ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1980, p. 629-637
0066-4804/80/10-0629/09$02.00/0

Vol. 18, No. 4

In Vivo Interaction of β-Lactam Antibiotics with the Penicillin-Binding Proteins of *Streptococcus pneumoniae*

RUSSELL WILLIAMSON, REGINE HAKENBECK,† AND ALEXANDER TOMASZ*

The Rockefeller University, New York, New York 10021

The interactions of several β-lactam antibiotics with the penicillin-binding proteins (PBPs) of *Streptococcus pneumoniae* have been studied using whole organisms treated with such antibiotics and subsequently with [^{3}H]benzylpenicillin. Differences in chemical structure were shown to cause major and selective changes in the affinities of the β-lactams for the PBPs. Only 4 of the 18 compounds tested induced a specific morphological effect (enlargement of the equatorial region) under the particular conditions tested. In 12 of the 18 β-lactams studied, a close correlation was found between the minimal inhibitory concentrations and the concentrations required to half-saturate PBP2b. However, such a correlation was no longer apparent when the bacteria were treated with the antibiotics at their minimal inhibitory concentrations. These findings are discussed in the context of various approaches that have been used to identify the growth-inhibitory targets of β-lactam antibiotics in bacteria.

The interactions of β-lactam antibiotics with the penicillin-binding proteins (PBPs) of several bacteria lead to a variety of biological consequences. Thus, at suitable concentrations, benzylpenicillin or cephaloridine, mecillinam, and cephalexin or piperacillin cause cell death (22), growth as round forms (24), or growth as filaments (6, 22), respectively, when added to *Escherichia coli*. Moreover, similar effects have been described with a variety of *Enterobacteriaceae* (3) and *Pseudomonas aeruginosa* (3, 13). Each of these antibiotics has been shown to specifically interact with one (or more) of the multiple PBPs of these organisms when tested using isolated membranes from the bacteria. Therefore these specific PBPs are presumably involved in processes or functions essential to the normal growth of the organisms.

In many of these and similar studies, the correlation between the observed effect and interaction with a PBP has been measured in terms of the amount of the antibiotic required to produce the effect and that required to half-saturate the particular PBP, although the exact relevance of such values may obviously depend on the accessibility of the PBPs in whole bacteria. We have therefore studied the interaction of several β-lactam antibiotics with exponential-phase *Streptococcus pneumoniae*. The correlations between the minimal growth inhibitory concentrations (MICs) of each compound and the morphogenic effect induced by four of these antibiotics are discussed. A similar approach has recently been described for several organisms by Chase and Reynolds (1).

† Present address: Max-Planck-Instut für Molekulare Genetik, D-1000 Berlin 33, Germany.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. pneumoniae* strains R6, a derivative of the Rockefeller University strain R36A, and CW1, an autolytic deficient transformant of the wild type (27), were used throughout these studies. Bacteria were grown without aeration at 37°C in C medium (9) at an initial pH of 8.0 and supplemented with yeast extract (0.1%; Difco). Growth was monitored with a Coleman nephelometer.

Susceptibility tests. The MICs of the various β-lactam antibiotics were determined by twofold serial dilution in C medium. Exponential-phase organisms (approximately 6×10^4 colony-forming units per ml) were inoculated into tubes containing the medium (1 ml) and incubated for 16 h at 37°C. The lowest concentration to inhibit growth of the bacteria was recorded as the MIC.

Morphology studies. Exponential-phase organisms (1 ml, about 6×10^4 colony-forming units per ml) of strain CW1 were incubated at 37°C with various concentrations (from 100 times less than the MIC up to the MIC) of the different β-lactam antibiotics. The morphologies of the bacteria were examined by phase-contrast microscopy after 4 and 20 h of incubation. The autolytic deficient mutant was used because the wild type autolysed in stationary phase.

