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Demonstration of a Missing Outer Membrane Protein in *tolG* Mutants of *Escherichia coli*

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A class of *Escherichia coli* mutants called *tolG* are specifically tolerant to bacteriocin JF246. Cell envelopes were prepared from three independent spontaneous *E. coli. tolG* mutants and the parental strain (*tolG*$^+$). Electrophoresis of these preparations in polyacrylamide gels containing sodium dodecyl sulfate showed that the *tolG* strains lacked a cell envelope protein found in the *tolG*$^+$ strain. It was estimated that this protein accounted for 10% of the total cell envelope proteins by densitometer tracings of gels stained with Fast Green. Membrane fractionation by isopycnic centrifugation in a sucrose density gradient showed that this protein was located in the outer membrane of *tolG*$^+$ cells. Genetic studies using conjugation, transduction and reversion showed that, in the limited number of recombinants or revertants studied, strains exhibiting the tolerant phenotype lacked the outer membrane protein, whereas the protein was present in bacteriocin-sensitive strains.

1. Introduction

Bacteriocins are a class of protein antibiotics that kill sensitive cells after adsorbing onto a specific receptor on the cell surface (Nomura, 1967). Different bacteriocins may have similar or distinct biochemical effects on sensitive cells (Nagel de Zwaig & Luria, 1967; Nomura, 1964,1967) but the events that lead to these biochemical effects are thought to involve an interaction of the bacteriocin with the bacterial cell membrane (Luria, 1964; Nomura, 1964). Colicin E3 is the bacteriocin whose mode of action is the most completely described. Comparison of *in vivo* (Konisky & Nomura, 1967) and *in vitro* (Boon, 1971; Bowman *et al.*, 1971*a*,*b*) biochemical effects of this bacteriocin suggests that the bacteriocin molecule itself is transported through the cell membrane to its biochemical target, the ribosomes. Nomura (1964) and Luria (1964) have independently postulated a mechanism of bacteriocin action in which the binding of a bacteriocin to its receptor initiates a specific stimulus that is amplified and transmitted to an intracellular biochemical target. The transmission system presumably involves the cell membrane. This model, which assumes that the bacteriocin–receptor complex remains on the cell surface, would have to be modified to account for colicin E3. However, the view remains that there is an essential interaction of bacteriocin with components of the cell membrane.

Bacteriocin-tolerant mutants are able to adsorb bacteriocin without being killed. A variety of these mutants have been isolated, (Foulds & Barrett, 1973; Hill &

Holland, 1967; Nagel de Zwaig & Luria, 1967; Nomura & Witten, 1967; Onodera *et al.*, 1970; Whitney, 1971) in the hope that they would be useful in the study of membrane structure and function as well as bacteriocin action. Characterization of some of the first mutants emphasized the pleiotropic effects of tolerant mutations, such as increased sensitivity to antibiotics, detergents and dyes (Nagel de Zwaig & Luria, 1967; Nomura & Witten, 1967). These observations form the basis for the view that the mutation leading to bacteriocin tolerance somehow affects the integrity of the bacterial cell membrane.

In spite of a number of studies, direct evidence detailing the molecular and biochemical effects of a tolerant mutation has not been reported. In no instance has the gene product of a tolerant gene been identified or characterized as a membrane component. Alterations in protein components of the membrane have been found in strains that carry a deletion in the *tolAB* region or the *tolC* region. The deletion in the *tolAB* region was shown to include a number of loci, such as *chlA* and *chlD*, which effect functions believed to be associated with the cell membrane (Onodera *et al.*, 1970). Membranes prepared from cells that carry this deletion lack several proteins (Onodera *et al.*, 1967) but it is not clear which, if any, of the missing proteins represents the gene product of the *tolAB* locus. Similarly, a deletion that extends into the *tolC* region also results in an alteration in the protein components of the membrane (Rolfe & Onodera, 1971). However, the extent of the deletion was not determined and may include one or more additional cistrons, again making difficult the assignment of the missing protein as the gene product of the *tolC* locus. Holland & Tucket (1972) have studied the protein components from membranes of *cetB* mutants tolerant to colicin E2. They found that this mutation results in the overproduction of a single protein apparently present in the wild type. In the mutant, this protein may account for over 5% of the total membrane protein.

