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Penicillin-Binding Protein 2 Is Required for Induction of the *Citrobacter freundii* Class I Chromosomal β-Lactamase in *Escherichia coli*

BRUNELLO OLIVA,[1] PETER M. BENNETT,[2] AND IAN CHOPRA[2]*

Microbiology Section, Department of Experimental Medicine, University of L'Aquila, Aquila, Italy,[1] and Department of Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom[2]

Received 10 November 1988/Accepted 12 April 1989

Involvement of *Escherichia coli* penicillin-binding proteins (PBPs) in induction of the cloned *ampC Citrobacter freundii* β-lactamase by 6-aminopenicillanic acid was investigated. The enzyme was not inducible at 42°C in a mutant thermosensitive for expression of PBP 2. The results imply that PBP 2 is involved in the process leading to induction of *ampC*.

Many gram-negative organisms possess chromosomal genes specifying class I β-lactamases (9). In some species, e.g., *Citrobacter freundii*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*, the enzyme is inducible, i.e., enzyme synthesis increases when a beta-lactam is added (9). The molecular basis of β-lactamase induction is poorly understood, but in *E. cloacae* and *C. freundii*, class I β-lactamase production is controlled by at least two genes, *ampR* and *ampD* (4). Beta-lactams may interact with *ampD*, thereby eliciting a signal sensed by *ampR*, leading to transcriptional activation of the β-lactamase *ampC* gene (4).

The cellular location of the *ampD* gene product is unknown, but if this product is cytoplasmic then beta-lactams would have to enter the cell to interact with it. Although beta-lactams can diffuse into liposomes (2), it is not known whether they cross the bacterial cytoplasmic membrane into the cytoplasm. If beta-lactams are restricted to the periplasm, their presence must be recorded and transmitted to the elements controlling *ampC* β-lactamase expression. This would involve a transmembrane signal. In *Bacillus licheniformis*, a membrane protein with properties similar to known penicillin-binding proteins (PBPs) is involved in the induction of its chromosomal β-lactamase (3). In view of this fact, we investigated whether *Escherichia coli* PBPs are involved in induction of the *C. freundii ampC* gene. This has been possible because the linked chromosomal *ampC* and *ampR* genes of *C. freundii* have been cloned and found to express inducible β-lactamase production in *E. coli* (5). Accordingly, we have been able to examine expression of the cloned *ampC* gene carried by plasmid pNU305 in known PBP mutants of *E. coli*.

Strains KN126 (*trpE tyr ilv sup-126*) (11), SP45 [a *pbpA* (Ts) mutant of KN126] (11), and SP63 (an *ftsI* mutant of KN126) (10) were transformed (6) with pNU305 (5), and β-lactamase (EC 3.5.2.6) activities were determined (1) before and after induction with 6-aminopenicillanic acid (6-APA) (5) at 28 or 42°C. For induction at 42°C, broth cultures were grown to mid-exponential phase at 28°C and the temperature was raised to 42°C on addition of the inducer 6-APA (2 mg/ml). At 28°C β-lactamase production was inducible in all strains, but at 42°C induction occurred only in KN126(pNU305) and SP63(pNU305) (Table 1). It was not possible to induce β-lactamase synthesis at 42°C in strain SP45(pNU305) (Table 1). Since we confirmed that the PBP profiles of the strains (see above) agreed with published reports (10, 11), the β-lactamase enzyme induction results (Table 1) imply a role for PBP 2 but not PBP 3 in induction of *ampC*.

We wondered whether the failure to induce β-lactamase at 42°C in the thermosensitive PBP 2 mutant (SP45) might arise by nonspecific effects on enzyme synthesis due to the combined action of inducing antibiotic (6-APA) and elevated temperature. To check this possibility, induction of β-galactosidase (EC 3.2.1.23) by isopropyl-thiogalactoside (0.5 mM) was examined (1) in the various strains carrying pNU305. KN126 and its derivatives contain a chromosomal *lac* determinant, and induction of β-galactosidase was performed at 28 and 42°C in both the presence and the absence of 6-APA. In all cases, isopropyl-thiogalactoside behaved as an effective inducer of β-galactosidase and the levels of enzyme synthesized were not influenced by the presence of 6-APA (data not shown).

The *E. coli* mutants used here synthesize PBPs that are unable to bind beta-lactams at the nonpermissive temperature (42°C) (10, 11). Hence, defective *ampC* induction at 42°C in a particular PBP mutant provides evidence for involvement of that protein during the normal induction process. On this basis, *E. coli* PBP 2 plays a role in *ampC* induction by 6-APA. Preliminary experiments in *P. aeruginosa* suggest involvement of PBPs 1b, 3, 4, and 5 for induction of *ampC* in this organism (7). Whether more than one PBP is also involved in *ampC* induction in *E. coli* is not yet known.

The mechanism by which beta-lactam-PBP interactions are recorded and recognized by *ampD* remains unknown. Signal transduction across the membranes of higher cells involves stimulation of membrane receptors by signal molecules followed by a cascade of biochemical processes that ultimately produce an intracellular signal, causing a change in the behavior of the cell (8). Whether a related mechanism operates for *ampC* induction in gram-negative bacteria remains to be established.

* Corresponding author.

TABLE 1. Expression of β-lactamase in various *E. coli* strains before and after exposure to 6-APA[a]

Strain	PBP no. (defect)	β-Lactamase activity[b]			
		28°C		42°C	
		No 6-APA	+6-APA	No 6-APA	+6-APA
KN126(pNU305)	None	0.50 ± 0.04	8.23 ± 1.75	0.61 ± 0.14	5.20 ± 0.84
SP45(pNU305)	2 (Ts)	0.53 ± 0.09	11.75 ± 2.28	0.56 ± 0.06	0.46 ± 0.10
SP63(pNU305)	3 (Ts)	0.37 ± 0.08	13.39 ± 2.97	0.57 ± 0.08	4.36 ± 1.04

[a] Bacteria were harvested from cultures and sonicated, and total β-lactamase activity was determined as described previously (1).
[b] Micromoles of nitrocefin destroyed per minute per milligram of protein ± one standard error of the mean.

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