Mutants of *Escherichia coli* That Are Resistant to Certain Beta-Lactam Compounds Lack the *ompF* Porin

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Carbenicillin-resistant mutants of *Escherichia coli* K-12 and B/r were found to produce greatly diminished levels of the porin coded by the *ompF* gene. Physiological and ecological implications of these findings are discussed.

During the course of other studies, one of us (M.M.) noticed that *Escherichia coli* K-12 mutants that are moderately resistant to β-lactam compounds containing two anionic groups—for example, sulbenicillin and carbenicillin—occurred spontaneously at rather high frequencies, so that several isolated colonies were routinely observed well within the inhibition zones around the β-lactam-containing disks in the disk diffusion tests for susceptibility. Since β-lactam antibiotics must first diffuse through the outer membrane of these bacteria, and more specifically through the transmembrane porin channels (12, 13), before they exert their antibacterial action, we examined the protein composition of the outer membrane of these mutants, and we found that they indeed lacked the porin species most efficient in producing permeability.

The resistant mutants were picked from colonies within the inhibition zones after incubation of L agar plates (3), which were spread with about 10^6 cells of wild-type *E. coli* K-12 or *E. coli* B/r strain CM6 (2), and on which filter paper disks each containing 500 µg of carbenicillin were placed. Agar dilution tests (Table 1) showed that these mutants were significantly more resistant to at least some β-lactam compounds. Figures 1 and 2 show the patterns of cell envelope proteins in representative mutants as well as in the wild-type strains. *E. coli* K-12 produces two species of porins, coded by *ompF* and *ompC* genes (17), and the extent of expression of these genes is controlled by the nature of the growth media (1, 10, 14) (cf. lanes a and b in Fig. 1). We observed that the mutants did not produce any *ompF* protein, even in a medium strongly favoring the expression of this gene; instead, *ompC* protein was strongly expressed (Fig. 1, lanes c through e). *E. coli* B/r produces only one porin, presumably coded for by the *ompF* gene (17). The amount of this porin was greatly diminished in the carbenicillin-resistant mutants (Fig. 1, lane h and i). There was no

significant difference between CM6 and mutant HN105 in terms of the level of β-lactamase or the pattern of penicillin-binding proteins (determined in accordance with the method in reference 7) (results not shown).

It has been established through both in vitro reconstitution approaches (8, 9) and mutant studies (2, 13) that porins in *E. coli* and *Salmo-*

TABLE 1. *Minimal inhibitory concentrations of various β-lactam compounds for parent and mutant strains[a]*

β-Lactam compound	Minimal inhibitory concn (µg/ml)			
	E. coli K-12		*E. coli* B/r	
	Parent (K-12)	Mutant (HN101)	Parent (CM6)	Mutant (HN105)
Dibasic				
Carbenicillin	8	128	4	32
Ticarcillin	4	32	4	32
Sulbenicillin	8	64	4	32
SCE-20	64	512	64	>512
Monobasic				
Cephacetrile	4	8	4	32
Cefazolin	1	2	1	8
Cephaloram[b]	8	64	1	32
Benzylpenicillin	16	64	8	16
Dipolar ionic				
Cephaloridine	2	2	2	32
Ampicillin[c]	1	2	0.5	4

[a] Minimal inhibitory concentrations were determined by spotting 0.02-ml bacterial suspensions containing about 10^5 cells of overnight broth culture on Difco antibiotic assay medium no. 2 plates containing twofold serial dilutions of antibiotics. The growth was read after 18 h at 37°C; readings of less than 10 colonies per spot were scored as negative. Sources of the antibiotics were Sigma Chemical Co. (benzylpenicillin, ampicillin, and carbenicillin), Eli Lilly & Co. (cefazolin, cephaloram, and cephaloridine), Takeda Chemical Industries (sulbenicillin and SCE-20), and Ciba-Geigy Corp. (cephacetrile).

[b] Cephaloram is an analog of cephalothin in which the thienyl group of cephalothin is replaced by a phenyl group.

[c] At a neutral pH, only a fraction of ampicillin molecules will be in the dipolar ionic form.

nella typhimurium produce channels for the diffusion of most nutrients. Since porin channels appear to lack any configurational specificity (8, 12), it is reasonable to assume that most small, hydrophilic antibiotics, including β-lactam compounds, reach the periplasmic space via the porin channel. Indeed, this role of the porin channel was proven in *S. typhimurium* for the penetration of cephaloridine (13) and since then has been confirmed for a number of other β-lactam compounds tested with *E. coli* strains (2; H. Nikaido, unpublished data). Thus, it was predicted that reduction in the amounts of porin would produce resistance to β-lactam compounds, and the isolation of *E. coli* B/r mutant HN105, which was moderately resistant to all β-lactam compounds tested (Table 1), confirmed this prediction.

With *E. coli* K-12, the situation is more complex. The mutants tested could not produce the *ompF* porin but overproduced the *ompC* porin (Fig. 1) and showed resistance only to some β-lactam compounds (Table 1). In a recent study, we could find no difference between the properties of *ompF* and *ompC* channels, except that in intact cells, *ompC* protein produced a far less efficient channel than *ompF* protein (H. Nikaido, E. Y. Rosenberg, and J. Foulds, unpublished data). Thus, the loss of the more efficient *ompF* channel will reduce the permeability of the outer membrane and still allow the slow