Antibiotics. Methicillin was obtained from Beecham Laboratories, Piscataway, N.J.; ampicillin, cefadroxil (BL-S578), cephalexin, and oxacillin were obtained from Bristol Laboratories, Syracuse, N.Y.; and cefotaxime (HR756) was obtained from Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J. Mecillinam was supplied by Hoffmann-La Roche Inc., Nutley, N.J.; piperacillin came from Lederle Laboratories Div., American Cyanamid Co., Pearl River, N.Y.; ben-

zylpenicillin, cephaloridine, and cephalothin were provided by Eli Lilly & Co., Indianapolis, Ind.; and cefoxitin was supplied by Merck, Sharp & Dohme, Rahway, N.J. Cefsulodin (SCE-129) and sulbenicillin were obtained from Takeda Chemical Industries Ltd., Osaka, Japan, and dicloxacillin, nafcillin and 6-aminopenicillanic acid (6-APA) came from Wyeth Laboratories Inc., Philadelphia, Pa. *para*-[^{3}H] benzylpenicillin, ethylpiperidinium salt (31 Ci/mmol) was the generous gift of E. O. Stapley, Merck, Sharp & Dohme, Rahway, N.J.

Labeling, separation, and detection of PBPs. Exponential-phase organisms (1 ml, about 8×10^7 cells per ml) were incubated with various amounts of the β-lactam antibiotics (sub- and supra-MIC values) at 37°C for 10 min, and then with an amount of [^{3}H]-benzylpenicillin sufficient to saturate the PBPs of untreated organisms (1 nmol) for a further 10 min (28). The control samples were preincubated with no antibiotic before exposure to the radiolabeled benzylpenicillin. An excess of unlabeled benzylpenicillin (1.7 mM) was then added, and the samples were immediately chilled in ice. The bacteria were recovered by centrifugation (1,100 × g for 2 min at 2°C), suspended in 50 μl of 50 mM sodium phosphate buffer (pH 7.0) containing 1.7 mM benzylpenicillin and 1% Sarkosyl NL-97, and incubated for 5 min at 37°C. This treatment resulted in complete lysis of the organisms and inactivation of the PBPs. The lysates were then prepared for slab gel polyacrylamide electrophoresis. The techniques used for discontinuous gel electrophoresis, staining, and detection of the PBPs by fluorography have been described earlier (28). Binding proteins were labeled PBP1 through 3, as introduced in previous communications (5, 28, 30).

RESULTS

The evaluation of 18 different β-lactams for their effectiveness as inhibitors of the pneumococcal PBPs was performed with an assay in which exponentially growing bacteria were first exposed to various β-lactams (at concentrations representing fractions or multiples of their corresponding MICs and subsequently to a saturating dose of [^{3}H]benzylpenicillin. The bacteria were then lysed, and the PBPs were separated by gel electrophoresis. Figure 1 illustrates the results of this type of assay. The fluorograms demonstrate binding of the [^{3}H]benzylpenicillin to the pneumococcal PBPs after pretreatment of the live cells with cefsulodin and cefoxitin. Figure 2 is a graphical representation of the same results. The relative affinities of the PBPs for the unlabeled benzylpenicillin (3 > 1a > 1b > 2a > 2b) were the same as those obtained with the [^{3}H]benzylpenicillin alone when tested in whole organisms, thereby suggesting that the procedure was not giving anomalous results. The effects of 10 penicillins and 8 cephalosporins on the subsequent binding of [^{3}H]benzylpenicillin were evaluated in a similar manner; the results are summarized in Table 1 along with the MICs for each antibiotic and the effects on the morphology of the organisms.

Only 4 of the 18 β-lactams caused a morphological change under the particular conditions tested (i.e., exposure of a *lyt* mutant to a maximum of 1 × the MIC). The single type of morphological change observed was swelling of the central zone of the bacteria (Fig. 3).

