JOURNAL OF BACTERIOLOGY, Aug. 1989, p. 4105–4111
0021-9193/89/084105-07$02.00/0

Vol. 171, No. 8

A Novel Mutation, *cog*, Which Results in Production of a New Porin Protein (OmpG) of *Escherichia coli* K-12

RAJEEV MISRA* AND SPENCER A. BENSON†

Department of Biology, Princeton University, Princeton, New Jersey 08544-1014

Received 13 December 1988/Accepted 26 April 1989

A mutant of *Escherichia coli* K-12 which produces a new outer membrane protein, OmpG, was isolated and genetically and biochemically characterized. The presence of OmpG allows growth on maltodextrins in the absence of the LamB maltoporin. The data obtained from in vivo growth and uptake experiments suggested that the presence of the OmpG protein results in an increase in outer membrane permeability for small hydrophilic compounds. In light of these findings, we suggest that OmpG is a porinlike protein. The mutation which results in the expression of OmpG has been termed *cog* (for control of OmpG) and mapped to 29 min on the *E. coli* chromosome. Diploid analysis shows that the mutant *cog-192* allele is recessive for both the Dex$^+$ and OmpG$^+$ phenotypes. We propose that the *cog* mutation destroys a negative regulatory function and therefore derepresses *ompG* expression.

The outer membrane of *Escherichia coli* K-12 serves as a selective permeability barrier and contains specialized transmembrane proteins. These transmembrane proteins, which include OmpF and OmpC, form water-filled channels or pores that allow the diffusion of small hydrophilic molecules across the membrane (for reviews, see references 4, 18, 25, and 26). Because of the pore-forming property of OmpF and OmpC, these proteins are known as porins. Synthesis of two other porins, NmpC and Lc, is activated under certain physiological conditions. The NmpC protein is produced in certain pseudorevertants of a porin-deficient (OmpF$^-$ OmpC$^-$) *E. coli* strain (28) and originates from a defective prophage, QSR, which lies at 12 min on the *E. coli* chromosome (13). The Lc protein is found in certain *E. coli* strains lysogenic for a lamboid bacteriophage, PA-2 (2, 29). Expression of Lc results in reduced expression of OmpF and OmpC (10). All four porins share significant sequence homologies, at both the nucleotide and amino acid levels (5), suggesting that these proteins have similar structures and a common ancestral origin. Recently, we have shown that the analogous residues of OmpF and OmpC are involved in determining the pore properties (3, 21, 22).

In addition to the porin proteins described above, there are at least three other proteins, LamB, PhoE, and Tsx, present in *E. coli* which form specialized pores (4, 25, 26). The channels formed by LamB, PhoE, and Tsx specifically facilitate the diffusion of maltose (and maltodextrins), phosphate compounds, and nucleosides, respectively. In addition, these pores, like that formed by OmpF and OmpC, allow nonspecific diffusion of small hydrophilic solutes. Normally, LamB and PhoE, unlike the general porin proteins, are present in very small quantities. However, their expression can be derepressed under inducing conditions, i.e., in the presence of maltose for LamB (33) and in a phosphate-limiting medium for PhoE (27) or in the presence of certain specific regulatory mutations (35). The Tsx protein is synthesized constitutively (19).

In this paper we describe the isolation and characterization of a mutation which results in the production of a new outer membrane protein, OmpG. The mutation controls the synthesis of OmpG, and therefore we term it *cog* (for control of OmpG). Our data suggest that OmpG is a porinlike protein which facilitates the diffusion of small hydrophilic solutes.

MATERIALS AND METHODS

Media and chemicals. Minimal medium (M63) and Luria broth were prepared as described previously (34). Defined maltodextrins (maltotriose to maltohexose) and ultra pure sucrose were purchased from Boehringer Mannheim Biochemicals. Maltodextrin and melibiose were purchased from Pfanstiehl Laboratories, Inc. Maltodextrin was further purified as described previously (21). [^{14}C]maltose was purchased from Amersham Corp.

Bacterial strains. Strains used in this study are described in Table 1.

Mutant isolation and genetic techniques. Spontaneous mutants able to grow on maltodextrins (Dex$^+$) were isolated from DME553 as described previously (21). Procedures involving P1 transduction, conjugation, and isolation of a Tn*10* linked to the *cog* mutation were performed by the methods of Miller (20) and Silhavy et al. (34). The genetic markers *zcj-81*::Tn*10*, *zcj-82*::Tn*10*, and *zcj-83*::Tn*10* were isolated from a Tn*10* pool (kindly provided by Nancy Trun) prepared on MC4100. To identify a Tn*10* insertion near *cog*, the tetracycline resistance (Tc^r) determinant from the Tn*10* pool was moved by P1 transduction into RAM123, which carries a mutant *cog* (*cog-192*) allele and therefore has a Dex$^+$ phenotype. The Tc^r transductions were screened for the Dex$^-$ phenotype. The linkage of *cog* and Tc^r was determined by using a purified Dex$^-$ Tc^r isolate. The linkages of the three different Tc^r markers to *cog* are approximately 15% (*zcj-83*::Tn*10*), 90% (*zcj-82*::Tn*10*), and 92% (*zcj-81*::Tn*10*).

Bacteriological and biochemical techniques. Unless specified, cultures were grown in Luria broth. [^{14}C]maltose uptake and growth rates on various sugars were determined as described previously (21). Crude colicin preparations and sensitivity assays were performed by the method of Pugsley and Schnaitman (29).

Whole-cell envelope fractions were prepared by the lysozyme sonication method as described by Morona and Reeves (24). Outer and inner membranes were separated by

* Corresponding author.

† Present address: Department of Microbiology, University of Maryland, College Park, MD 20742-4451.