A class of bacteriocin-tolerant mutants called *tolG* has recently been described and genetically characterized (Foulds, 1974; Foulds & Barrett, 1973). *tolG* mutants differ from the parental type in their sensitivity to EDTA, eosin yellow and novobiocin. They are tolerant to bacteriocin JF246, but sensitive to colicins A, C, E1, E2, E3, K and Ia (Foulds & Barrett, 1973). The *tolG* locus lies between *pyrD* and *fabA* (Foulds, 1974), at about 21·5 minutes on the *E. coli* linkage map of Taylor & Trotter (1972). In this study we report the absence of a single protein from the outer membrane of certain *tolG* mutants.

2. Materials and Methods

(a) *Bacterial strains and media*

Bacterial strains used in this study, their sources and some characteristics are listed in Table 1.

Media described by Foulds & Barrett (1973) were used for growth of the cultures.

(b) *Chemicals*

The following chemicals were obtained commercially from Sigma Chemical Co., St. Louis: Sodium dodecyl sulfate, Tris and Coomassie Brilliant Blue; Fast Green was from Eastman, Rochester, N.Y.; acrylamide was obtained from Baker Chemical Co., Phillipsburg, N.J.; N,N′-methylene bisacrylamide (Bis) and N,N,N′,N′-tetramethylethylene diamine were from Canalco, Rockville, Md. L-[^{14}C]leucine (uniformly labeled) and L-[4,5-^{3}H]leucine were purchased from New England Nuclear Boston. All other chemicals used were reagent grade from various commercial sources.

TABLE 1

E. coli *K12 strains*

Strain	Sex	Genotype†	Reference or source
JF404	HfrH	*thyA*	Foulds & Barrett (1973)
JF404-2a	HfrH	*thyA, tolG2*	Foulds & Barrett (1973)
JF404-6a	HfrH	*thyA, tolG6*	Foulds & Barrett (1973)
JF404-9a	HfrH	*thyA, tolG9*	Foulds & Barrett (1973)
AT3143	F⁻	*proC24, pdxC3, pyrC30, ilv-277, met-65, his-53, cyc-1, xyl-14, lacY29, str-97, tsx-63, λ⁻*	A. L. Taylor strain
CSH75	F⁻	*ara, leu, lacY, proC, purE, gal, trp, his, argG, malA, strA, xyl, mtl, ilv, metA or B, thi*	ColdS pring Harbor
JF465	F⁻	*purE⁺*	‡
JF466	F⁻	*purE⁺, tolG2*	‡
JF467	F⁻	*purE⁺, tolG2*	‡
JF468	F⁻	*purE⁺, tolG2*	‡
JF555	F⁻	*pyrD34, tolG2, trp45, mtl-2, xyl-7, malA1, strA-118*	Foulds (1974)
JF568	F⁻	*proC24, aroA357, ilv-277, met-65, his-53, cyc-1, xyl-14, lacY29, str-97, tsx-63, λ⁻*	§

† The genetic nomenclature used is that of Taylor & Trotter (1972).

‡ *purE⁺, strA* recombinant strains derived from strain CSH75 following conjugation with strain JF404-2A. Except for the changes indicated, all other genetic markers were those of the recipient.

§ Transductant derived from strain AT3143 in 2 steps. First, the *pyrC30* allele was replaced by the wild type allele using phage P1 grown on strain JF404. Next, the *aroA357* allele was introduced by cotransduction using phage P1 grown on *E. coli* K12 strain RE138 (*pdxC⁺, aroA357*).

(c) *Growth of cells*

One loopful of culture from a stock slant was transferred into 50 ml of Proteose Peptone–Beef Extract medium (PPBE) in a 250-ml flask and incubated at 37°C with vigorous aeration to an A_{650} of 0·3 (approx. $1{\cdot}5 \times 10^8$ cells/ml). This culture was then diluted 100-fold into 500-ml portions of PPBE medium in 2-l flasks and grown at 37°C to an A_{650} of 0·3 on a rotary shaker. Cultures were harvested by centrifugation at 4°C and washed once with cold saline solution. The pellets were resuspended in 20 ml cold 50 mM-Tris·HCl (pH 7·8) and the total viable cell count determined.

