Journal of Industrial Microbiology, 1 (1987) 335–340
Elsevier

SIM 00040

Short Communication

Escherichia coli strains with defined mutations which alter cellular permeability

Barbara A. Sampson and Spencer A. Benson

Department of Molecular Biology, Princeton University, Princeton, NJ 08544, U.S.A.

Received 25 July 1986
Revised 18 September 1986
Accepted 30 September 1986

Key words: Escherichia coli; Outer membrane permeability; Porin

SUMMARY

We describe the construction and analysis of an isogenic series of *Escherichia coli* K12 strains that vary in their outer membrane permeability. They carry mutations that alter the amount and the type of porin present in the outer membrane. The permeability profiles of these strains suggest that both the amount and the type of porin present in the outer membrane affects permeability. Several of the strains carry mutations that extend the permeability of the outer membrane to include a variety of compounds that are normally excluded from entering the cell.

INTRODUCTION

The Gram-negative bacterium *Escherichia coli* has been a model system for the study of bacterial physiology, biological regulatory systems, and genetics, and for advances in the recombinant DNA and biotechnology fields. One great advantage in utilizing this organism to study biological processes of both basic and applied interest is the tremendous knowledge base we have regarding this organism and the variety of genetic, biochemical, and recombinant DNA approaches that can be brought to bear on problems of interest.

One constraint in using *E. coli* is the limited permeability of the envelope structure. *E. coli* has a tri-laminar envelope structure composed of an inner membrane, a peptidoglycan layer, and an outer membrane, which serves as the first permeability barrier to the environment. The outer membrane functions as a selective permeability barrier, allowing small (<600 Da) hydrophilic molecules to cross the membrane through proteinaceous water-filled channels formed by a specialized class of proteins termed the porins [for review see Ref. 10]. *E. coli* K12 has two major porin species, OmpF and

Correspondence: Dr. S.A. Benson, Department of Molecular Biology, Princeton University, Princeton, NJ 08544, U.S.A.

OmpC, encoded by the *ompF* and *ompC* genes. The two porins have similar, though not identical, pore sizes with the OmpC protein having a slightly smaller pore [8]. Compounds with a molecular weight of > 600 Da, charged molecules, oligopeptides, and hydrophobic compounds do not readily pass through the channels present in these proteins [10]. Consequently, many compounds of pharmaceutical and industrial interest are prevented from entering the cell. We have previously described the isolation of porin mutants with altered outer membrane permeability [2]. Here we have capitalized upon these as well as other mutants to construct a series of isogenic strains that vary in their outer membrane permeability and allow many compounds which do not normally traverse the outer membrane to cross.

MATERIALS AND METHODS

Media and chemicals

Bacteriological media and chemicals are as previously described [5,11]. Maltooligosaccharide (dextrin) was purchased from Pfanstiehl Laboratories, Waukegan, IL. It was further purified by dialysis to remove maltodextrins with a molecular weight less than 1000 Da. Antibiotic discs were purchased from Difco or BBL.

Bacterial strains

Bacterial strains are described in Table 1.

Construction of isogenic bacterial strains

All bacterial strains were constructed by P1 transduction using the bacteriophage $P1^{vir}$ [5,11]. Strain BAS2003 carries the *ΔlamB*106 mutation. The *lamB* gene product is an inducible outer membrane protein required for the uptake of maltodextrin and functions as the receptor for the bacteriophage λ [4]. Consequently, BAS2003 is unable to grow on minimal media containing maltodextrin as the carbon source (Dex^-) and is resistant to λ (λ^r) [4]. Strains BAS2004 and BAS2005 were constructed by transducing BAS2003 to Dex^+ with P1 lysates prepared on strains carrying the *ΔlamB*106 and the *ompC*3256 or the *ompF*3255 allele. The *ompF* and *ompC* mutations each confer a Dex^+ phenotype presumably by allowing dextrin to enter through the altered pore [2]. Furthermore, they increase the permeability of the outer membrane to detergents and antibiotics.