Several observations were apparent in the data summarized in Tables 1 and 2. (i) First, for each β-lactam tested, there appeared to be significant differences in the antibiotic concentrations needed to half-saturate one PBP or another. Different β-lactams differed in the degrees of this type of variation. For instance, the difference between the concentrations of benzylpenicillin needed to half-saturate the PBPs showing the lowest (PBP2b) versus the highest (PBP3) affinity for this drug was only about sixfold. In contrast, this difference was almost 100-fold in the case of cefadroxil (see 50% benzylpenicillin binding inhibition [I_{50}] values for PBP2b versus PBP3).

(ii) Another type of drug-to-drug variation was also clear in that β-lactams differed widely in the fractions or multiples of the MICs needed to half-saturate the various PBPs. In the case of penicillin, half-saturation of even the most penicillin-sensitive PBP (PBP3) required as much as 2× the MIC. However, as little as 0.04× the MIC of cefoxitin could half-saturate PBP1b. Particularly noteworthy was the case of cephalosporin C, a relatively poor inhibitor of pneumococcal growth (MIC, 114 nmol/ml). This β-lactam was able to half-saturate even the least sensitive PBPs (PBP2a and -2b) at 0.2× the MIC, whereas the PBP most sensitive to this β-lactam (PBP1b) reached half-saturation at a small (0.01×) fraction of the MIC.

(iii) The antibiotics also differed in their order of affinities to the various PBPs. PBP3 had the highest affinity for 9 of the 18 antibiotics (see values in bold face in Table 1), and, with the exception of benzylpenicillin, the other 9 of these could achieve half-saturation of this PBP at concentrations that were fractions of the corresponding MICs, e.g., 0.01×, 0.03×, or 0.07× the MIC in the cases of cefoxitin, cefadroxil, or cephalosporin C, respectively.

(iv) For a given PBP, β-lactams showed great variation in their specific inhibitory activities (compared on the basis of their molar concentrations). For instance, in the case of PBP3, of the 18 β-lactams compared, cephaloridine and cefotaxime had the highest I_{50} values, 0.0066 and 0.0086 nmol/ml, respectively. Mecillinam (I_{50}, 50 nmol/ml) and 6-APA (I_{50}, 160 nmol/ml) had the lowest specific inhibitory activities. Figure 4 shows the relationships between the MICs and

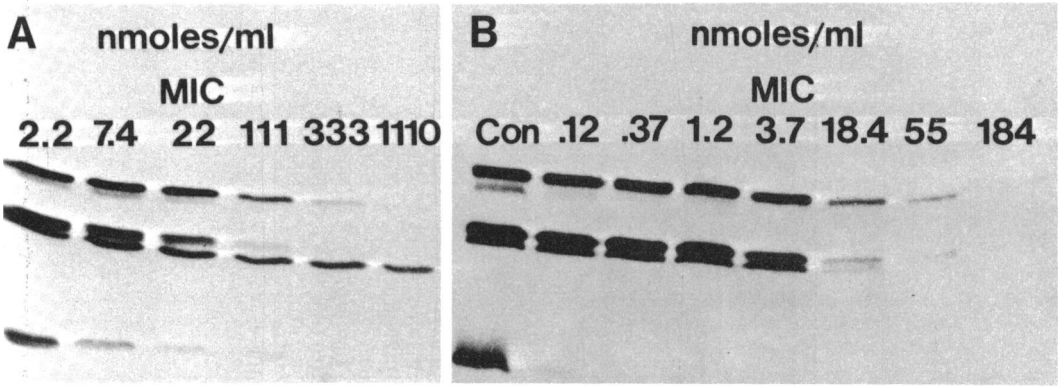

FIG. 1. *Competition of cefsulodin and cefoxitin for the PBPs of S. pneumoniae. The PBPs were detected with [^{3}H]benzylpenicillin after pretreatment of whole organisms with various concentrations of either (A) cefsulodin or (B) cefoxitin.*

