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Penicillin-Binding Proteins and Induction of AmpC β-Lactamase

CHRISTINE C. SANDERS,[1]* PATRICIA A. BRADFORD,[2] ANTON F. EHRHARDT,[1] KAREN BUSH,[2]† KEVIN D. YOUNG,[3] THOMAS A. HENDERSON,[3] AND W. EUGENE SANDERS, JR.[1]

Center for Research in Anti-Infectives and Biotechnology, Department of Medical Microbiology, Creighton University School of Medicine, Omaha, Nebraska 68178[1]; Wyeth Ayerst Research, Lederle Laboratories, Pearl River, New York 10965[2]; and Department of Microbiology and Immunology, School of Medicine, University of North Dakota, Grand Forks, North Dakota 58202[3]

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In competition assays for radiolabeled penicillin, penicillin-binding proteins (PBPs) 4, 7a, and 7b showed very high affinities for strong inducers of AmpC β-lactamase. Loss of PBP 4 resulted in diminished inducibility. This suggests that if PBPs are involved in induction of AmpC β-lactamase, there is probably a redundancy in function among the different PBPs.

The process involved in induction of AmpC β-lactamase has been under study for a number of years. Recent studies have shown that many of the genes involved in induction are also involved in recycling of the bacterial cell wall (1, 2, 7–9, 11, 12, 16–19, 22). Studies are now only beginning to address how an inducing β-lactam, like cefoxitin, alters recycling of muropeptides in a manner different from that of noninducing β-lactams (2, 8). It is possible that unique interactions with penicillin-binding proteins (PBPs) may be related to the specificity of β-lactamase induction. Therefore, a study was designed to assess the relationship between PBP binding and inducer potency for AmpC β-lactamase.

In these studies the inducibility and PBP patterns of *Escherichia coli* SNO3 and its various derivatives (8, 16, 18, 19) were examined. Induction was performed as described previously with pBP131, which carries *ampR ampC* from *Enterobacter cloacae* 14 (12). PBP patterns were determined with membranes prepared as described by Spratt with competition assays for [^{3}H] benzylpenicillin performed as described previously (14, 20, 24, 26). The PBP patterns of *E. coli* SNO302, the *ampD* mutant derivative of *E. coli* SNO3, in competition experiments with ampicillin, piperacillin, aztreonam, imipenem, cefoxitin, and cefotetan are shown in Fig. 1. The major difference between the good inducers imipenem, cefoxitin, and cefotetan and the poor inducers ampicillin, piperacillin, and aztreonam was a higher affinity of PBPs 1a, 4, 7a, and 7b for the good inducers (Table 1). This difference in affinity was especially marked for PBPs 4, 7a, and 7b. Although PBP 7a showed a high affinity for piperacillin, a poor inducer, this was still lower than the affinities of PBPs 2 and 3 for piperacillin. Similar differences were observed when PBP patterns obtained with these drugs were examined in tests with *E. coli* SNO302 in the presence of plasmids containing genes encoding wild-type or temperature-sensitive AmpD (pGKS146-3 and pGKS147-3), with *E. coli* HfrH (the wild type of *E. coli* SNO3), and with its *ampG* mutant *E. coli* HfrH-01 (data not shown).

These results suggested that binding to PBP 4 and/or 7 may initiate induction of AmpC β-lactamase. Therefore, inducibility of *E. coli* SNO3R (recombination-proficient derivative of *E. coli* SNO3), its PBP 4$^-$ derivative, *E. coli* SNO3RΔ4, its PBP 7$^-$ derivative, *E. coli* THO3R7, and its PBP4/7$^-$ derivative, *E. coli* THO3R7Δ4, was determined. These PBP$^-$ derivatives were prepared by P1*vir* transduction (21). *pbpG* was cloned into pUC19 (25), insertionally inactivated with Ω-CM (3), and recombined into the *E. coli* CS109 chromosome (13, 15). *pbpG*::Ω-CM was then P1 transduced into *E. coli* SNO3R to produce THO3R7. PBP 4$^-$ and PBP 4/7$^-$ strains were constructed by P1 transduction of *dacB*::Kan into *E. coli* SNO3R and THO3R7, respectively. PBP alterations were confirmed as previously described (5). As shown in Table 2, inactivation of PBP 7 in *E. coli* THO3R7 did not alter inducibility, while inactivation of PBP 4 with or without inactivation of PBP 7 diminished inducibility.

If PBP binding is involved in induction of AmpC β-lactamase, it would be likely that the PBPs involved would be nonessential PBPs (i.e., PBPs 4, 5, 6, and 7). Loss of function of these via drug binding would not lead to cell death, and there is redundancy in function among the nonessential PBPs (4). Ideally, their affinities for inducing β-lactams should be higher than those of essential PBPs (i.e., PBPs 1, 2, and 3). This