Strains BAS2001 and BAS2002 were constructed by introducing either an *ompF-lacZ* (BAS2001) or an *ompC-lacZ* (BAS2002) gene fusion into BAS2003. The gene fusions were introduced by selecting for growth on media containing lactose as the carbon source (Lac^+). Strain BAS2000 was constructed by introducing the *ΔlamB*106 mutation in-

Table 1

Bacterial strains

Strain	Genotype or relevant genes	Source
MC4100	F^- *araD*139 *Δ(argF-lac)U*169 *rpsL*150 *relA flbB*5301 *ptsF*25 *deoC*1	M. Casadaban
MH1160	MC4100 *ompR*101	M. Hall
DME557	MC4100 *ΔlamB*106 *pyrD*39 Φ (*ompF'-lac*$^+$) Hyb16-13	S. Benson
BAS2000	MC4100 *ΔlamB*106 *ompR*101	This study
BAS2001	MC4100 *ΔlamB*106 Φ (*ompF'-lac*$^+$) Hyb16-13	This study
BAS2002	MC4100 *ΔlamB*106 Φ (*ompC'-lac*$^+$) Hyb10-25	This study
BAS2003	MC4100 *ΔlamB*106	This study
BAS2004	MC4100 *ΔlamB*106 *ompC*3256	This study
BAS2005	MC4100 *ΔlamB*106 *ompF*3255	This study
BAS2006	MC4100 *ΔlamB*106 *ompC*3256 *ompF*3255	This study

to strain MH1160 which carries a mutation in the positive regulatory gene *ompR* [3]. This mutation blocks expression of *ompF* and *ompC*. The mutation was introduced in two steps. First, the strain was made Mal$^-$ (unable to grow on the sugar maltose) by introducing a *malE*::Tn*10* by selection for tetracycline resistance (Tc^r). We then introduced the *ΔlamB*106 mutation by co-transduction with a *malE*$^+$ allele. The *lamB* gene is tightly linked to the *malE* gene, but is not required for growth on maltose [4]. Transductants which had acquired the *ΔlamB*106 mutation were identified by their Dex$^-$ λ^r phenotype.

Strain BAS2006 was constructed by the following series of transductions. The *ompC*3256 allele was introduced into strain DME557 by selecting for the Dex$^+$ phenotype conferred by the *ompC*3256 allele. The *ompF*3255 allele was then introduced by co-transduction with a *pyrD*$^+$ allele. The *pyrD* locus is approximately 50% linked by P1 co-transduction to the *ompF* gene. PyrD$^+$ transductants which had acquired the *ompF*3255 allele were identified as white colonies (*lacZ*$^-$) on glucose minimal media containing the chromogenic substrate 5-bromo-5-chloro-3-indolyl-β-D-galactoside (XG). This substrate is a sentitive indicator of β-galactosidase activity. To confirm that each strain carried the indicated alleles, we tested sensitivity to bacteriophages K20 and SS4 (these bacteriophages use the porins OmpF and OmpC, respectively, as their receptors), detergents, and antibiotics (Table 2), and confirmed the presence or absence of the porin proteins in the cellular envelope by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1).

We noted that our standard transduction procedure of adding sodium citrate (66-mM final concentration) to block re-adsorption of the P1 phage often resulted in lysis of the recipient cells if they carried the *ompR*101, the *ompC*3256, or the *ompF*3255 mutations. To overcome this problem we reduced the amount of sodium citrate 3-fold.

Detergent, antibiotic, EDTA and $CuSO_4$ sensitivity

Sensitivity to EDTA, detergents and $CuSO_4$ was tested by plating 0.1 ml of an overnight culture onto L agar in 3-ml of H-top agar [11]. Paper discs (6-mm diameter; Schleicher & Schuell analytical paper) were positioned on the lawn of cells, and 15-μl of the test compound were placed on the disc. Plates were incubated overnight at 37°C, and then the zones of growth inhibition were measured with a metric template. Sensitivity to the antibiotics was tested in an identical fashion except commercial antibiotic discs were used.

Preparation of washed envelopes and SDS-PAGE

The procedures for the preparation of washed envelope samples and SDS-PAGE were as described previously [2], except that 10% non-urea gels with a bis-to-acrylamide ratio of 1:30 were used to separate the major outer membrane proteins. Five-μl samples were applied to the gels and electrophoresed overnight.