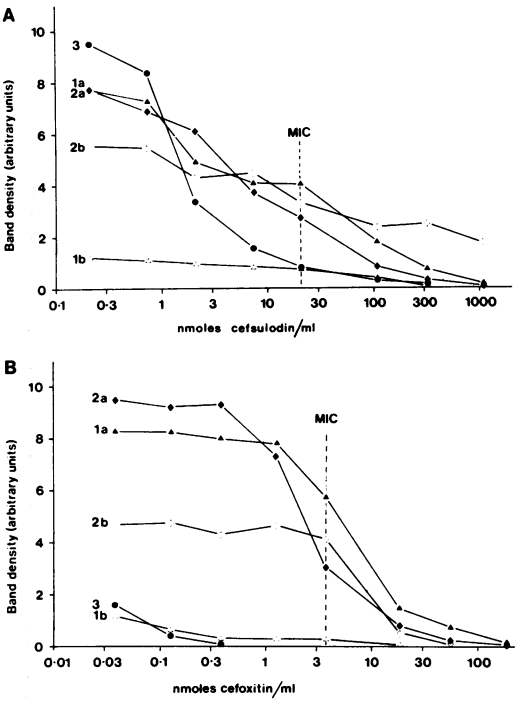

FIG. 2. *Saturation curves of PBPs after pretreatment with (A) cefsulodin or (B) cefoxitin. The relative densities of the PBP bands in Fig. 1 were measured by microdensitometry.*

the corresponding I_{50} values of each antibiotic listed in Table 1 for PBP2b and 3. Twelve of the 18 antibiotics had a surprisingly good correlation between these values in the case of PBP2b, but not for any of the other PBPs.

(v) Some of the β-lactam antibiotics used in this study were structurally similar, and an attempt was made to correlate chemical structure with the MICs and PBP affinity patterns.

Benzylpenicillin, ampicillin, and sulbenicillin have the same structure except for substitution on the benzylic carbon of the side chain. The MICs for ampicillin and sulbenicillin were some 4- and 51-fold greater than that of benzylpenicillin, but the affinities of ampicillin for the PBPs, particularly 1b and 2a, were generally greater, whereas those of sulbenicillin were much lower, although not in proportion to the increase in MIC (Table 1). The small differences in structure caused marked changes in the relative order of affinities of the PBPs (benzylpenicillin, 3 > 1a > 1b > 2a > 2b; ampicillin, 1b > 2a > 3 > 1a > 2b; sulbenicillin, 3 > 1b > 2b > 1a > 2a).

Dicloxacillin and oxacillin differ only in the disubstitution of the benzyl ring, which results in the former having a sixfold-lower MIC. The affinities of four of the PBPs (1a, 1b, 2a, and 2b) were also lower for oxacillin than dicloxacillin. However, the interaction with PBP3 was some sixfold greater with oxacillin than dicloxacillin; in contrast, that of PBP1a was reduced by a factor of five. These differences produced a major change in the relative order of affinities of dicloxacillin (1a > 2a > 1b > 2b > 3) and oxacillin (3 > 2a > 2b > 1b > 1a).

Cefoxitin and cephalothin are similar except for the substitution of a 7-α-methoxy group and an amino group on the Z side chain of cefoxitin, and this caused a sevenfold decrease in the MIC. There was also a dramatic decrease of some 425-fold in the affinity of PBP1a for cefoxitin, the affinities of PBPs 1b, 2a, and 2b were some tenfold less, and that of PBP3 was about twofold more. Thus, the greatest change in the relative affinities of the PBPs for cephalothin (1a, 1b > 3 > 2a > 2b) and cefoxitin (3 > 1b > 2a > 1a > 2b) was in the interaction with PBP1a.