(d) *Preparation of total membranes*

Cells were disrupted by passing the suspension once through a precooled French pressure cell (piston diameter, 1 in; American Instrument Co., Silver Spring, Md) at the maximum working pressure of 18,000 lb/in². Some cell suspensions were passed through the French press twice. The clear mixture of broken cells was centrifuged at 1000 *g* for 20 min to remove the trace of intact cells and debris. The supernatant solution was then centrifuged at 30,000 *g* for 2 h and the pellets, containing primarily cell envelope materials, were resuspended and washed once with 50 mM-Tris·HCl (pH 7·8) to reduce the contamination by cytoplasmic components. The final pellets were carefully resuspended in a small amount of 50 mM-Tris·HCl (pH 7·8) using a 23 gauge needle and syringe at a final protein concentration of 5 to 10 mg/ml. Protein concentration was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. For storage, envelope materials were frozen in liquid nitrogen or a solid CO_2–acetone mixture and kept at −20°C.

(e) *Preparation of cytoplasmic and outer membtranes*

Cytoplasmic and outer membrane fractions were prepared as described by Osborn *et al.* (1972). Cells were harvested in the early logarithmic phase of growth (approx. 1×10^8 cells/ml) by centrifugation at 15,000 *g* for 5 min at 4°C. When large amounts of cells were required, the Sorvall Szent-Györgvi–Blum continuous flow system was used at a speed of 20,000 *g* and a flow rate of 400 ml/min. The cells were resuspended immediately following centrifugation in cold 0·75 M-sucrose, 10 mM-Tris·HCl (pH 7·8) at a final A_{650} of 10. The mixture was chilled in ice. Lysozyme solution was added to a final concentration of 120 μg/ml and, following a 2-min incubation at 0°C, 2 columns of cold 1·5 mM-EDTA (pH 7·5) were added to the suspension with a peristaltic pump, at a constant flow rate, over 5 to 15 min. Spheroplasts formed were disrupted sonically by four, 15-s treatments with 15-s intervals between treatments. The sonicated materials were centrifuged for 20 min at 1000 *g* to remove intact cells. The membrane materials were collected by ultracentrifugation at 104,000 *g* for 1 h, washed with 0·25 M-sucrose, 3·3 mM-EDTA (pH 7·5) and fractionated by isopycnic sucrose density-gradient centrifugation in the SW41 rotor at 5°C at 37,000 revs/min for 14 h in a step-wise gradient of 30% to 55% sucrose. Visible membrane bands from gradients were collected, washed and resuspended in 50 mM-Tris·HCl (pH 7·8) at a final protein concentration of approximately 2 to 10 mg/ml.

(f) *Preparation of radioactive cell envelopes*

Strains were grown in 500 ml of minimal medium supplemented with 0·2% glucose and all growth requirements except leucine. L-[^{3}H]leucine was added to JF465 *tolG*$^+$ at a final concentration of 10 μg/ml (3 μCi/ml) and L-[^{14}C]leucine added to JF466 *tolG* at a final concentration of 10 μg/ml (0·4 μCi/ml). When the cell density reached approximately 2×10^8 cells/ml, the cultures were harvested by centrifugation, washed once with saline containing 0·01% L-leucine and resuspended in 20 ml of saline containing 0·01% leucine. The 2 suspensions were combined and thereafter treated as a single sample. The doubly-labeled cell mixture was disrupted by treatment with the French Press apparatus and proteins prepared from cell envelopes were solubilized and applied to polyacrylamide gels as described below. Following electrophoresis the gels were frozen and fractionated using an Aliquo-gel fractionator (Gilson Medical Electronics). Each fraction, corresponding to approximately 1 mm of gel, was extracted with 0·25 ml of 1% sodium dodecyl sulfate for 48 h at 37°C, and counted in 5 ml of a toluene scintillation liquid containing 5% Biosolv (Beckman Instruments, Palo Alto, California).