TABLE 1. Bacterial strains

Strain	Characteristics	Source or reference
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U139 rpsL150 relA1 flbB5301 ptsF25 deoC1 thi-1 rbsR*	6
MCR106	MC4100 Δ*lamB106*	7
DME553	MCR106 Δ*ompF80*	S. Benson
RAM105	DME553 *zei-06*::Tn*10*	21
RAM191	MCR106 Δ*ompC178 zei-198*::Tn*10*	21
RAM123	DME553 *cog-192*	This study
RAM194	RAM123 Δ*ompC178 zei-198*::Tn*10*	This study
MBM7007	F⁻ *araC*(Am) *araD* Δ(*argF-lac*)*U139 trp*(Am) *malB*(Am) *rpsL relA thi*	34
RAM453	MBM7007 (Mal⁺) Δ*lamB106*	This study
RAM455	RAM453 *zcj-83*::Tn*10*	This study
RAM456	RAM455 *cog-192*	This study
RAM457	RAM455 *recA*::Kan^r	This study
RAM458	RAM456 *recA*::Kan^r	This study
RAM459	RAM457(F'123 *trp*⁺)	This study
RAM460	Same as RAM459	This study
RAM461	RAM458(F'123 *trp*⁺)	This study
RAM462	Same as RAM461	This study

centrifuging whole-cell envelopes on a sucrose gradient essentially as described by Ito et al. (14). Briefly, whole-cell envelopes, isolated by the method described above, were layered onto 5.5 ml of 53% (wt/vol) sucrose–3 mM EDTA with a cushion of 70% (wt/vol) sucrose–3 mM EDTA (1 ml) at the bottom and centrifuged at 60,000 rpm for 4 h in a Beckman 75 Ti rotor. Inner and outer membranes were recovered from two distinct bands obtained just below the 53% and 53 to 70% sucrose interface regions, respectively. Membrane samples were diluted 10-fold with 10 mM $MgCl_2$ and pelleted by centrifuging at 34,000 rpm for 1 h in a Beckman 50 Ti rotor.

Proteins were analyzed on linear sodium dodecyl sulfate (SDS)-polyacrylamide gels (11% acrylamide) as previously described (17). In specified gels, 4 M solid urea was added in the running gel buffer to achieve a better resolution of OmpG from OmpF and OmpC. SDS extraction of the outer membrane proteins was performed by the method of Rosenbusch (31).

RESULTS

Isolation and genetic analysis of the *cog* mutation. We have previously described a selection procedure (21) to isolate mutations which confer a Dex⁺ phenotype (ability to grow on maltodextrin medium) in the absence of LamB, a maltoporin which is required for growth on maltodextrins (9). This selection was devised to isolate mutations which alter outer membrane permeability. Starting with a LamB⁻ OmpF⁻ OmpC⁺ strain (RAM105 or DME553) three classes of mutations conferring a Dex⁺ phenotype were obtained. We have previously reported the analysis of the *ompC* (21, 22) and *imp* (23; B. A. Sampson, R. Misra, and S. A. Benson, Genetics, in press) mutations. In this paper we describe the characterization of a third class of mutations, which we have designated as *cog* (for control of OmpG), since the genetic defect appears to be in the control function (see below). The *cog*(Dex) mutations, obtained without mutagenesis, were an infrequent class of mutations. We estimate the frequency of appearance of the *cog*(Dex) mutation to be approximately 0.5×10^{-10}. We have chosen a representative isolate, RAM123, carrying the *cog-192* allele for the detailed analysis.

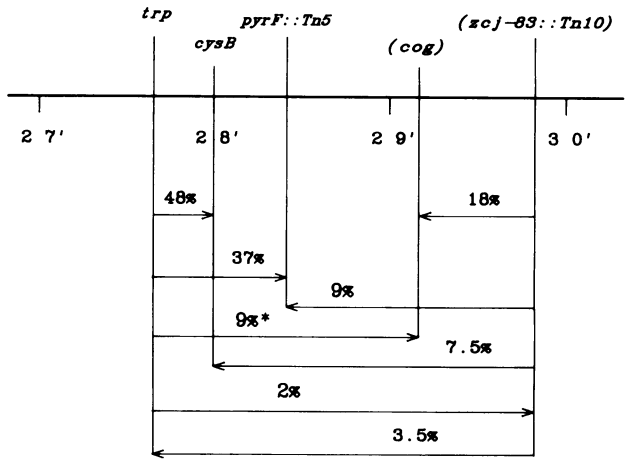

FIG. 1. Mapping of the *cog* mutation. The P1 cotransduction frequencies (from over 200 transductants screened) from two-factor crosses are shown. The donor and recipient strains are indicated by the base of the arrow and the arrowhead, respectively. The gene order, *trp–cysB–pyrF*::Tn*5*–*cog-192*–*zcj-83*::Tn*10*, was unambiguously confirmed by three-factor crosses (Table 2). Symbol: *, average of two separate transductions, one in which the donor was *cog-192* (Dex⁺) and the recipient was *cog*(Wt) (Dex⁻) (6%) and the other in which the donor was *cog*(Wt) (Dex⁻) and the recipient was *cog-192* (Dex⁺) (12%). Parentheses around a gene symbol indicate that the position of that marker is not precisely determined.