Cefadroxil and cephalexin differ in the addition of a hydroxyl group at the 4-position of the

TABLE 1. *Effect of pretreatment with β-lactam antibiotics on the penicillin-binding proteins of S. pneumoniae*

β-Lactam antibiotic	MIC (nmol/ml)	I_{50}[a] (nmol/ml) for PBP:					Morphological response
		1a	1b	2a	2b	3	
Benzylpenicillin (1)	0.017	0.115 (6.8)	0.14 (8.2)	0.195 (11.5)	0.22 (12.9)	**0.034** (2.0)	None
Cefotaxime (2)	0.026	0.015 (0.6)	0.0072 (0.3)	**0.0052** (0.2)	0.056 (2.2)	0.0086 (0.3)	None
Piperacillin (3)	0.046	0.94 (20.4)	0.62 (13.5)	0.185 (4.0)	0.051 (1.1)	**0.042** (0.9)	Swollen (0.5)[b]
Cephaloridine (4)	0.057	0.05 (0.9)	0.018 (0.3)	0.042 (0.7)	1.45 (25.4)	**0.0066** (0.1)	Swollen (0.5)[b]
Nafcillin (5)	0.061	0.27 (4.4)	**0.061** (1.0)	0.19 (3.1)	0.42 (6.9)	0.32 (5.8)	Swollen (0.5)[b]
Ampicillin (6)	0.070	0.06 (0.9)	**0.0066** (0.1)	0.023 (0.3)	0.105 (1.5)	0.045 (0.6)	None
Dicloxacillin (7)	0.102	**0.34** (3.3)	0.39 (3.8)	0.36 (3.5)	0.60 (5.9)	0.97 (9.5)	None
Methicillin (8)	0.48	9.2 (19.2)	5.2 (10.8)	**0.54** (1.1)	1.15 (2.4)	2.05 (4.3)	Swollen (0.5)[b]
Cephalothin (9)	0.49	**<0.016** (<0.03)	**<0.016** (<0.03)	0.19 (0.4)	0.86 (1.8)	0.08 (0.2)	None
Oxacillin (10)	0.63	1.75 (2.8)	1.6 (2.5)	0.42 (0.7)	1.45 (2.3)	**0.17** (0.3)	None
Sulbenicillin (11)	0.87	4.6 (5.3)	1.55 (1.8)	4.9 (5.6)	3.7 (4.3)	**0.66** (0.8)	None
Cefoxitin (12)	3.68	6.8 (1.9)	0.145 (0.04)	2.4 (0.7)	8.0 (2.2)	**<0.037** (<0.01)	None
Cefadroxil (13)	4.3	4.6 (1.1)	8.0 (1.9)	1.3 (0.3)	12.0 (2.8)	**<0.14** (<0.03)	None
Cephalexin (14)	8.75	7.2 (0.8)	4.5 (0.5)	2.2 (0.3)	28.0 (3.2)	**0.29** (0.03)	None
Mecillinam (15)	8.8	1,320.0 (150.0)	185.0 (21.0)	**30.0** (3.4)	155.0 (17.6)	50.0 (5.7)	None
Cefsulodin (16)	22.2	23.0 (1.0)	37.0 (1.7)	6.7 (0.3)	52.0 (2.3)	**1.55** (0.07)	None
6-APA (17)	105.0	2,900.0 (27.6)	480.0 (4.6)	**30.0** (0.3)	370.0 (3.5)	160.0 (1.5)	None
Cephalosporin C (18)	114.4	13.0 (0.1)	**<1.14** (<0.01)	20.0 (0.2)	19.0 (0.2)	8.5 (0.07)	None

[a] Concentration of antibiotic required to inhibit the subsequent binding of [^{3}H]benzylpenicillin by 50%. Values in parentheses indicate multiples of the MIC.
[b] Values in parentheses indicate the lowest multiple of the MIC that resulted in the morphological effect.