(g) *Gel electrophoresis*

We used the sodium dodecyl sulfate gel electrophoresis in a discontinuous buffer system of Neville (1971). The running gel contained 11% acrylamide, 0·1% bisacrylamide and 0·05% ammonium persulfate in 0·42 M-Tris·HCl (pH 9·18); the stacking gel contained 3% acrylamide, 0·2% bisacrylamide and 0·05% ammonium persulfate in 0·054 M-Tris·H_2SO_4 (pH 6·1). In each gel tube, 3 ml of running gel and 0·5 ml of stacking gel were cast. Before the gels set, approximately 200 μl of *t*-amyl alcohol was added to the top of each tube. After the gels hardened the *t*-amyl alcohol was removed. The buffer system for protein samples consisted of 0·04 M-Tris·HCl, 0·04 M-H_3BO_3, 0·1% sodium dodecyl sulphate, 6% sucrose, 0·15% dithiothreitol and 0·001% bromphenol blue as tracking dye, pH 8·64. Protein samples were solubilized by adding 20 μl 0·5 M-Na_2CO_3 (pH 10), 50 to 80 μg protein sample, 20 μl 20% sodium dodecyl sulfate, 20 μl β-mercaptoethanol and buffer mixture in sequence to make a final volume of 200 μl. The sample mixtures were boiled at 100°C for 2 min, cooled to room temperature and applied to the gel. Gels were run at 1·5 m*A*/tube at room temperature with tap water cooling. Electrophoresis was terminated when the tracking dye had just left the bottom of the gel. Gels were removed from tubes, stained with 0·05% Coomassie Blue in 10% acetic acid for 15 h and destained in 10% acetic acid at 37°C.

(h) *Quantitative estimation of gel proteins*

Densitometer tracings were made of sodium dodecyl sulfate–polyacrylamide gels stained with Fast Green to provide an estimate of the relative quantity of protein in each band (Gorovsky *et al.*, 1970).

Following electrophoresis, gels were stained overnight with a solution containing

0·1% Fast Green and methanol/acetic acid/water (5:1:4, by vol.). Gels were destained in a solution containing 10% acetic acid, 25% methanol until the gel background was clear. Next the gels were soaked at room temperature for 12 to 18 h in 50% methanol to reduce their size. The gels were scanned at 637 nm using a Gilford 2400 spectrophotometer equipped with a linear transport apparatus. Peak areas were measured by triangulation.

(i) *Genetic techniques*

The procedures for conjugation and transduction were as described previously (Foulds & Barrett, 1973). Independent spontaneous *tolG*$^+$ revertants were selected by plating approximately 5×10^8 cells from sepatate cultures of strain JF404-2a (*tolG*) on PPBE agar containing 50 μg novobiocin/ml. Only one revertant was chosen from each plate.

(j) *Phosphoglucose isomerase*

Phosphoglucose isomerase activity was determined using the method of Slein (1955).

3. Results

(a) *Characterization of proteins in envelope fraction*

One passage through the French press apparatus resulted in a decrease in the viable count of at least 10^{-4}, indicating that the disruption of cells was essentially complete. The contamination of the final envelope preparation with cytoplasmic proteins was estimated by comparison of phosphoglucose isomerase activity in the broken cell suspension with that found in the final envelope preparation. We found approximately 2000 units of enzyme activity per mg protein in the broken cell preparation and 50 to 60 units/mg protein, representing less than 1% of the total initial activity, in the final envelope preparation indicating little contamination from cytoplasmic components.

(b) *Gel electrophoresis of envelope proteins*

The solubilized envelope proteins from three independent *tolG* mutants and the *tolG*$^+$ parental strain were analyzed by discontinuous polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. A major protein band (band G) solubilized from envelope prepared from the *tolG*$^+$ strain was missing in similar preparations from each of three *tolG* mutants (Plate I, gels A,B,C and D). The molecular weight of the protein in band G was estimated to be 33,000 by comparison of its electrophoretic mobility with four proteins (myoglobin, chymotrypsinogen, ovalbumin and serum albumin) of known molecular weight. Although the molecular weight of several proteins can be estimated by this method (Weber & Osborn, 1969) this result should be interpreted with caution since the electrophoretic mobility of glycoproteins in a similar system did not correspond to their molecular weight. (Segrest *et al.*, 1971).

Visual examination of the stained gels revealed no trace of band G in proteins solubilized from *tolG* envelope preparations, even where large amounts of protein were used (Plate I, gels E and F).