The *cog-192* mutation was mapped with the help of linked Tn*10*s (*zcj-81*::Tn*10*, *zcj-82*::Tn*10*, and *zcj-83*::Tn*10*; see Materials and Methods for the isolation of these Tn*10*s). These Tn*10*s were first mapped by Hfr crosses. The results showed that all three Tn*10*s were closely linked to the *trp* operon at 27.5 min on the *E. coli* chromosome (data not shown). To further map the position of the Tn*10*s, we determined P1 cotransduction linkages to *trp* by two-factor crosses; the approximate linkages for *zcj-83*::Tn*10*, *zcj-82*::Tn*10*, and *zcj-81*::Tn*10* were 3, 18, and 20%, respectively. In the subsequent experiments, we used *zcj-83*::Tn*10* to map the *cog* mutation. Initial mapping of *cog-192* was done by various two-factor crosses (Fig. 1). The data obtained from three-factor crosses with *trp* as an outside marker suggested that *cog* was present between *trp* and *zcj-83*::Tn*10* (Table 2). The orientation of *zcj-83*::Tn*10* with respect to *trp* was determined by three-factor crosses with the *pyrF*::Tn*5* marker (*pyrF* maps at 28.3 min on the *E. coli* chromosome). The data obtained (Table 2) suggested that the gene order is *trp–pyrF*::Tn*5*–*zcj-83*::Tn*10*. Using *zcj-83*::Tn*10* as an outside marker, we then ordered *cog* with respect to *pyrF*::Tn*5*. The results (Table 2) suggested that the gene order is *pyrF*::Tn*5*–*cog-192*–*zcj-83*::Tn*10*. This gene order was reconfirmed by a three-factor mapping experiment in which *trp* was used as an outside marker (Table 2). In the final three-factor mapping experiment, *pyrF*::Tn*5* was used as an outside marker, and the gene order of *pyrF*::Tn*5*–*cog-192*–*zcj-83*::Tn*10* was again confirmed (Table 2; Fig. 1). From the P1 cotransductional linkages of *cog-192* to *trp* and *pyrF*::Tn*5*, we estimate the map position of the mutation to be approximately 29 min on the *E. coli* chromosome. This places *cog-192* in or near the defective prophage Rac, which maps between 29 and 31 min on the *E. coli* chromosome (1). Since only certain *E. coli* strains carry the Rac prophage in their genome, the strains used in this study were tested to determine whether they carry the Rac prophage. Southern analysis with a Rac-specific DNA probe (data not shown)

TABLE 2. Three-factor mapping of the *cog* mutation

Recipient strain	Donor strain	Selected marker[a]	Unselected marker[b]
Trp$^-$ Dex$^+$ Tet^r	Trp$^+$ Dex$^-$ Tet^s	Trp$^+$ (433)	Dex$^+$ Tet^r (355, 82%), Dex$^-$ Tet^r (63, 14.5%), Dex$^-$ Tet^s (15, 3.5%) Dex$^+$ Tet^s (0, 0%)
Trp$^-$ Dex$^-$ Tet^s	Trp$^+$ Dex$^+$ Tet^r	Trp$^+$ (511)	Dex$^-$ Tet^s (446, 87.3%), Dex$^+$ Tet^s (34, 6.7%), Dex$^+$ Tet^r (19, 3.7%), Dex$^-$ Tet^r (12, 2.3%)
Tet^s Ura$^-$ Trp$^-$	Tet^r Ura$^+$ Trp$^+$	Tet^r (240)	Ura$^-$ Trp$^-$ (214, 89.2%), Ura$^+$ Trp$^-$ (20, 8.3%), Ura$^+$ Trp$^+$ (6, 2.5%), Ura$^-$ Trp$^+$ (0, 0%)
Tet^s Dex$^+$ Ura$^+$	Tet^r Dex$^-$ Ura$^-$	Tet^r (1,093)	Dex$^+$ Ura$^+$ (973, 89%), Dex$^-$ Ura$^+$ (80, 7.3%), Dex$^-$ Ura$^-$ (40, 3.7%), Dex$^+$ Ura$^-$ (0, 0%)
Trp$^-$ Ura$^-$ Dex$^-$	Trp$^+$ Ura$^+$ Dex$^+$	Trp$^+$ (513)	Ura$^-$ Dex$^-$ (347, 67.6%), Ura$^+$ Dex$^-$ (97, 18.9%), Ura$^+$ Dex$^+$ (65, 12.7%), Ura$^-$ Dex$^+$ (4, 0.8%)
Ura$^-$ Dex$^-$ Tet^r	Ura$^+$ Dex$^+$ Tet^s	Ura$^+$ (670)	Dex$^-$ Tet^r (324, 48.4%), Dex$^+$ Tet^r (288, 43%), Dex$^+$ Tet^s (54, 8%), Dex$^-$ Tet^s (4, 0.6%)

[a] Numbers represent the total number of colonies screened.

[b] Numbers in parentheses are the number of colonies with the marker and percentage of the total number. The presence of the *zcj-83*::Tn*10*, *cog-192*, and *pyrF*::Tn*5* alleles confers the Tet^r, Dex$^+$, and Ura$^-$ phenotypes, respectively.

confirmed that our strains carry Rac. The F'132 used in diploid analysis also contains the Rac prophage.

The *cog-192* allele results in the appearance of a new membrane protein, OmpG. To determine the effect of the *cog-192* mutation on the outer membrane, whole-cell envelopes were prepared from strains carrying *cog*$^+$ and *cog-192* alleles and analyzed by SDS-polyacrylamide gel electrophoresis with polyacrylamide-urea gels (Fig. 2). Strains carrying the *cog-192* allele produced a new membrane protein, which we have designated OmpG. This peptide has an apparent molecular mass of 36 kilodaltons. The presence of the *cog-192* allele always correlated with the presence of OmpG and the Dex$^+$ phenotype.

We observed that the amount of OmpG in the membrane varied depending on the growth medium. For example, the level of OmpG was repressed two- to threefold when strains were grown in Luria broth or minimal medium containing glucose, whereas its expression was derepressed when the strains were grown in minimal medium containing either glycerol or maltose (data not shown).