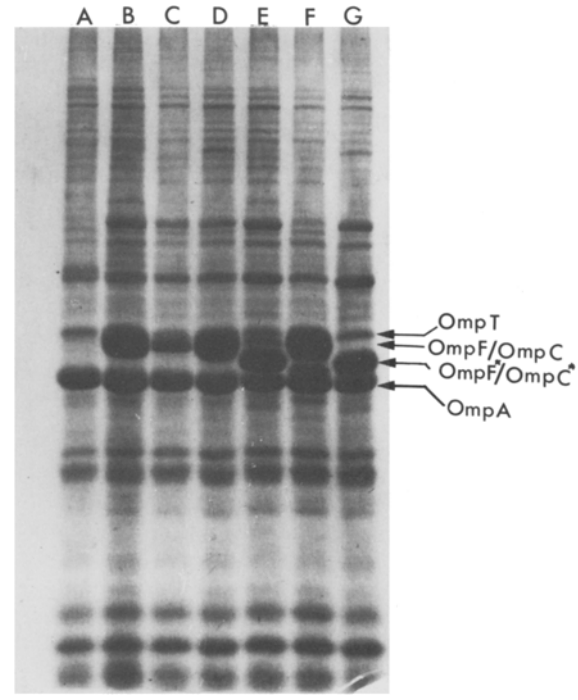

Fig. 1. SDS-PAGE of the cellular envelopes. Samples were prepared and electrophoresed as described in 'Materials and Methods'. The positions of the major outer membrane protein species are marked. Lane A, BAS2000 (F$^-$C$^-$); Lane B, BAS2001 (F$^-$C$^+$); Lane C, BAS2002 (F$^+$C$^-$); Lane D, BAS2003 (F$^+$C$^+$); Lane E, BAS2004 (F$^+$C*); Lane F, BAS2005 (F*C$^+$); Lane G, BAS2006 (F*C*).

Table 2

Sensitivity to various compounds

Compound (concn. or amount)	Inhibition zone (mm)						
	BAS2000	BAS2001	BAS2002	BAS2003	BAS2004	BAS2005	BAS2006
Porin composition[a]	F^-C^-	F^-C^+	F^+C^-	F^+C^+	F^+C^*	F^*C^+	F^*C^*
$CuSO_4$, chelating agents, Eosin Y and detergents							
$CuSO_4$ (1.19 mg)	14	8	8	9	11	10	12
EDTA (470 μg)	0	0	0	0	0	0	0
Na citrate (3.9 mg)	8	0	0	0	0	0	0
Eosin Y (150 μg)	0	0	0	0	9	0	8
Deoxycholate (750 μg)	7[b]	0	0	0	13	9	16
Triton X-100 (750 μg)	8[b]	0	0	0	17	8[b]	17
SDS (750 μg)	10[b]	0	0	0	23	14	24
Antibiotics							
Tetracyclines							
chlorotetracycline (30 μg)	19	16	15	16	19	21	21
oxytetracycline (5 μg)	14	11	14	14	14	14	16
tetracycline (5 μg)	10	9	11	11	13	13	13
Aminoglycosides							
gentamicin (10 μg)	15	16	16	14	16	16	16
kanamycin (5 μg)	10	10	12	10	10	11	9
neomycin (5 μg)	8	8	8	7	8	8	8
Betalactams							
ampicillin (50 μg)	15	18	21	21	21	26	28
carbenicillin (100 μg)	23	22	25	24	28	29	32
cephalothin (30 μg)	0	0	12	11	12	15	17
penicillin (2 U)	0	0	0	0	0	0	0
Macrolides							
clindamycin (2 μg)	0	0	0	0	8	9	9
erythromycin (15 μg)	0	0	0	0	13	21	24
novobiocin (30 μg)	0	0	0	0	22	13	22
Peptide							
bacitracin (10 U)	0	0	0	0	14	22	22
polymixin (50 μg)	8	8	9	8	8	8	9
Others							
chloramphenicol (5 μg)	0	0	7	9	16	18	18
furazolidone (100 μg)	17	16	16	15	16	16	17
nalidixic acid (5 μg)	12	10	13	11	19	18	17
nitrofurantoin (100 μg)	18	17	16	15	16	15	17
oxolinic acid (2 μg)	19	16	18	16	21	20	22
rifampin (15 μg)	11	9	8	8	11	25	22
trimethaprim (5 μg)	21[b]	18	24	16	21	22	23

[a] The * designation indicates that an altered porin is present; in the case of OmpF, the strain carries the *ompF*3255 allele and, in the case of OmpC, the strain carries the *ompC*3256 allele. [b] Indicates a faint zone of growth inhibition.