The loss of a major protein from envelope preparations was confirmed by a double label experiment. Strains JF466 (*tolG*) and JF465 (*tolG*$^+$) were grown in minimal medium containing ^{14}C and ^{3}H-labeled L-leucine, respectively. After the cells were washed by centrifugation, the two types of cells were mixed and treated as a single sample during preparation of envelope proteins and gel electrophoresis. The results, presented in Figure 1, show a single peak of ^{3}H-labeled material missing from the

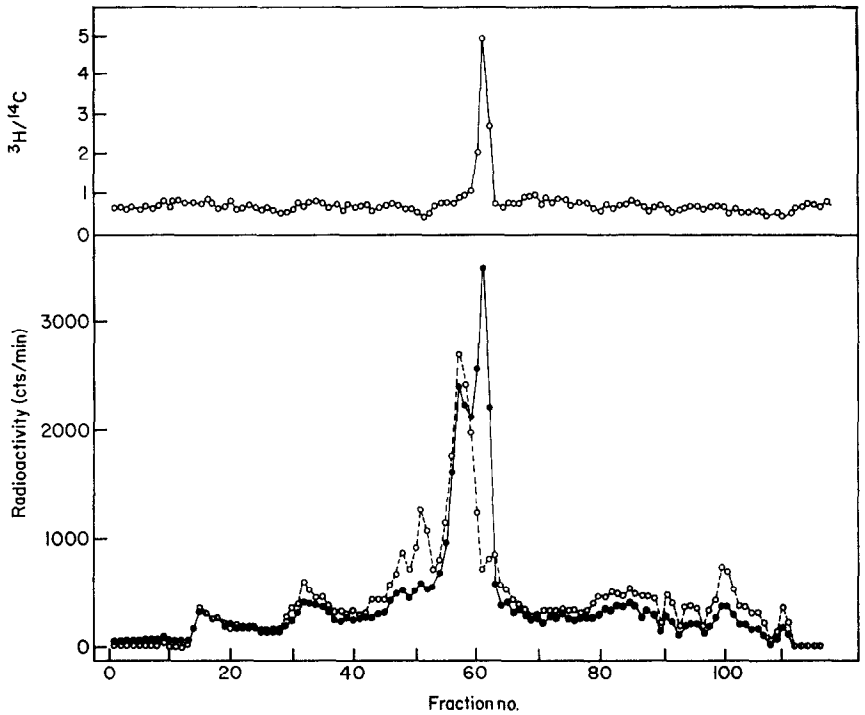

FIG. 1. Polyacrylamide gel electrophoresis of mixed envelope proteins from strain JF465 (*tolG*$^+$) labeled with [^{3}H]leucine (—●—●—) and strain JF466 (*tolG*) labeled with [^{14}C]leucine (—○—○—). Fraction 1 corresponds to the top of the gel (cathode), whereas fraction 116 corresponds to the bottom (anode).

The upper portion of the Figure plots the ratio of $^3H/^{14}C$ in each fraction.

region of the gel where band G was found. No other difference in $^3H/^{14}C$ was detected in the gel.

Examination of the stained gels revealed 31 bands staining as protein in addition to band G. Visual comparison of gels prepared from *tolG* and *tolG*$^+$ revealed no differences apart from band G in the pattern of protein bands or density of staining (Plate I), a result supported by the double-label experiment described in Figure 1.

The intensity of staining of a protein band in an acrylamide gel by Fast Green is related to the amount of protein present (Gorovsky *et al.*, 1971). Comparison of densitometer tracings of gels stained with Fast Green showed that band G accounted for between 8% and 12% of the total cell envelope protein.

(c) *Genetic studies*

To confirm the correlation between the *tolG* mutation and the protein missing from envelope preparations, the *tolG* locus was transferred by conjugation and transduction. Envelope proteins were prepared from the recombinants and examined by gel electrophoresis.

For the conjugation studies, strain JF404-2a was used as a donor in gradient type (uninterrupted) mating (de Hann *et al.*, 1969) with strain CSH 75. We found 17% of *purE*$^+$ *strA* recombinants carried the unselected marker *tolG*. Three *tolG* and one *tolG*$^+$ recombinants that carried no other unselected donor markers were labeled

