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NOTES

Effect of β-Lactam Antibiotics on In Vitro Peptidoglycan Cross-Linking by a Particulate Fraction from *Escherichia coli* K-12 and *Bacillus megaterium* KM

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The binding constants of several β-lactam antibiotics towards penicillin-binding components in *Escherichia coli* K-12 (Spratt, Eur. J. Biochem. **72**:341-352, 1977) and the antibiotic concentrations required to inhibit the peptidoglycan transpeptidase of *E. coli* 50% were compared. Penicillin-binding component 1B may have been the transpeptidase working in vitro. The structure-activity relationships of β-lactam antibiotics and the mechanisms of action in *E. coli* and *Bacillus megaterium* are discussed.

Since the demonstration of peptidoglycan cross-linking by the transpeptidase in *Escherichia coli* strains Y-10 (5) and B (1), the peptidoglycan transpeptidase has been believed to be the primary target of β-lactam antibiotics. It is inhibited at low concentrations of the antibiotics, and the inhibition is irreversible (5, 8). Most studies on the effect of β-lactams were done with UDP-*N*-acetylmuramyl-L-alanyl-D-glutamyl-*meso*-2,2'-diaminopimelic acid-D-[^{14}C]alanyl-D-[^{14}C]alanine as substrate. This method does not necessarily give the correct degree of cross-linking (8). We have determined the efficacies of various β-lactam antibiotics with our improved method (8). The determinations will provide some information about the mechanisms of action of the antibiotics in *E. coli* and also help to assign the transpeptidase to one of the penicillin-binding components (PBCs) (2, 11) in it.

E. coli KN126 (12) was obtained from T. Nagata (Kyoto University, Kyoto, Japan) and grown in 2-liter Erlenmeyer flasks at 30°C in a medium consisting of 2% dehydrated nutrient broth (Kyokuto, Tokyo, Japan) and 0.5% yeast extract (pH 7). Cells were harvested, washed, and stored as described (8). Preparation of the particulate fraction from frozen cells was done as described previously (8). The standard reaction mixture contained 0.125 M tris (hydroxymethyl) aminomethane-hydrochloride (pH 8.5), 0.0125 M $MgCl_2$, 2.8×10^{-5} M [^{14}C]UDP *N*-acetylglucosamine (150 μCi/μmol), 1.5×10^{-4} M UDP-*N*-acetylmuramyl-pentapeptide (prepared as described [8]), 16.7% (vol/vol) glycerol, and particulate fractions (ca. 170 μg as protein) in 30 μl. The mixture was incubated at 25°C for 30 min. The assays of polymerization and cross-linking were done as described (8). The degree of cross-linking is expressed as the percentage of cross-linked disaccharide peptide(s) (8). All β-lactam antibiotics used were commercially available except cephalosporin G, which was kindly prepared by A. Sato of these laboratories according to the method reported previously (3). UDP-*N*-acetyl-D-[*U*-^{14}C]glucosamine (300 mCi/mmol) was purchased from Radiochemical Centre (Amersham, England).

All the penicillins tested (Table 1) inhibited the transpeptidase in *E. coli* KN126 at reasonably low concentrations, which would explain their minimum inhibitory concentrations. Meanwhile, cephalosporin derivatives varied considerably in this respect. Though cephaloridine was a good inhibitor of the enzyme, cephalexin and cephradine were such poor inhibitors of the transpeptidase that they cannot be believed to exert their physiological effects through inhibition of the enzyme. However, these cephalosporin derivatives were potent inhibitors of *Bacillus megaterium* transpeptidase.

The introduction of an amino group to the benzylic carbons of penicillin G and cephalosporin G reduced their abilities to inhibit the transpeptidase. Also, the removal of an acetyl or acetoxy group from the acetoxymethyl group at position 3 of the cephalosporins reduced the inhibitory activity against the transpeptidase (unpublished data). Thus, the resultant compounds cephaloglycin, cephalexin, and cephra-

TABLE 1. *Effect of β-lactam antibiotics on in vitro cross-linking in E. coli KN126 and B. megaterium KM.*

β-Lactam	Concn required to inhibit cross-linking 50% (μg/ml)	
	E. coli	*B. megaterium*
Penicillin G	1	<0.17
Penicillin V	4.6	—[a]
Ampicillin	7.5	0.2
Cephalosporin G	60	0.33
Cephaloglycin	167	0.5
Cephalexin	500	1
Cephradine	1,000	2.5
Cephaloridine	13.5	0.16
Cephalothin	23.5	0.25
Cefamandole	8.0	0.30
Cefuroxime	3.0	0.15
Cefoxitin	1.5	—

[a] —, Not determined.

