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Binding of β-Lactam Antibiotics to Penicillin-Binding Proteins of *Staphylococcus aureus* and *Streptococcus faecalis*: Relation to Antibacterial Activity

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The binding of 14 structurally diverse β-lactam antibiotics to penicillin-binding proteins of *Staphylococcus aureus* and *Streptococcus faecalis* was studied, and the results were examined in the context of the antibacterial activity of the compounds. Penicillin-binding proteins 1 (molecular weight, 87,000) and 3 (molecular weight, 75,000) of *S. aureus* and penicillin-binding proteins 1 (molecular weight, 105,000) and 3 (molecular weight, 79,000) of *S. faecalis* bound β-lactam antibiotics at concentrations comparable to minimum inhibitory concentrations and might therefore be essential. The low affinity of *S. faecalis* penicillin-binding proteins, relative to that of *S. aureus* penicillin-binding proteins, toward most β-lactam antibiotics is probably responsible for the resistance of the former organism to most of these compounds.

Staphylococci and streptococci are gram-positive bacteria which play an important role in infectious disease (5). The gram-positive cocci are notoriously susceptible to penicillins and cephalosporins, with the exception of β-lactamase-producing staphylococci in the case of certain penicillins (1) and penicillin-tolerant organisms (10, 15). *Streptococcus faecalis* is an exception to this rule, showing susceptibility to some penicillins and usually showing resistance to cephalosporins (14). This differential β-lactam susceptibility suggested to us that staphylococci and *S. faecalis* might possess different essential penicillin-binding proteins (PBPs).

PBPs are cytoplasmic membrane proteins which specifically bind penicillin and other β-lactam antibiotics and which are involved in cell wall biosynthesis. Their biochemical properties and physiological functions have been studied extensively in *Escherichia coli* (11), in which essential PBPs have also been unequivocally identified (13). Although *E. coli* PBPs adequately represent enterobacterial and probably pseudomonal PBPs (3), they provide very little information on other bacterial PBPs (4). In the present study, the PBP binding pattern of several structurally diverse β-lactam antibiotics was examined in *Staphylococcus aureus* and *S. faecalis* and subsequently correlated with antibacterial activity against the two organisms.

S. aureus SC 2399 and *S. faecalis* SC 9011 were from the Squibb Culture Collection. They were grown in brain heart infusion medium (Difco Laboratories) at 37°C on a Gyrotory shaker (280 rpm; New Brunswick Scientific Co.) for 16 h and subsequently (10% inoculum) for 4 h (4). Cells were harvested by centrifugation and sonicated for 9 min, and membranes were solubilized (2% Triton X-100) as previously described (4).

Solubilized membranes were incubated (100 μg of protein, determined by the method of Lowry et al. [9]) with the appropriate amount of β-lactam antibiotic (freshly prepared aqueous solution; final concentrations, 0, 0.1, 0.5, 2.0, 10, 30, and 100 μg/ml) at 30°C for 10 min. Ten nanomoles of [8-^{14}C]penicillin G (Amersham Corp.; specific activity, 54 μCi/μmol) was added, and the incubation was continued for another 10 min. Protein was precipitated with 4 volumes of cold acetone and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7; running gel, 10% acrylamide-0.27% bisacrylamide) followed by fluorography (8). Protein-bound [^{14}C]penicillin G was determined by visual examination of the X-ray film.

The results are summarized in Tables 1 and 2. PBP molecular weights and numbering system are those of reference 4. PBP patterns of the two organisms (Fig. 1) were similar to those reported previously for different strains (2, 6). In *S. aureus* (Table 1), PBP5 (DD-carboxypeptidase [6]) appeared to be the least sensitive to β-lactam antibiotics, showing strong affinity only for cefoxitin. This affinity for 7-methoxylated cephalosporins appears to be a general property of bacterial carboxypeptidases (unpublished data). PBP1, PBP2, and PBP3, on the other hand, were sensitive to all β-lactam antibiotics examined, with the notable exception of mecillinam (12). Antibacterial activity appeared to correlate with binding to PBP1 and PBP3.

TABLE 1. *Binding of β-lactam antibiotics to S. aureus PBPs*

Compound	μg/ml to completely inhibit penicillin G binding				MIC (μg/ml)[a]
	PBP1 (87K)	PBP2 (80K)	PBP3 (75K)	PBP5 (41K)	
Cefaclor	0.1	>100	0.1	>100	0.2
Cefamandole	0.1	0.5	0.5	100	0.1
Cefatrizine	0.5	10	0.1	>100	0.8
Cefotaxime	0.5	0.5	10	≥100	1.6
Cefoxitin	10	2.0	10	0.5	0.8
Cefsulodin	2.0	10	30	>100	6
Cefuroxime	0.5	0.1	10	>100	1.6
Cephaloridine	0.1	0.1	0.1	100	<0.05
Cephalothin	0.5	0.5	0.5	100	0.1
Cephradine	0.5	>100	0.1	>100	0.8
Mecillinam	100	>100	2.0	>100	12.5
Mezlocillin	0.1	0.1	0.1	≥100	0.4
Penicillin G	0.1	0.1	0.1	≥100	<0.05
Piperazillin	0.1	0.1	0.1	>100	0.4
Thienamycin	2.0	≥100	10	10	2.4

[a] MIC, Minimum inhibitory concentration; 10^4 colony-forming units of *S. aureus*.