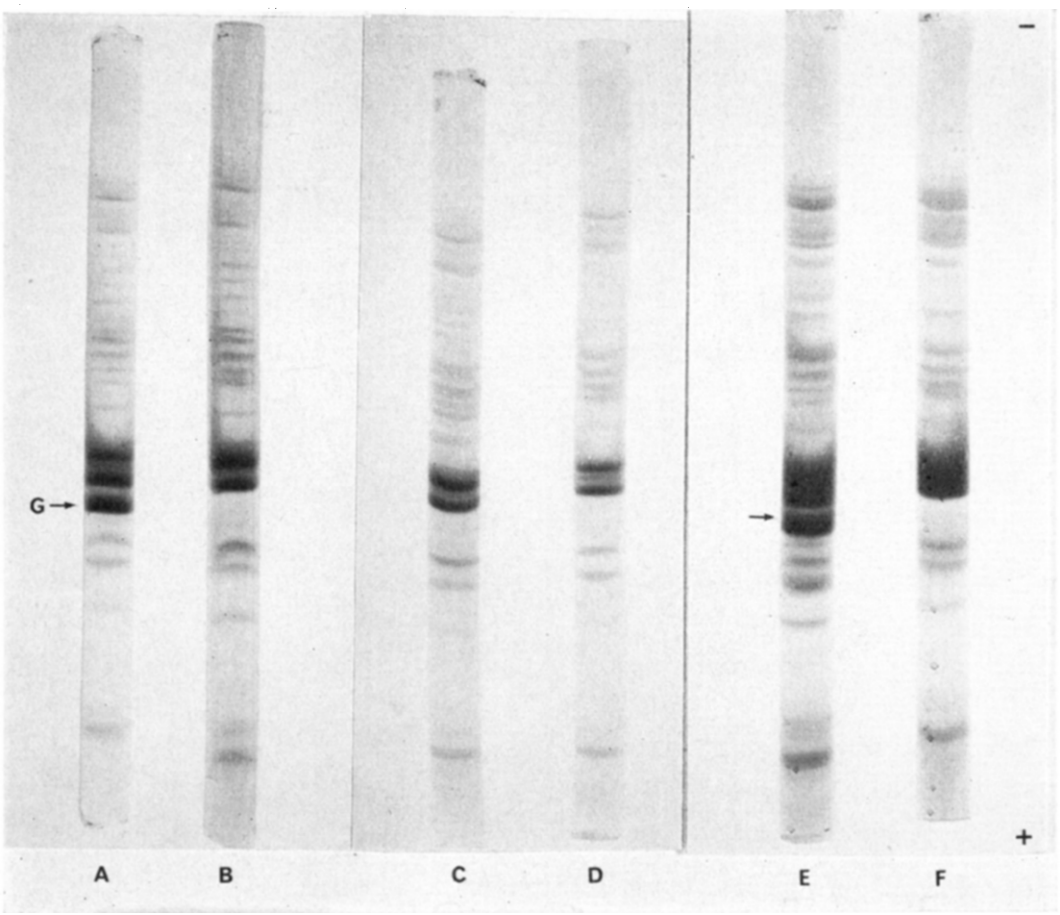

PLATE I. Polyacrylamide gel electrophoresis of envelope proteins from a *tolG*$^+$ strain and three *tolG* mutants. Gels A, B, C, and D contain 80 μg protein/gel. Gels E and F contain 300 μg protein/gel. Gels A and E contain envelope proteins from strain JF404 (*tolG*$^+$), gels B and F from strain JF404-2a, gel C from JF404-6a, gel D from JF404-9a. The arrow indicates the position of band G, absent from gels containing envelope proteins prepared from *tolG* strains.

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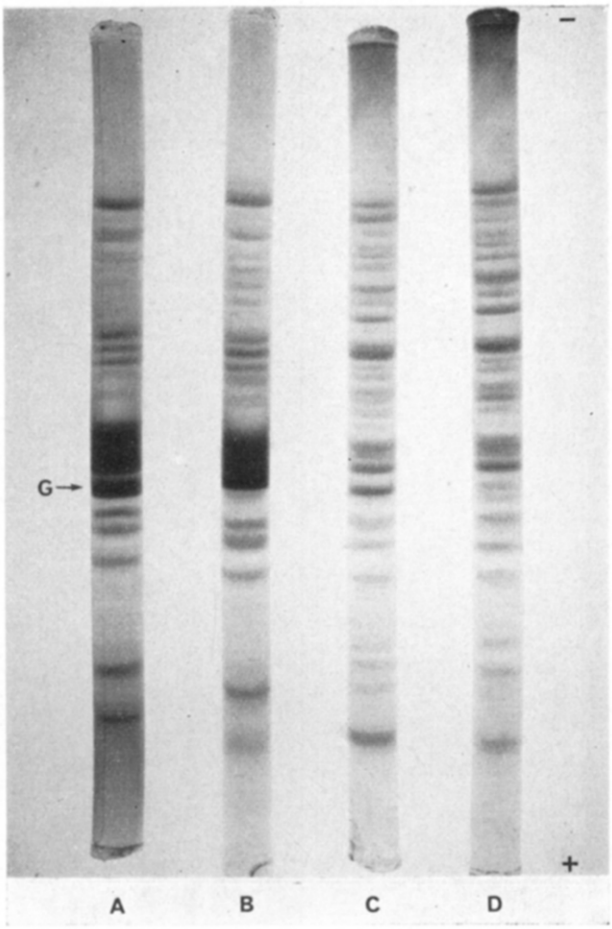