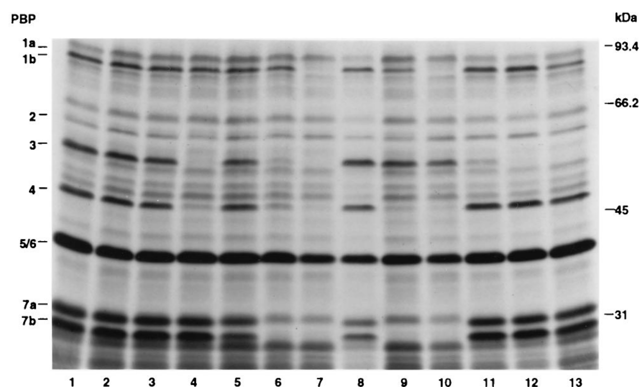

FIG. 1. Affinity of PBPs of *E. coli* SNO302 for ampicillin, cefotetan, cefoxitin, and aztreonam. Lane 1, control (no drug); lanes 2, 3, and 4, ampicillin at 0.01, 0.1, and 1.0 μg/ml; lanes 5, 6, and 7, cefotetan at 0.01, 0.1, and 1.0 μg/ml; lanes 8, 9, and 10, cefoxitin at 0.01, 0.1, and 1.0 μg/ml; lanes 11, 12, and 13, aztreonam at 0.01, 0.1, and 1.0 μg/ml.

* Corresponding author. Mailing address: Center for Research in Anti-Infectives and Biotechnology, Department of Medical Microbiology, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178. Phone: (402) 280-1881. Fax: (402) 280-1225.

† Present address: Astra Research Center Boston, Cambridge, MA 02139.

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TABLE 1. Binding of various β-lactam antibiotics[a] to penicillin-binding proteins of *E. coli* SNO302

PBP	Concn (μg/ml) required to inhibit binding of [^{3}H]benzylpenicillin by 50%					
	Amp	Pip	Atm	Cfx	Ctt	Imi
1a	2.0	0.7	0.54	0.12	0.12	0.01
1b	2.3	0.1	>1	>1	>1	0.09
2	0.9	0.09	>1	>1	>1	0.03
3	0.15	<0.01	<0.01	0.2	0.004[c]	>1
4	0.08	0.4	>1	0.02	0.032	0.02
5/6	>10	>10	>1	0.55	0.59	>1
7a	>1	0.12	>1	<0.01	0.032	0.2
7b	>1	—[b]	>1	0.002[c]	≤0.009	0.1

[a] Abbreviations: Amp, ampicillin; Pip, piperacillin; Atm, aztreonam; Cfx, cefoxitin; Ctt, cefotetan; Imi, imipenem.
[b] —, PBP not detected.
[c] Estimated value.

would guarantee that induction would be initiated at a concentration lower than that inhibiting growth or killing the cell. The results of this study suggest that several nonessential PBPs, PBPs 4, 7a, and 7b, may be involved in induction of AmpC β-lactamase. Failure to eliminate inducibility in derivatives lacking these PBPs does not rule out a potential role for them in AmpC induction given the potential for other nonessential PBPs to assume their function, e.g., PBPs 5 and 6. This possibility needs further study.

There are many similarities between PBP 4 and PBP 7 other than their high affinities for potent inducers of AmpC β-lactamase (6, 10, 23). Both are DD-endopeptidases that cleave the cross-linkages between D-alanine and diaminopimelic acid in the gram-negative cell wall, although only PBP 4 acts on both soluble muropeptides and insoluble high-molecular-weight murein sacculi (6, 10, 23). Thus, PBP 4 has a role in recycling of cell wall and may, in this regard, also be involved in induction of AmpC β-lactamase. In contrast to other PBPs of *E. coli*, neither PBP 4 nor PBP 7 is an integral membrane protein (6, 10, 23). PBP 4 and PBP 7 share 15% identical amino acids, two of the four motifs, SXXK and SXN, are perfectly aligned, and the KTG sequences are misaligned by only one amino acid (6). A major difference between PBP 4 and PBP 7 is that only the former possesses DD-carboxypeptidase activity.

Overall, the results of this study suggest a role for PBP 4 in the induction of AmpC β-lactamase. Although a role for PBP 7 cannot be ruled out at this time, its purported lack of involvement in cell wall recycling makes it unlikely that it is involved with induction of AmpC β-lactamase (23). Additional studies will be needed to assess changes in inducibility related to changes in each of the nonessential PBPs, taken alone and in combination. Alterations in the PBP patterns of potent inducers in studies with derivatives lacking one or more of the other nonessential PBPs are also needed to assess changes in affinity with loss of PBPs. Finally, changes in the products formed during recycling of the cell wall need to be assessed in these studies to relate PBP binding, PBP function, and inducibility. It is already clear with the data available to date that induction of AmpC β-lactamase is a complex process with many component parts.

TABLE 2. Inducibility of *E. coli* SNO3R(pBP131) and its derivatives lacking one or more PBPs

Inducer	Inducibility[a] of *E. coli* strain			
	SNO3R	PBP 7⁻	PBP 4⁻	PBP 4/7⁻
None	96	101	73	92
Ampicillin	256	221	152	133
Piperacillin	155	139	138	133
Aztreonam	81	56	58	51
Cefoxitin	2,057	2,561	1,670	1,211
Cefotetan	2,110	1,651	1,775	1,407
Imipenem	2,726	2,955	1,101	1,232

[a] Results are expressed as nanomoles of cephalothin hydrolyzed per minute per milligram of protein.

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