uptake of nutrients for survival. Why is it then that the K-12 mutants are resistant only to some β-lactam compounds? This is presumably because there is a large difference among β-lactam compounds in rates of penetration through the outer membrane (12, 18, 19). The additional negative charge present on dibasic β-lactam compounds, as well as hydrophobicity, severely reduced permeability coefficients through porin channels (12; H. Nikaido, E. Y. Rosenberg, and J. Foulds, unpublished data), and it is conceivable that the outer membrane penetration step very nearly comprises the rate-limiting step in the action of these agents even against wild-type *E. coli*. If so, moderate reduction in outer membrane permeability will make the mutant significantly more resistant to these β-lactam compounds; indeed, the mutant HN101 was found to be strongly resistant only to those agents expected to penetrate slowly because of the dibasic structure (i.e., carbenicillin, ticarcillin, sulbenicillin, and SCE-20) or because of strong hydrophobicity (i.e., cephaloram and benzylpenicillin) (Table 1). Compounds with high penetration rates, such as cephacetrile, cefazolin, cephaloridine, and ampicillin (12, 19; Nikaido et al., unpublished data), remained nearly fully effective in the mutant, and in fact, mutants resistant to cefazolin could not be isolated easily in *E. coli* K-12 (S. Tomioka and M. Matsuhashi, unpublished data).
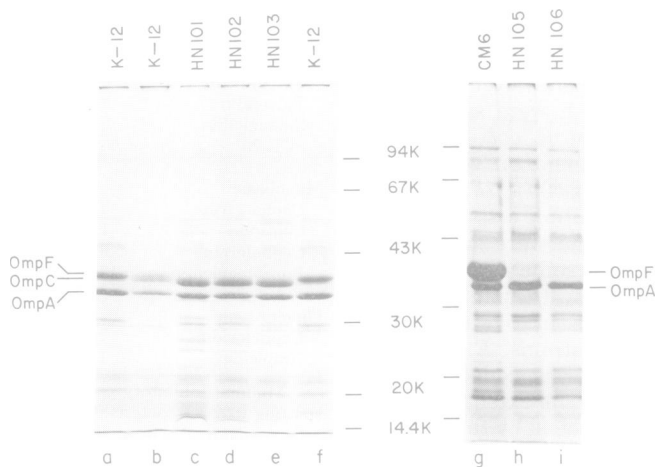

FIG. 1. *Protein patterns of the cell envelope preparations from various strains. E. coli B/r strain CM6 and its carbenicillin-resistant mutants HN105 and HN106 (lanes g, h, and i, respectively) were grown in L broth (glucose omitted). E. coli K-12 and its mutants HN101, 102, and 103 were grown either in L broth (glucose omitted) (lane b) or in Difco nutrient broth containing 0.2% sodium succinate (lanes a, c, d, e, and f); for the parent K-12 strain, the L broth allows about equal expression of ompF and ompC proteins, whereas ompF protein predominates in nutrient broth-succinate-grown cells, as reported earlier (1). Cell envelope fractions were prepared by sonication of exponential-phase cells (2), solubilized, and analyzed by polyacrylamide slab gel electrophoresis in sodium dodecyl sulfate (6). Positions of molecular-weight marker proteins, as well as those of ompF, ompC, and ompA proteins, are indicated.*
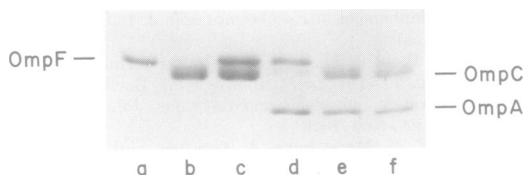
FIG. 2. *Higher resolution analysis of the protein patterns of the K-12 derivatives. To separate the two porins more widely, the polyacrylamide slab gel electrophoresis was carried out with a 25-cm-long gel, and the electrophoresis was continued after the dye front reached the bottom. Only the region containing the porins and ompA protein is shown. Lane a, Purified ompF protein from JF701 (5); lane b, purified ompC protein from JF703 (5); lane c, mixture of the purified porins; lane d, K-12; lane e, HN102; lane f, HN103 (all grown in Difco nutrient broth containing 0.2% sodium succinate).*

Similar considerations also apply to other bactericidal and bacteriostatic agents. If the agent has low intrinsic permeability through the porin channels, it should be possible to isolate resistant mutants of *E. coli* K-12 lacking the *ompF* protein. It is already known that some mutants resistant to tetracyclines and chloramphenicol are of this type (4, 14, 16). Although the rates of penetration of these agents through the outer membrane have not been reported, these rates are expected to be quite slow, because tetracycline is large and negatively charged, and chloramphenicol is strongly hydrophobic (11).

All these considerations suggest that the resistance phenotype of the K-12 mutants studied in this work is due to the absence of the *ompF* porin. To confirm this interpretation, we compared a known *ompF* mutant, JF703 (5), with its isogenic parent, JF568 (5), in an agar dilution assay; the assay showed that the *ompF* mutant was indeed resistant to carbenicillin, sulbenicillin, and ticarcillin but not to cephacetrile and cephaloridine. This observation is obviously consistent with our conclusion. However, the minimal inhibitory concentrations of the dibasic β-lactam compounds were increased only 2- to 4-fold for this mutant, in contrast to the 8- to 16-fold increase seen for HN101. The basis for these quantitative differences is currently under investigation.

It has been proposed that bacterial strains containing multiple porin species may have an ecological advantage, because mutants without one porin species still have a reasonably permeable outer membrane and are able to survive in the presence of phages and colicins that utilize this particular porin as the receptor (15). Similarly, the presence of multiple porin species might help the organism survive in an environment containing deleterious agents of low intrinsic permeability by the production of mutants lacking the more efficient porin channel.

While this work was in progress, we learned that Y. Komatsu, K. Murakami, and T. Nishikawa (personal communication) isolated an *E. coli* K-12 mutant which was strongly resistant to moxalactam and appeared to lack porin(s). Interestingly, moxalactam is a dibasic β-lactam compound, and the mutant was also moderately resistant to carbenicillin.

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