PLATE II. Polyacrylamide gel electrophoresis of proteins from fractionated cytoplasmic (cm) and outer membranes (om). Gels contain 200 μg protein/gel. A, JF404 (*tolG*$^+$) om; B, JF404-6a (*tolG*) om; C, JF404 cm; D, JF404-6a cm. The arrow indicates the position of band G.

JF465, JF466, JF467 and JF468. Gel electrophoresis of envelope proteins prepared from these strains showed all three *tolG* recombinants lacked band G, whereas the band was present in the envelope proteins of the *tolG*$^+$ recombinant tested.

The cotransduction frequency of *tolG* and *pyrD* is about 60% (Foulds, 1974). We selected *pyrD*$^+$ transductants of strain JF555 (*pyrD tolG*) using phage P1 grown on strain JF568 (*pyrD*$^+$ *tolG*$^+$). Gel electrophoresis of envelope proteins prepared from three *pyrD*$^+$ *tolG*$^+$ transductants and one *pyrD*$^+$ *tolG* transductant showed that band G, missing in strain JF555, was present in all three *pyrD*$^+$ *tolG*$^+$ transductants tested but missing in the *pyrD*$^+$ *tolG* transductant.

tolG strains are sensitive to novobiocin. When approximately 10^8 cells of strain JF404-2a were plated on PPBE agar containing novobiocin, approximately 20 to 200 colonies appeared. Four of these colonies were picked and purified by single colony streaks. All four novobiocin-resistant revertant strains had regained sensitivity to bacteriocin JF246 (*tolG*$^+$). Gel electrophoresis of envelope proteins from all four revertant strains showed the presence of band G.

(d) *Gel electrophoresis of proteins prepared from fractionated cytoplasmic and outer membranes*

tolG strains were converted to spheroplasts less efficiently than *tolG*$^+$ strains. The procedure described by Osborn *et al.* (1972) for the fractionation of cytoplasmic and outer membranes, emphasized that culture age, time spent harvesting cells, rate of addition of EDTA and aeration were important factors for spheroplast conversion. With careful attention to these factors, as well as lysozyme concentration, treatment of *tolG* mutants resulted in only 45 to 70% conversion to spheroplasts, whereas treatment of *tolG*$^+$ strains resulted in 96 to 99% conversion to spheroplasts.

Isopycnic sucrose density-gradient centrifugation of membranes prepared from strain JF404-6A (*tolG*) and JF404 (*tolG*$^+$) gave four visible bands, as described by Osborn *et al.* (1972). The top two bands were the source of cytoplasmic membrane proteins used for gel electrophoresis and the bottom (most dense) band was the source of outer membrane proteins. The protein patterns after electrophoresis are shown in Plate II. Band G, present in the *tolG*$^+$ strain outer membrane, was missing in the proteins from the outer membrane fraction prepared from the *tolG* mutant. A band with the same apparent mobility as band G was found in the cytoplasmic membrane fraction of the *tolG*$^+$ strain. A band with similar mobility but of much reduced intensity in the stained gels, was found in the cytoplasmic membrane fraction from the *tolG* strain. A mixture of proteins prepared from the cytoplasmic membrane of a *tolG* strain and the outer membrane of a *tolG*$^+$ strain was applied to a single gel and subjected to electrophoresis. The mixture contained 180 μg of cytoplasmic membrane protein and 20 μg of outer membrane protein. The stained gel showed that the band present in reduced amounts in the cytoplasmic membrane of *tolG* mutants had an electrophoretic mobility identical to band G.

4. Discussion

A mutation at one of several different loci can result in bacteriocin tolerance, indicating that several genes and presumably several proteins are involved in the expression of the biochemical events initiated by adsorption of a bacteriocin. A bacteriocin-tolerant mutation often results in increased sensitivity to agents such as

antibiotics, detergents and dyes. These pleiotropic effects have been interpreted as an indication that a mutation at a *tol* locus results in an alteration in the bacterial cell membrane. However, there has been no direct identification of the gene product of a tolerance gene.