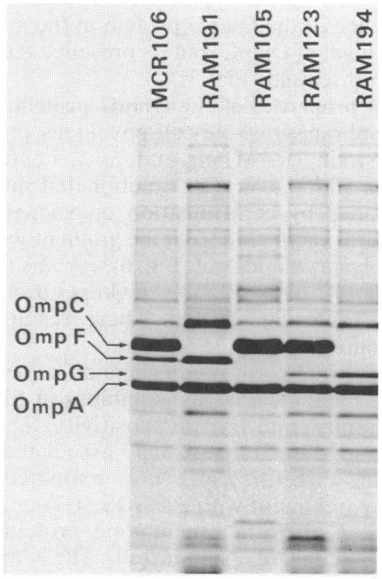

FIG. 2. SDS-polyacrylamide gel electrophoresis (polyacrylamide-urea gel) of whole-cell envelopes from strains indicated at the top of the figure. The strains were grown in Luria broth. Positions of OmpC, OmpF, OmpG, and OmpA are indicated by arrows.

The *cog-192* allele is recessive. To determine whether the *cog-192* mutation is dominant or recessive to the wild-type allele, we performed the diploid analysis. A *cog*$^+$/*cog-192* merodiploid strain was constructed by introducing F'123 (*cog*$^+$) from a Trp$^+$ donor strain into a Trp$^-$ Dex$^+$ OmpG$^+$ recipient strain which contains the mutant *cog* (*cog-192*) allele. As a control, F'123 was introduced into an isogenic Trp$^-$ strain containing the *cog*$^+$ allele. Both the donor and recipient strains carried a *recA* mutation. We then tested the purified Trp$^+$ merodiploids (*cog*$^+$/*cog-192*) for their ability to grow on maltodextrin medium and for the presence of the OmpG protein. All of the Trp$^+$ merodiploids were Dex$^-$ and no longer expressed the OmpG protein in the membrane (Fig. 3). These results demonstrated that the mutant *cog-192* allele is recessive to the wild-type *cog*$^+$ allele.

OmpG is a porinlike protein. Since the presence of the OmpG protein correlated with the Dex$^+$ phenotype, we tested the possibility that OmpG is a porin protein. The in

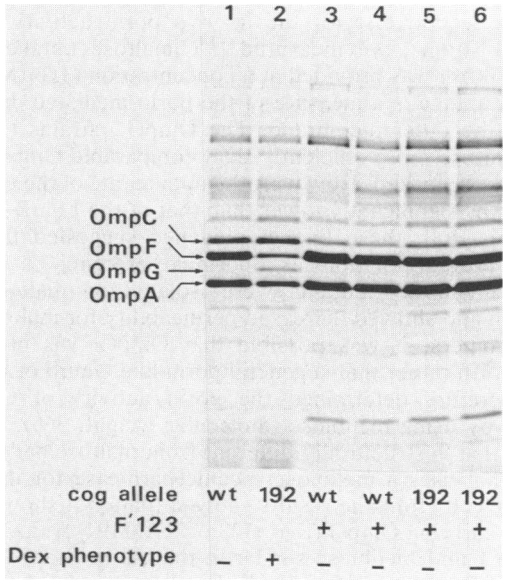

FIG. 3. Diploid analysis of *cog*$^+$ and *cog-192* alleles. Whole-cell envelopes prepared from haploid (lane 1, RAM457; lane 2, RAM458) or merodiploid (lane 3, RAM459; lane 4, RAM460; lane 5, RAM461; lane 6, RAM462) strains were analyzed as in Fig. 2. The wild type or mutant *cog* alleles, F'123, and the resulting Dex phenotypes are indicated.

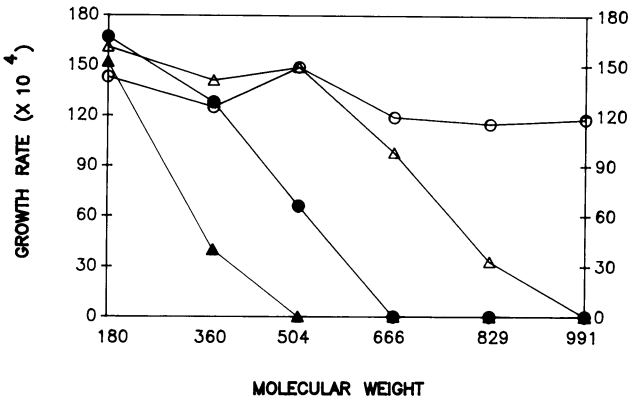

FIG. 4. Growth rates of the various strains (DME553 [▲], RAM191 [●], RAM123 [△], and MC4100 [○]) on defined maltodextrins. Growth rate were determined as described previously (21). The substrates (glucose [molecular weight, 180], maltose [molecular weight, 340], maltotriose [molecular weight, 504], maltotetrose [molecular weight, 666], maltopentose [molecular weight, 829], and maltohexose [molecular weight, 991]) were provided at 1 mM. Growth rates are represented as the inverse of the doubling time in minute.