TABLE 2. *Binding of β-lactam antibiotics to S. faecalis PBPs*[a]

Compound	μg/ml to completely inhibit penicillin G binding					MIC (μg/ml)[b]
	PBP1 (105K)	PBP2 (86K)	PBP3 (79K)	PBP4 (74K)	PBP5 (42K)	
Cefaclor	30	0.1	≥100	≥100	>100	25
Cefamandole	100	0.5	≥100	≥100	10	25
Cefatrizine	100	0.5	≥100	100	>100	25
Cefotaxime	>100	0.1	>100	>100	100	50
Cefoxitin	≥100	0.1	>100	100	0.1	>100
Cefsulodin	>100	10	>100	>100	2.0	>100
Cefuroxime	>100	0.5	>100	>100	≥100	50
Cephaloridine	2.0	0.1	100	100	0.5	6.3
Cephalothin	100	0.1	100	≥100	100	25
Cephradine	100	0.5	100	100	100	50
Mecillinam	>100	100	>100	>100	100	>100
Mezlocillin	2.0	0.5	2.0	10	10	0.4
Penicillin G	2.0	0.5	10	100	0.1	1.0
Piperacillin	10	0.5	10	10	30	0.8
Thienamycin	≥100	100	≥100	>100	0.5	>50

[a] PBP6 (35K), present in some membrane preparations, had a β-lactam sensitivity identical to that of PBP5 (42K) and might therefore be a degradation product of PBP5.

[b] MIC, Minimum inhibitory concentration; 10^4 colony-forming units of *S. faecalis*.

PBP2, which was ruled out as an essential PBP on the basis of the cefuroxime results, is probably more than one component, since binding of β-lactam antibiotics is often biphasic (Fig. 1).

In *S. faecalis* (Table 2), PBP4 appeared to be the least sensitive to β-lactam antibiotics. PBP5 (DD-carboxypeptidase [2]) was sensitive to some β-lactam antibiotics, but its sensitivity could not always be correlated with antibacterial activity. For example, it binds to cefoxitin at antibiotic concentrations of three orders of magnitude less than the minimum inhibitory concentration (0.1 versus >100 μg/ml). Thus, the recent suggestion, based on PBP binding profiles of a number of structurally related penicillins, that PBP5 is a killing site (2) is probably incorrect. PBP2 was sensitive to all β-lactam antibiotics examined, with the notable exception of mecillinam. PBP1 was sensitive to cephaloridine, penicillin G, piperacillin, and mezlocillin, with complete binding occurring at antibiotic concentrations of less than 10 μg/ml. All four compounds also had good antibacterial activities (minimum inhibitory concentrations equal to or less than 6 μg/ml). Since the three penicillins also bound to PBP3 and had minimum inhibitory concentrations lower than that of cephaloridine, which did not bind to PBP3, this component might also be an essential PBP in *S. faecalis*.

Among *E. coli* PBPs, PBP1 (molecular weight, 91,000 [91K]) and PBP3 (66K) are involved, respectively, in peptidoglycan cross-link-

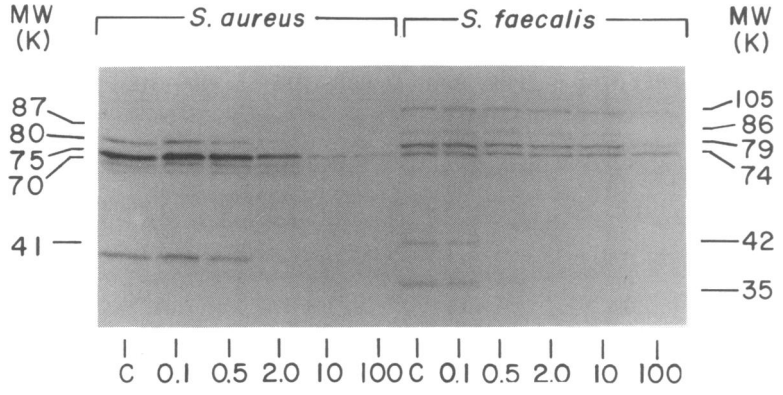

FIG. 1. *Fluorogram of a sodium dodecyl sulfate slab gel of solubilized membranes from S. aureus and S. faecalis after a 10-min incubation with thienamycin (concentrations, 0.1, 0.5, 2.0, 10, and 100 μg/ml) followed by [^{14}C]penicillin G binding. C, Untreated control ([^{14}C]penicillin G binding only); MW, molecular weight.*

ing and septum formation, whereas PBP2 (gram negative specific [12]) is involved in cell shape (11). It is tempting to speculate that PBP1 and PBP3 have universal functions and occurrence and that it is not a mere coincidence that *S. aureus* and *S. faecalis* also have two essential PBPs.

In conclusion, *S. aureus* and *S. faecalis* both appear to have two essential PBPs, with molecular weights of 87K and 75K (*S. aureus*) and of 105K and 79K (*S. faecalis*). These essential PBPs are more sensitive to β-lactam antibiotics in *S. aureus* than in *S. faecalis*, and the differential susceptibility of the two organisms to these antibiotics is most likely a reflection of differential PBP affinity.

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