RESULTS AND DISCUSSION

The isogenic strain series was constructed as described in 'Materials and Methods'. It consists of strains in which we have altered the outer membrane permeability by both the amount and type of porin protein present in the outer membrane (Fig. 1). The least permeable strain BAS2000 (F^-C^-) carries a mutation (*ompR*101) which prevents expression of both *ompF* and *ompC* [3]. Strain BAS2001 (F^-C^+) expresses only the OmpC porin, whereas BAS2002 (F^+C^-) expresses only the OmpF porin. In each of these strains, expression of one of the porins is prevented by either an *ompF-lacZ* (BAS2001) or an *ompC-lacZ* (BAS2002) gene fusion. Strain BAS2003 (F^+C^+) expresses both porins. Strains BAS2004 (F^+C^*) and BAS2005 (F^*C^+) each carry a wild type porin and a mutant porin that results in increased outer membrane permeability. Strain BAS2006 (F^*C^*) carries mutations in both *ompF* and *ompC*, each of which increases outer membrane permeability.

To determine the permeability properties of these strains, we assayed sensitivity to 29 different agents including detergents, chelators, $CuSO_4$, and a variety of antibiotics. The results of these tests are shown in Table 2.

Several general patterns of sensitivities emerge. Mutants lacking both porins and those containing altered porin proteins show increased sensitivity to many of the agents tested. These include detergents, $CuSO_4$ and various antibiotics. The increase in sensitivity is most dramatic for the detergents in those strains carrying altered porin proteins. The porin-less strain shows only a slight increase in sensitivity to these agents. It is generally accepted that it is the LPS moiety of the outer membrane that prevents detergents from entering Gram-negative bacteria [10]. Interestingly, neither the porin-less strain nor the strains with altered porins exhibit increased sensitivity to EDTA, suggesting that the LPS structure is not altered in these mutants. A slight sensitivity to high levels of sodium citrate is observed with strain BAS2000.

All of the strains appear to have equivalent levels of aminoglycoside sensitivity. This suggests that the mode of entry for this class of antibiotics does not require expression of the major porins OmpF and OmpC. This is consistent with previously published reports [6]. In contrast, the mode of entry for the β-lactams appears to be via the porin channels for the following reasons. Strains which carry the altered porins have increased sensitivities to the majority of β-lactams tested. Furthermore, BAS2001 which contains only the smaller OmpC pore is more resistant than BAS2002 or BAS2003, both of which carry the OmpF pore (Table 2). These findings are most consistent with the hypothesis that the β-lactams enter through the porin proteins as has been previously suggested by several laboratories [9,10,12].

The macrolide antibiotics and the peptide antibiotics require the altered porins to enter the cell. One exception is the antibiotic polymixin. This antibiotic binds to LPS and disrupts outer membrane structure [13]. Thus, it is not surprising that we failed to detect differences in sensitivity to this agent. Interestingly, there appears to be a porin preference for the large antibiotics erythromycin, novobiocin, bacitracin, and rifampin. Erythromycin, bacitracin, and rifampin appear to enter preferentially through the altered OmpF porin, whereas novobiocin appears to enter preferentially through the altered OmpC pore. This is unusual since the OmpC and OmpF porins have been reported not to have significant substrate specificity [7]. However, recent results have suggested that the two porins do exhibit specificity in their ability to allow certain peptides to cross the outer membrane [1]. Other antibiotics such as chloramphenicol and nalidixic acid appear to enter at an increased rate through the altered porins without preference.

Since we do not know in molecular terms the exact nature of the alterations present in the *ompF*3255 and *ompC*3256 mutations, it is difficult to state confidently whether the increase in sensitivity to a given agent results from an increase in the size of the channel within an altered porin or from a more general permeability change caused by the insertion of the altered porin into the outer membrane architecture. We favor the possibility that increased permeability of the outer membrane in the

mutants results from a combination of these effects. This contention is supported by the observations that strains carrying the altered porins show increased sensitivity to detergents, dyes, and metals which presumably cross the outer membrane in a porin-independent fashion. At the same time, they show increased sensitivity to the β-lactam antibiotics and large antibiotics which presumably cross the outer membrane through the porins.

The strains described here have several unique properties that make them both interesting and potentially useful. They have a defined porin composition that can be exploited to render the cells permeable to agents that would not normally enter *E. coli*. They can be used to screen agents such as fermentation broths for novel antibacterial compounds without the exclusion limits normally imposed by wild type *E. coli*. They can also be used to determine whether potential antibacterial agents enter in a porin-dependent or -independent manner. Finally, by using the various mutants it may be possible to determine the type of antibiotic compound present in a fermentation broth.

ACKNOWLEDGEMENT

This work was supported by a grant from the National Institute of Health GM-34810-01.

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