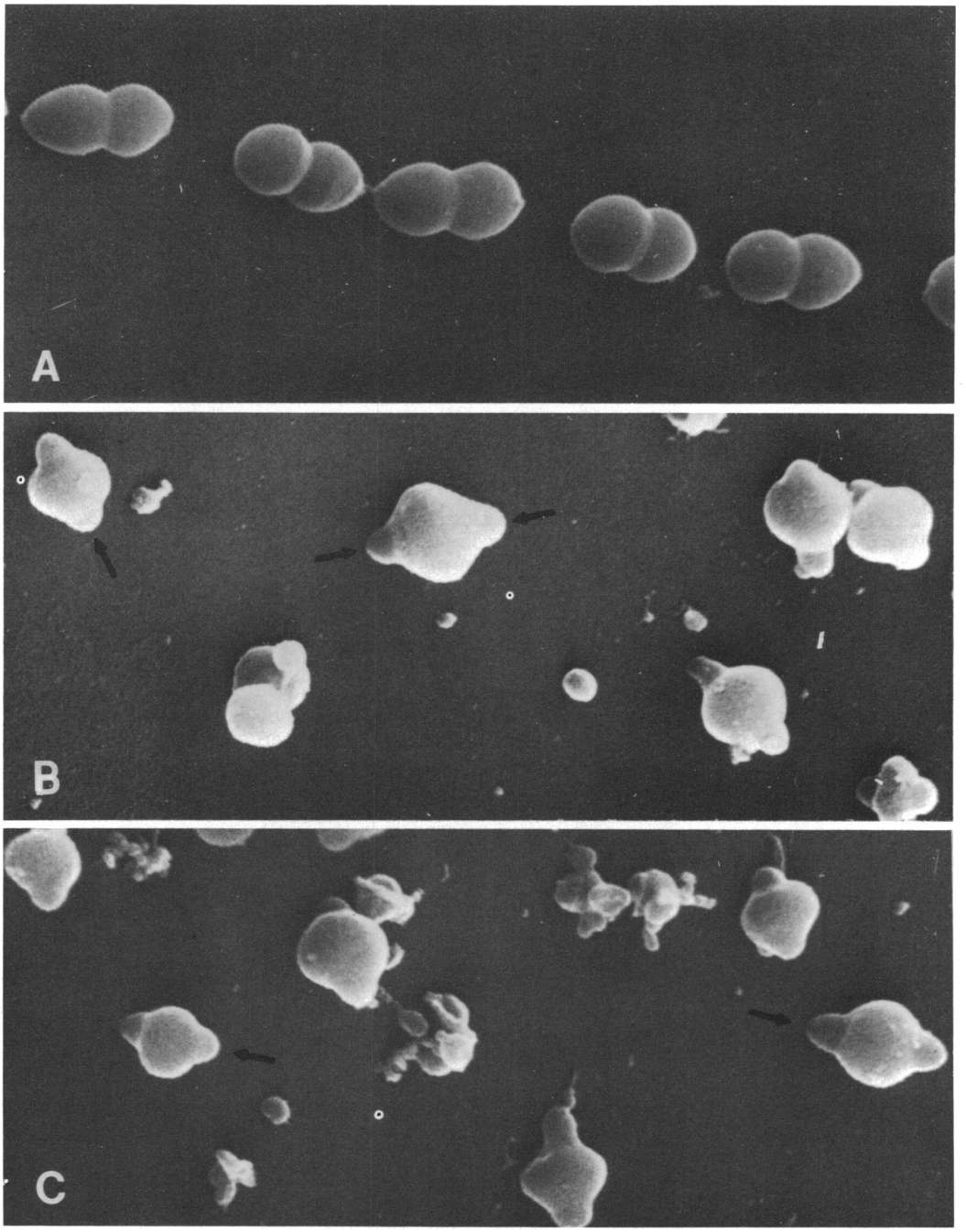

FIG. 3. *Scanning electron micrographs of S. pneumoniae. (A) Control organisms; (B and C) organisms grown in the presence of half the MIC of piperacillin for 4 h. Magnification, ×30,000. The arrows indicate cell poles.*

benzyl ring in the former. However, this appeared to have little effect on either the MIC or affinities of these antibiotics. All the values for cefadroxil were some twofold less than those of cephalexin, indicating increased affinity, in line with a decreased MIC. The exception was PBP1b, which had a twofold decrease in affinity for cefadroxil. Thus, the relative affinities of

