a discontinuous gel system. The stacking gel consisted of 3% acrylamide, 1.1% bisacrylamide and 0.1% SDS in 0.125 M Tris buffer, pH 6.8. The separating gel had an acrylamide concentration of 7% and bisacrylamide concentration of 2.6% with 0.1% SDS in 0.0375 M Tris buffer, pH 8.8. TEMED and ammonium persulfate were added to a concentration of 0.0003 ml/ml and 0.5 mg/ml, respectively, for initiating and catalyzing the polymerization reaction. The gels were run in a buffer

containing 0.15% Tris, 7.2% glycine and 0.1% SDS. The gels were 6 mm in diameter and 12 cm long. They were pre-run for at least half an hour before applying the sample. 50% sucrose was added to the sample to permit layering between the buffer and the top of the gel. The gels were run at 2.5 mA/gel for 3 hours. The proteins in the gel were stained with either Amido Black 10B (10) or coomassie blue (11,12).

A sucrose-SDS gradient was devised as a preliminary separation of membrane proteins before subjecting membrane preparations to analysis by SDS-gel electrophoresis. Membrane preparations of strain X36 grown in the presence of ^{14}C-leucine and mutant 101 labelled with ^{3}H-leucine were obtained by French pressing combined cell suspensions of both strains X36 and 101 and centrifuging as described above. The final membrane preparations, containing about 10% of the total incorporated radioactivity, were carefully resuspended in a dissolving buffer (of 0.8% Tris, 1% SDS, 1% sucrose, pH 6.8) overnight at 4°C. The radioactivity in CPM of these membrane protein suspensions was about 1.94×10^5 cpm/ml of ^{3}H and about 1.31×10^4 cpm/ml of ^{14}C. Sedimentation of the membrane proteins was carried out in a 1-5% sucrose gradient which also contained 1% SDS. Gradients were centrifuged at 24,000 rpm for 16 hours in the SW 25.1 Spinco rotor at 21°C. Fractions were collected from a hole pierced in the bottom of the centrifuge tube and samples taken from the individual fractions for counting in a scintillation counter. A gradient run was arbitrarily divided into two fractions A and B and the individual tube fractions comprising fraction B were pooled together and dialyzed overnight against a Tris buffer (0.15%) at 4°C. The contents (membrane proteins) of the dialysis tube were precipitated by the addition of ammonium sulphate (60% of saturation) and kept on ice for 30 minutes before being centrifuged at 18,000 rpm for 1 hour. The pellet was resuspended in dissolving buffer for SDS gels (11). Total protein content (13) was determined and about 150 µg was added per gel for analysis by gel electrophoresis.

RESULTS AND DISCUSSION

Strain 101 is tolerant to colicin E1 only and sensitive to the killing action of colicins E2, E3, K, A and phage BF23 (8) indicating a mutation in the tol C locus (4,5,8). The genetic analysis of this mutant by both phage P1 transduction and by bacterial matings (5,8) has confirmed the close linkage of the tol C locus (and the deletion of strain 101) to the met C bacterial marker between arg G (at 61 minutes) and met C (at 58 minutes).

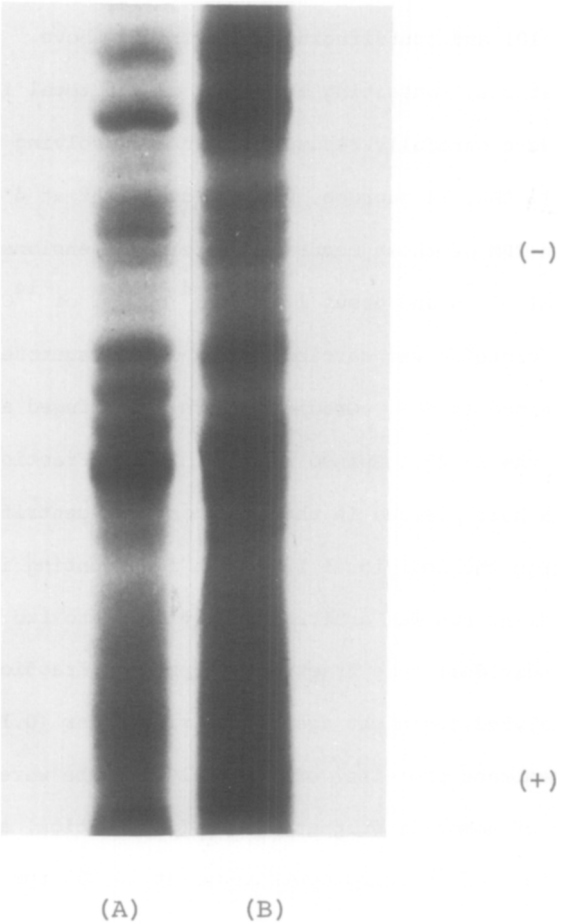

Figure 1. Polyacrylamide gel electrophoresis of membrane proteins of the parent strain (A) and the tol C deletion mutant (B).