Comparison of the cell envelope proteins from *tolG* strains with those prepared from *tolG*$^+$ strains shows clearly that *tolG* strains lack a single major cell envelope protein. The missing protein is correlated with bacteriocin tolerance, for it was not segrated by conjugation or transduction. In addition, *tolG*$^+$ revertants selected by resistance to novobiocin simultaneously acquired the missing protein and sensitivity to bacteriocin JF246. These results suggest that the *tolG* locus may be the structural gene for the missing protein. However, the *tolG* locus could be a gene that controls the synthesis, modification or insertion of the protein into the cell envelope. In this respect, it is striking that the protein was completely absent from cell envelope proteins prepared from each of three independently isolated *tolG* mutants. This suggests either a control function for the *tolG* locus or that any alteration leading to tolerance necessarily alters the structure of the protein sufficiently to prohibit its incorporation into the cell envelope.

When cytoplasmic and outer membranes were fractionated, we found that band G was present in the outer membrane fraction prepared from a *tolG*$^+$ strain. Osborn *et al.* (1972) found the cytoplasmic membrane fraction was about 10% contaminated by outer membrane components as judged by the lipopolysaccharide content. This contamination provides an explanation for the presence of a protein band in the cytoplasmic membrane fraction with an electrophoretic mobility similar to that of the band G. However, the band in question was visualized in the cytoplasmic membrane fractions prepared from both a *tolG*$^+$ and, to a lesser extent, from a *tolG* strain. There are several possible explanations for this puzzling observation. For example, we may not have put sufficient total cell envelope material on the gel to visualize a minor component of the cytoplasmic membrane. The band in the cytoplasmic membrane fraction may reflect partial degradation of a membrane protein that occurred during fractionation. Alternatively, the band may represent a protein that is lost during preparation of the total envelopes. Electrophoresis of a mixture of proteins showed that this minor band present in the cytoplasmic membrane of *tolG* mutants had an electrophoretic mobility identical to band G. However, it is premature to assume that the minor band present in the cytoplasmic membrane of *tolG* strain is identical to that found in the outer membrane of *tolG*$^+$ strains. Experiments using antibody prepared against purified band G should provide substantial information on this interesting point.

Koplow & Goldfine (1974) have reported the decreased protein content of outer membranes prepared from a heptose-deficient mutant of *E. coli*. Examination of outer membrane proteins prepared from these mutants by SDS polyacrylamide gel electrophoresis showed several major outer membrane proteins were present in reduced amounts. One of these proteins (labeled band C by Kaplow & Goldfine) is probably identical with the protein missing in *tolG* mutants. They find a lower density for the outer membrane fraction prepared from heptose-deficient mutants. The density of outer membranes prepared from *tolG* was unchanged as compared to the *tolG*$^+$ strain.

The role of the outer membrane in the actions of colicins is not clear. Stable L-forms of *E. coli* B are sensitive to colicins K and E2 (Smarda & Schumann, 1966). These

strains are reported to lack both the peptidoglycan and outer membrane layers of the cell envelope, suggesting that there are colicin receptors on the cytoplasmic membrane. However, other studies (Weltzein & Jesaitis, 1971) have shown colicin receptors, including the receptor for colicin K, are associated with the outer membrane. Studies using radioactively labeled bacteriocin JF246 (Foulds, unpublished observations) have shown that the outer membrane fraction purified from *E. coli* strain K12 both binds and neutralizes bacteriocin JF246. There is also significant binding of bacteriocin JF246 by the cytoplasmic membrane, but it is not possible to rule out that this is simply due to contamination of cytoplasmic membrane with outer membrane.

Bacteriocin JF246 inhibits all macromolecular synthesis in sensitive cells, possibly by a direct or indirect effect on the cytoplasmic membrane. We can only speculate on the role of the protein missing from *tolG* mutants. Following adsorption of bacteriocin JF246 onto an outer membrane receptor, the bacteriocin–receptor complex may be involved in transmitting a biochemical effect to the cytoplasmic membrane. Alternatively, the missing protein may be responsible for transmitting the bacteriocin molecule itself, or a portion of it, through the outer membrane to a site within the cell envelope where the bacteriocin can then interact with the cytoplasmic membrane at a direct site of action or to permit the colicin to be further transported into the cytoplasm.

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