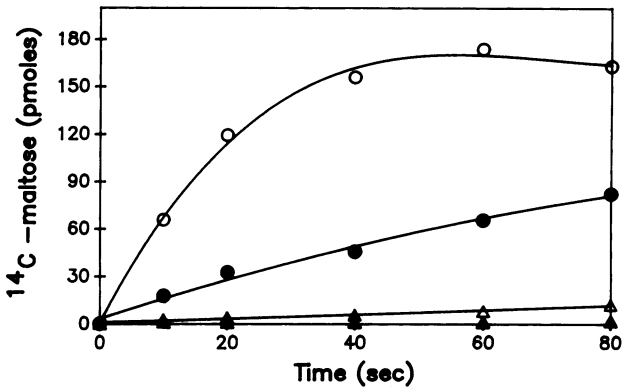

FIG. 5. [^{14}C]maltose uptake assays on DME553 (▲), RAM191 (△), RAM123 (●), and MC4100 (○). Assays were done as described previously (21). [^{14}C]maltose was provided at 3.3 μM.

vivo porin activity of strains with various porin compositions was determined by the following method. The growth rates of the various strains grown on sugars of defined molecular weights from 180 to 991 (glucose to maltohexose) were measured. These sugars were provided at a concentration (1 mM) at which diffusion across the outer membrane is rate limiting for growth. The results (Fig. 4) show that the presence of OmpC and OmpF supported growth on maltose and maltotriose, respectively, whereas the OmpG$^+$ strain grew on maltose through maltotetrose with growth rates similar to that of a LamB$^+$ strain. The OmpG$^+$ strain was also able to grow on maltopentose, although its growth rate was much lower than that of a LamB$^+$ strain. The OmpG$^+$ strain was unable to grow on maltohexose.

As a second test for the in vivo porin activity of the various strains, we measured [^{14}C]maltose uptake rates. [^{14}C]maltose was provided at a concentration (3.3 μM) such that its uptake is a measure of the porin-mediated diffusion across the outer membrane. The OmpG$^+$ strains took up [^{14}C]maltose more efficiently than comparable OmpF$^+$ and OmpC$^+$ strains did. However, the uptake rate of the OmpG$^+$ strain was significantly lower than that of the LamB$^+$ strain (Fig. 5). Both these line of evidence suggested that the OmpG protein functions as a porinlike protein.

Since OmpG$^+$ mutants were isolated on maltodextrin medium and showed increased permeability for maltose and its oligomers, it was possible that OmpG was in fact a maltoporin rather than a general porin like OmpF or OmpC. We therefore determined the porin activity of OmpG$^+$ strains by using melibiose (molecular weight, 360), a compound that is structurally different from maltose and, moreover, that uses a melibiose-specific permease for its entry into the cell (16). The results showed (data not shown) that strains carrying OmpG (RAM123 or RAM194) had a growth rate on 1 mM melibiose similar to that of an OmpF$^+$ strain (RAM191) and considerably higher than that of a OmpC$^+$ strain (RAM105). These results were very similar to that obtained with maltose (Fig. 4) and show that OmpG is a general porin protein.

Properties of the OmpG$^+$ strain. To further explore the porin properties of the OmpG peptide, we tested OmpG$^+$ strains for their sensitivity to various antibiotics and colicins. When compared with an OmpG$^-$ strain, the OmpG$^+$ strains did not show any significant change in sensitivity to ampicillin, carbenicillin, benzylpenicillin, chloramphenicol, rifampin, erythromycin, or novobiocin. The porin proteins OmpF and OmpC facilitate the transport of certain E-group colicins (E2 and E3) and colicin L, which requires OmpF for its transport into the cell (30). The colicin sensitivity of an OmpG$^+$ strain (RAM194) and various control strains was determined by endpoint dilution spot tests with crude colicin preparations. The OmpG$^+$ strain grown under conditions in which OmpG expression was derepressed showed no sensitivity to colicins E2 and E3. On the other hand, the OmpC$^+$ and OmpF$^+$ strains were sensitive to colicin extracts diluted 50- and 100-fold, respectively. The OmpG$^+$ strain was also resistant to colicin L. Thus, OmpG does not facilitate the transport of colicins E2, E3, and L. Expression of *ompF* and *ompC* is positively controlled at the transcription level by *ompR* and *envZ* (11). We tested whether mutations in these regulatory genes affect the synthesis of OmpG. Null mutations in *ompR* and *envZ* did not affect the expression of OmpG or the Dex$^+$ phenotype (data not shown). We noted that the presence of the OmpG protein in the membrane did not affect the level of OmpC, but its presence reduced OmpF levels two- to threefold (Fig. 3).

Biochemical properties of the OmpG protein. To localize OmpG in membranes, whole-cell envelopes prepared from an OmpG$^+$ strain (RAM194) and as a control from an OmpC$^+$ strain (RAM105) were fractionated into outer and inner membranes by centrifugation on sucrose gradients. Membrane bands recovered from the gradient were analyzed on an SDS-polyacrylamide gel (Fig. 6). As was the case with OmpC and OmpA, over 90% of the OmpG protein fractionated with the outer membrane. These results show that OmpG is an outer membrane protein.

The OmpG protein and other major outer membrane proteins were characterized for solubility in SDS, solubilization temperature, and trypsin sensitivity. The porin proteins OmpF and OmpC are strongly associated with peptidoglycan and therefore are not extracted from the membranes by treatment with 2% SDS at 37 or 56°C. Under these conditions, all other membrane proteins, including OmpA, were completely solubilized. The OmpG protein, like OmpA, was completely solubilized with 2% SDS at both temperatures (Fig. 7).

When envelope samples are heated to 37°C, OmpF and OmpC are not denatured into monomers (31). OmpG, like

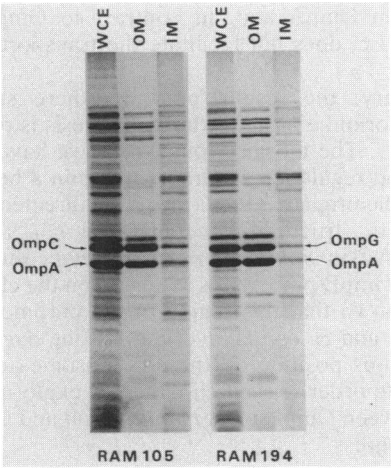

FIG. 6. Fractionation of whole-cell envelopes prepared from RAM105 (OmpC$^+$) and RAM194 (OmpG$^+$) into outer and inner membranes by centrifugation on a sucrose gradient. Membrane bands were collected from the bottom of the tube, and fractions representing the outer and inner membranes were pooled and analyzed on an SDS-polyacrylamide gel. Lanes: WCE, whole-cell envelope; OM, outer membrane; IM, inner membrane. Strains were grown on minimal medium containing glycerol.