Because the physiological evidence (2,3,4,5,8) and the work of Bhattacharyya and co-workers (7) strongly suggested that tol C mutants were membrane mutants we decided to investigate whether or not the absence of certain membrane proteins could be observed in mutant 101 which carried an extensive deletion within the tol C locus. It was possible to demonstrate with polyacrylamide gel electrophoresis the absence of a particular protein band from the deletion mutant 101 when compared with its parent strain (Figure 1).

Additional corroborative evidence regarding this apparent absence of a membrane component in the tol C deletion mutant was obtained by comparing radioactively-labelled preparations of membranes from both the parent and mutant strain. The experiment was to demonstrate a distinct presence of a membrane component in the parent strain with its corresponding absence in the tol C deletion mutant. The sucrose-SDS gradient was used as a preparative procedure to initiate the separation of membrane proteins on the basis of their size and to see if such a

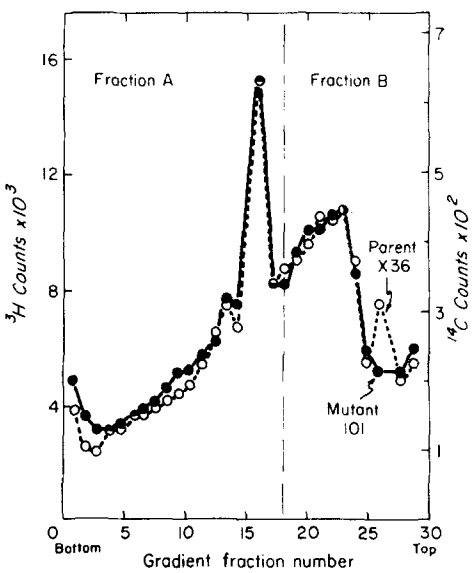

Figure 2. Profile of a sucrose-SDS gradient run of a combined membrane preparation containing both the parent X36 (^{14}C-labelled, O———O) and mutant 101 (^3H-labelled, ●———●) membranes. The procedure provides an initial separation of radioactively labelled membrane proteins.

difference between the strains could be observed. The results of the gradient run (Figure 2) provide an indication of membrane protein differences between the two strains (Fraction B of Figure 2). Further proof of the absence of certain proteins in the membrane of the tol C deletion mutant 101 was obtained by examining fraction B from the sucrose-SDS gradient on SDS gel electrophoresis (Figure 3). When the ratios of $^{14}C/^{3}H$ were calculated and plotted, fraction B off the gradient contained a single peak indicating the presence of a membrane component in the parent but absent in the mutant strain (Figure 3). These observations taken together further confirm the view that the tol C locus codes directly or indirectly for proteins that are integral components of the bacterial cell membrane (4,5). It should be noted, that at present, only one end of the deletion in the tol C locus has been precisely determined. Experiments are now in progress to further investigate the gene products from the tol C locus and establish their function and role in the organization of cell membranes. Finally, this combined procedure of an SDS gradient separation followed by an SDS gel electrophoresis of different gradient fractions, should provide an effective method for the more general problem of separating membrane proteins.

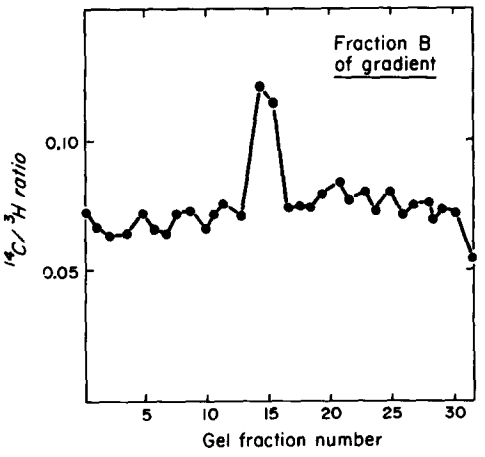

Figure 3. SDS gel electrophoresis of pooled fraction B taken off the sucrose-SDS gradient run of Figure 2. The gel were fractionated into about 30 equal slices and the amount of radioactivity determined per slice. The ratio of $^{14}C/^{3}H$ was calculated for each slice and plotted against gel fraction number.

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