dine, in that order, decreased in inhibitory activity. This was also true for the penicillin G-ampicillin relationship. The introduction of an amino group, however, does not impair the affinity toward PBC-3 and even may increase it (12). Thus, the most widely used oral cephalosporin, cephalexin, remains a potent antimicrobial agent against *E. coli*, although its inhibitory activity against the major in vitro peptidoglycan transpeptidase of *E. coli* is substantially reduced. The wide range of modifications on β-lactams, including those discussed above, did not significantly affect inhibitory activity against *B. megaterium* transpeptidase. Perhaps the specificity of the *B. megaterium* transpeptidase is broad, whereas that of the *E. coli* transpeptidase is narrow, and the antibiotic's loss in activity against the transpeptidase has to be compensated with an affinity toward other targets; or perhaps the affinity toward a second possible target in *B. megaterium* is quite low, causing the modifications tested so far to be unsuccessful in inhibiting primarily the second function instead of that of the transpeptidase.

Recently, Spratt has reported binding constants of various β-lactam antibiotics for PBCs in *E. coli* KN126 (12; B. G. Spratt, Second Tokyo Symposium on Microbial Drug-Resistance, 1977). By comparing concentrations of these β-lactams required to inhibit cross-linking by 50% with the binding constants reported, it may be possible to assign the major in vitro transpeptidase to one of the PBCs. Among six PBCs reported, PBCs 1A, 4, and 5/6 have been judged not essential for growth (4, 6, 13, 14). Since PBC 2 is not the major transpeptidase in vitro (9, 10), we can limit our discussion to PBCs 1B and 3. Unfortunately, complete comparison is impossible due to the lack of some necessary data. However, an assignment can be made mainly based on the fact that cephalexin and cephradine did not inhibit the transpeptidase significantly even at 167 μg/ml. Corresponding to this fact, both have unusually large binding constants toward PBC 1B. The binding constant for cephradine is not reported but can be estimated from the curve for PBC 1 (12). PBC 1A cannot be the major in vitro transpeptidase because of its high affinity toward cephalosporins, including cephalexin. That the transpeptidase working in vitro is PBC 1B is compatible with the previous notion of the protein. PBC 1 is thought to be the site at which β-lactams bind to inhibit cell elongation and cause lysis (10). The inhibition of the transpeptidase would reduce the degree of cross-linking in the cell wall, and the resultant less cross-linked cell wall would become more susceptible to lysis by cellular lytic enzymes. As for the inhibition of elongation, we could observe the inhibition of polymerization in the concentration range in which sharp inhibition of cross-linking was also observed (Fig. 1). The same phenomenon has been reported in cell-free systems of *Micrococcus luteus* (7) and *B. megaterium* (8). Mirelman et al.

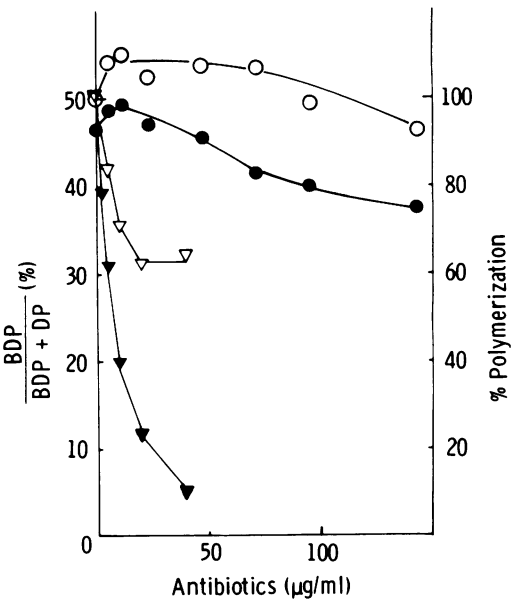

FIG. 1. *Effects of ampicillin and cephalexin on the in vitro incorporation of UDP-N-acetylglucosamine into peptidoglycan and cross-linking by a particulate fraction from E. coli KN126. Symbols: circles, cephalexin; triangles, ampicillin; open, incorporation (expressed as percent polymerization); closed, cross-linking (expressed as percent bisdisaccharidepeptide[s] [BDP]). The incorporation in the control tube (no addition) was 9,939 cpm.*

(7) assumed that the elongation of the peptidoglycan strand occurs by both transpeptidation and transglycosylation. Of course, this transpeptidation may be catalyzed by other transpeptidase(s) than PBC 1B, and it is quite tempting to suppose that this is done by PBC 3. But this seems not to be the case, since cephalexin, which has a high affinity toward PBC 3, did not inhibit polymerization until it inhibited the formation of cross-linking at higher concentrations (Fig. 1). That PBC 1B is one of the essential enzymes involved in cross-linking was proposed by Tamaki et al. (15) on a different basis.

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