OmpF and OmpC, ran at its denatured position only when envelope samples were heated to 100°C (Fig. 7).

When present in the envelope fraction, the porin proteins OmpF and OmpC are resistant to cleavage by trypsin, whereas OmpA is extremely sensitive to trypsin. To test the trypsin sensitivity of OmpG, samples of whole-cell envelopes prepared from OmpG$^+$ and OmpG$^-$ strains were treated with trypsin. The membrane fractions were isolated and analyzed on an SDS-polyacrylamide gel (Fig. 8). The OmpG protein appeared to be cleaved by trypsin to a slightly lower-molecular-weight fragment (OmpG*), which remained

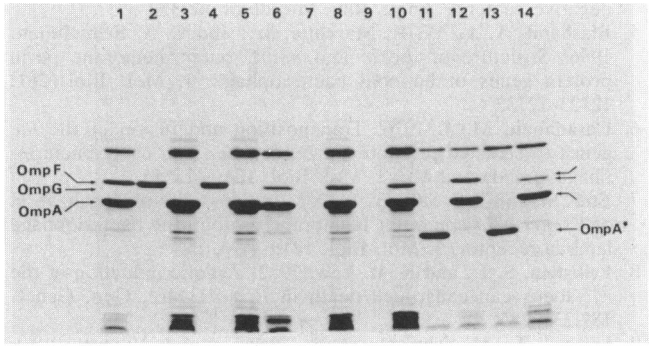

FIG. 7. Peptidoglycan association and heat solubilization of OmpF and OmpG. Samples of the outer membrane from RAM191 (OmpF$^+$; lanes 1 to 5) and RAM194 (OmpG$^+$; lanes 6 to 10) were suspended in SDS-sample buffer (31) and incubated at 37°C (lanes 2, 3, 7, and 8) or 56°C (lanes 4, 5, 9, and 10) for 30 min. Membranes were isolated, and samples of the pellet (peptidoglycan-associated materials; lanes 2, 4, 7, and 9) and supernatant (lanes 3, 5, 8, and 10) were analyzed on an SDS-polyacrylamide gel. Lanes 1 and 6 contain untreated samples. Samples of the outer membrane from RAM191 (lanes 11 and 12) and RAM194 (lanes 13 and 14) were suspended in SDS-sample buffer and analyzed on an SDS-polyacrylamide gel after being heated to 37°C (lanes 11 and 13) or 100°C (lanes 12 and 14) for 5 min. Positions of OmpF, OmpG, and OmpA are shown. OmpA* is a heat-modifiable form of OmpA.

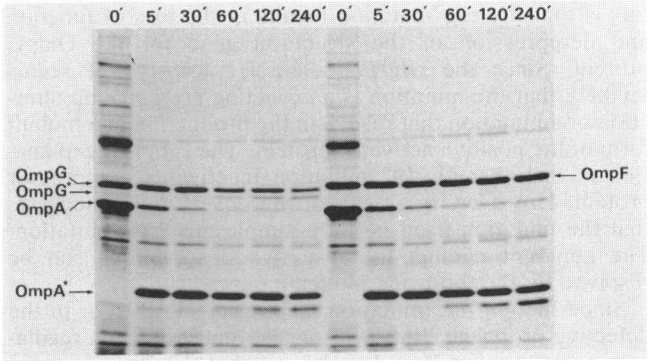

FIG. 8. Trypsin sensitivity test. Whole-cell envelopes from RAM194 (first six lanes) and RAM191 (last six lanes) were treated with 200 μg of trypsin per ml in 50 mM Tris hydrochloride (pH 7.5). At the times indicated above the lanes, samples were withdrawn and the reaction was stopped by addition of excess trypsin inhibitor. Membranes were pelleted, solubilized in SDS-sample buffer, and analyzed on an SDS-polyacrylamide gel. The positions of OmpG, OmpA, and OmpF are shown. OmpG* and OmpA* are the tryptic fragments of OmpG and OmpA, respectively.

attached to the envelope. This low-molecular-weight fragment was not present in an OmpG$^-$ OmpF$^+$ control strain. The rate at which OmpG was converted to its apparent lower-molecular-weight tryptic fragment, OmpG*, was much lower than the rate of conversion of OmpA to OmpA* (Fig. 8).

DISCUSSION

In this paper we report the isolation and characterization of a mutation, *cog*, which maps at 29 min on the *E. coli* chromosome. The *cog* mutation results in the production of a new outer membrane protein, OmpG, and confers a Dex$^+$ phenotype in the absence of LamB. Results of in vivo experiments suggest that OmpG is a porinlike protein.

We have developed a powerful genetic selection system to isolate mutations which alter outer membrane permeability. This involves selection for a Dex$^+$ phenotype in the absence of the LamB protein. Using this selection, we have previously reported the isolation of mutations in at least four different loci which confer a Dex$^+$ phenotype (23). The majority of mutations conferring a Dex$^+$ phenotype occur in the *ompF* gene (3, 23). Mutations of *ompC* [*ompC*(Dex)] were found only in the absence of the functional *ompF* gene (21, 22). The third class of mutations, *imp*, were isolated least frequently (23) and conferred a porin-independent Dex$^+$ phenotype. The final class of mutations, *cog*, like the *ompC*(Dex) mutations, were obtained only in the absence of the functional *ompF* gene. The *cog* mutations were isolated almost as frequently as the *ompC*(Dex) mutations. It is not clear why we failed to obtain *ompC*(Dex) or *cog*(Dex) mutations in the presence of the functional *ompF* gene.

The frequency at which the *cog* mutations were isolated (approximately 0.5×10^{-10}) suggests that the alteration is not a null mutation. If simply inactivating the *cog* gene is sufficient to confer a Dex$^+$ phenotype, we would expect the frequency at which *cog* mutations are obtained to be several orders of magnitude higher (in the range of 10^{-6} to 10^{-7}). Thus, we suggest that the *cog* mutations that yield a Dex$^+$ phenotype are specific base pair substitutions, deletions, or insertions.

The *cog-192* allele is recessive and results in the expression of a new protein. One simple genetic explanation for

this is that the *cog* mutation results in the loss of function and derepression of the structural gene for the OmpG protein. Since the *cog-192* allele is recessive, it seems unlikely that the mutation is a *cis*-acting promoter up alteration or a mutation that results in the production of a mutant form of the positive activator protein. The simplest explanation is that the *cog-192* mutation inactivates a repressor protein. However, the rarity of the *cog* mutation suggests that the mutation itself is not a simple missense mutation. The apparent conflict in these two observations can be resolved by the following model.

Since the *cog-192* mutation maps very close to or in the defective prophage, Rac, we suggest that it lies in a regulatory component of Rac. It is known that the Rac prophage possesses a repressor which controls the expression of a number of prophage genes (15). It thus seems reasonable that the gene for OmpG may also be under the control of this repressor and that loss of the repressor results in the expression of OmpG. This would account for the recessive nature of the *cog-192* mutation. However, the notion that the *cog-192* mutation is a simple knockout of this regulatory gene is in conflict with the low frequency at which we obtained such a mutation. To reconcile this apparent conflict, we suggest that simple loss of the regulatory molecule may lead to the expression of bacteriophage *kil* function (8), which would be lethal to the cell. Therefore, the mutations we obtain would have to be specific alterations which destroy both the repressor and *kil* functions of the Rac prophage. The genome structure of Rac and other lambdoid phages (15) is consistent with this hypothesis, and our experimental data support the contention that simple loss of a prophage maintenance repressor is lethal to the host.

We believe that the Dex^+ phenotype and increased outer membrane permeability of $OmpG^+$ $OmpF^-$ $OmpC^-$ strains, as assayed by increased growth rates on maltodextrins and [^{14}C]maltose uptake, are due to the presence of OmpG in the membrane and not to a general membrane defect. The notion that OmpG, a porin protein, is encoded by a prophage is reminiscent of the situation for at least two other cryptic porins, NmpC and Lc. At present, however, the lack of any physical evidence prevents us from directly demonstrating that the OmpG protein is derived from the Rac prophage or a source independent of the Rac genome. At this stage, we do not know the map position of the structural gene for OmpG.

Biochemically, the OmpG protein shares similarities with OmpF and OmpC. All three proteins facilitate the transport of small hydrophilic molecules across the outer membrane. It should be noted that under the experimental conditions of this investigation, OmpG facilitated the transport of uncharged sugars ([^{14}C]maltose and maltodextrins) much more efficiently than OmpF or OmpC did. In light of this finding, we can conclude that OmpG is a porinlike protein and may have pores that are functionally larger than those of the OmpF and OmpC porins. We can eliminate the possibility that OmpG is a maltoporin, since it also supports growth on melibiose in the absence of other porins. At this stage, we have no data to suggest that, like OmpF and OmpC, OmpG is a oligomeric protein.

In certain respects, unlike OmpF and OmpC, OmpG behaves like OmpA. We found that significant amounts of OmpG and OmpA could be stripped from membranes treated with sodium sarcosinate (R. Misra, unpublished data) and were less tightly bound to peptidoglycan; certain porin proteins share this property (12, 19). In addition, OmpG is sensitive to cleavage by trypsin, although it is more resistant than OmpA and, in contrast to OmpF, OmpC, NmpC, and Lc, does not facilitate the transport of colicins E2 and E3.

In summary, the results presented here suggest that OmpG is a porinlike protein whose synthesis is regulated by the *cog* locus. The most curious issues we have raised are the origin and regulation of OmpG. To gain a better understanding of the origin of OmpG, we have directed our efforts to cloning its structural gene and the *cog* locus. It is interesting that in *Salmonella typhimurium*, the structural gene for the OmpD porin maps at 32 min on the chromosome (32). It is known that this region of the chromosome in *S. typhimurium* and *E. coli* is inverted, putting *cog* and *ompD* in an analogous position on the chromosome and with the identical gene order. Research aimed at exploring the relationship between OmpD of *S. typhimurium* and OmpG of *E. coli* is in progress.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM34810 from the National Institutes of Health.

We thank Valley Stewart, Charles Yanofsky, and Carl Schnaitman for providing bacterial strains and Greg Phillips for providing *recA*::Kan^r. We also thank Jim Slauch for suggesting the use of melibiose.

LITERATURE CITED

1. **Bachmann, B. J.** 1987. Linkage map of *Escherichia coli* K-12, edition 7, p. 807–876. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
2. **Bassford, P. J., Jr., D. L. Diedrich, C. A. Schnaitman, and P. Reeves.** 1977. Outer membrane proteins of *Escherichia coli*. VI. Protein alternation in bacteriophage-resistant mutants. J. Bacteriol. **131**:608–622.
3. **Benson, S. A., J. L. Occi, and B. A. Sampson.** 1988. Mutations that alter the pore function of the OmpF porin of *Escherichia coli* K12. J. Mol. Biol. **203**:961–970.
4. **Benz, R.** 1988. Structure and function of porins from gram-negative bacteria. Annu. Rev. Microbiol. **42**:359–393.
5. **Blasband, A. J., W. R. Marcotte, Jr., and C. A. Schnaitman.** 1986. Structure of the *lc* and *nmpC* outer membrane porin protein genes of lamboid bacteriophage. J. Mol. Biol. **261**: 12723–12732.
6. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoter in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104**:541–555.
7. **Emr, S., and T. J. Silhavy.** 1980. Mutations affecting localization of *Escherichia coli* outer membrane protein, the bacteriophage lambda receptor. J. Mol. Biol. **141**:63–90.
8. **Feinstein, S. I., and K. B. Low.** 1982. Zygotic induction of the *rac* locus can cause cell death in *E. coli*. Mol. Gen. Genet. **187**:231–235.
9. **Ferenci, T., M. Schwentorat, S. Ullrich, and J. Vilmart.** 1980. Lambda receptor in the outer membrane of *Escherichia coli* as a binding protein for maltodextrins and starch polysaccharides. J. Bacteriol. **142**:521–526.
10. **Fralick, J., and D. Diedrich.** 1982. Studies on the expression of outer membrane protein 2 in *Escherichia coli*. Mol. Gen. Genet. **188**:139–142.
11. **Hall, M. N., and T. J. Silhavy.** 1981. The *ompB* locus and the regulation of the outer membrane porin proteins of *Escherichia coli* K-12. J. Mol. Biol. **146**:23–43.
12. **Hancock, R. E. W., and A. M. Carey.** 1980. Protein D1—a glucose-inducible, pore-forming protein from the outer membrane of *Pseudomonas aeruginosa*. FEMS Microbiol. Lett. **8**:105–109.
13. **Highton, P. J., Y. Chang, W. R. Marcotte, Jr., and C. A.**

Schnaitman. 1985. Evidence that the outer membrane protein gene *nmpC* of *Escherichia coli* K-12 lies with the defective qsr' prophage. J. Bacteriol. **162**:256–262.

14. **Ito, K., T. Sato, and T. Yura.** 1977. Synthesis and assembly of the membrane proteins in *E. coli*. Cell **11**:551–559.
15. **Kaiser, K., and N. E. Murray.** 1979. Physical characterization of the "Rac prophage" in *E. coli*. Mol. Gen. Genet. **175**:159–174.
16. **Lin, E. C.** 1987. Dissimilatory pathways for sugars, polyols, and carboxylates, p. 244–284. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. vol. 1. American Society for Microbiology, Washington, D.C.
17. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the major outer membrane proteins of *Escherichia coli* K-12 into four bands. FEBS Lett. **58**:254–258.
18. **Lugtenberg, B., and L. van Alphen.** 1983. Molecular architecture and functioning of the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **737**:51–115.
19. **Maier, C., E. Bremer, A. Schmid, and R. Benz.** 1987. Pore forming activity of the Tsx protein of *Escherichia coli*. Demonstration of a nucleotide-specific binding site. J. Biol. Chem. **263**:2493–2499.
20. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Misra, R., and S. A. Benson.** 1988. Isolation and characterization of OmpC porin mutants with altered pore properties. J. Bacteriol. **170**:528–533.
22. **Misra, R., and S. A. Benson.** 1988. Genetic identification of the pore domain of the OmpC porin of *Escherichia coli* K-12. J. Bacteriol. **170**:3611–3617.
23. **Misra, R., B. A. Sampson, J. L. Occi, and S. A. Benson.** 1987. Identification of mutants with altered membrane permeability, p. 436–442. *In* P. Actor, L. Daneo-Moore, M. L. Higgins, M. R. J. Salton, and G. D. Shockman (ed.), Antibiotic inhibition of bacterial cell surface assembly and function. American Society for Microbiology, Washington, D.C.
24. **Morona, R., and P. Reeves.** 1982. The *tolC* locus of *Escherichia coli* affects the expression of three major outer membrane proteins. J. Bacteriol. **150**:1016–1023.
25. **Nakae, T.** 1986. Outer-membrane permeability of bacteria. Crit. Rev. Microbiol. **13**:1–62.
26. **Nikaido, H., and M. Vaara.** 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev. **49**:1–32.
27. **Overbeeke, N., and B. Lugtenberg.** 1980. Expression of outer membrane protein e of *Escherichia coli* K-12 by phosphate limitation. FEBS Lett. **112**:229–232.
28. **Pugsley, A. P., and C. A. Schnaitman.** 1978. Identification of three genes controlling production of new outer membrane pore proteins in *Escherichia coli* K-12. J. Bacteriol. **135**:1118–1129.
29. **Pugsley, A. P., and C. A. Schnaitman.** 1978. Outer membrane proteins of *Escherichia coli*. VII. Evidence that bacteriophage-directed protein 2 functions as a pore. J. Bacteriol. **133**:1181–1189.
30. **Reeves, P.** 1979. The genetics of outer membrane proteins, p. 256–291. *In* M. Inouye (ed.), Bacterial outer membranes. John Wiley & Sons, Inc., New York.
31. **Rosenbusch, J. P.** 1974. Characterization of the major outer membrane protein from *Escherichia coli*: regular arrangement on the peptidoglycan and unusual dodecyl sulfate binding. J. Biol. Chem. **249**:8019–8029.
32. **Sanderson, K. E., and J. A. Hurley.** 1987. Linkage map of *Salmonella typhimurium*, p. 877–918. *In* F. C.Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
33. **Schwartz, M.** 1987. The maltose regulon, p. 1482–1502. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
34. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35. **Tommassen, J., and B. Lugtenberg.** 1980. Outer membrane protein e of *Escherichia coli* K-12 is co-regulated with alkaline phosphatase. J. Bacteriol. **143**:151–157.