TABLE 2. *Inhibition of binding of [^{3}H]benzylpenicillin to the PBPs of S. pneumoniae by β-lactam antibiotics at their MICs*

β-Lactam antibiotic	% Inhibition[a] of binding to PBP:				
	1a	1b	2a	2b	3
Benzylpenicillin	27	25	23	12	38
Cefotaxime	58	91	90	40	83
Piperacillin	0	8	27	48	51
Cephaloridine	55	82	63	8	89
Nafcillin	9	59	20	41	17
Ampicillin	53	61	63	42	61
Dicloxacillin	12	18	18	11	17
Methicillin	28	39	49	37	29
Cephalothin	100	100	84	41	89
Oxacillin	24	15	56	20	82
Sulbenicillin	26	37	26	35	61
Cefoxitin	31	79	68	11	100
Cefadroxil	48	32	77	31	89
Cephalexin	52	59	73	30	86
Mecillinam	11	5	31	9	25
Cefsulodin	48	35	65	40	92
6-APA	20	19	59	13	41
Cephalosporin C	98	100	90	93	90

[a] Percentage of decrease in the amount of [^{3}H]benzylpenicillin bound after pretreatment with the particular antibiotic compared with the amount bound in the absence of the antibiotic.

each compound were similar (cefadroxil, 3 > 2a > 1a > 1b > 2b; cephalexin, 3 > 2a > 1b > 1a > 2b).

Two of the antibiotics tested had extremely high MICs, 105 nmol/ml for 6-APA and 114.4 nmol/ml for cephalosporin C, yet their interactions with the PBPs were significantly different. Whereas 6-APA appeared to have relatively low affinities for most of the PBPs, cephalosporin C had extremely high affinities in terms of its MIC, although in molar proportions, even cephalosporin C had low affinity as compared with other β-lactam antibiotics with much lower MICs.

DISCUSSION

A major purpose of these studies was to establish a correlation between the biological responses to β-lactam antibiotics and the biochemical event of binding to PBPs in a gram-positive organism. Similar studies using membrane preparations have been reported for a variety of gram-negative bacteria (3, 13, 16, 20–22). One of the approaches used in such studies was based on the known selective morphological effect of certain β-lactams in *E. coli* and the analysis of the biochemical basis of this selectivity on the interaction with the PBPs. Thus, the selective effect of mecillinam was correlated with binding to PBP2 (24), and the less selective effects of cephalexin and piperacillin were correlated with binding to PBP3 (6, 22).

In pneumococci, only one morphological response has been observed (enlargement of the equatorial region). Such a change was caused by only 4 of the 18 antibiotics tested under the conditions used, but there was no obvious correlation to inhibition of any particular PBP. For instance, for four of the PBPs (1b, 2a, 2b, and 3) to which the morphogenic antibiotics had strong affinities, there were examples of other antibiotics (e.g., ampicillin) that bound to each of these PBPs at similar or even lower concentrations without apparent effect.

Several attempts have been made to correlate the antibacterial effects of β-lactam antibiotics (inhibition of growth, killing, and lysis) with inhibition of one or more PBPs. Such studies have indicated PBP1b as the major function in *E. coli*, the inhibition of which leads to rapid killing and lysis (22, 23) and induction of uncontrolled murein hydrolase activity (8). Genetic studies with mutants of *E. coli* with a thermosensitive PBP1b and defective in PBP1a supported this notion (26). Additional studies have implicated PBP3 of *E. coli* also as an important antibacterial target (22, 23). Investigations with mutants of *E. coli* defective in PBPs 4, 5, and 6 (7, 10, 11, 25), which retained the same sensitivity to β-lactam antibiotics as the parental strain, indicated that these PBPs did not represent physiologically important functions.

The binding proteins are presumably proteins involved in synthesis of peptidoglycan, and it has been suggested that PBPs 1b and 3 of *E. coli* may represent the penicillin-sensitive transpeptidases (22). However, several studies have failed to establish a consistent correlation between the antimicrobial effectiveness (MIC)

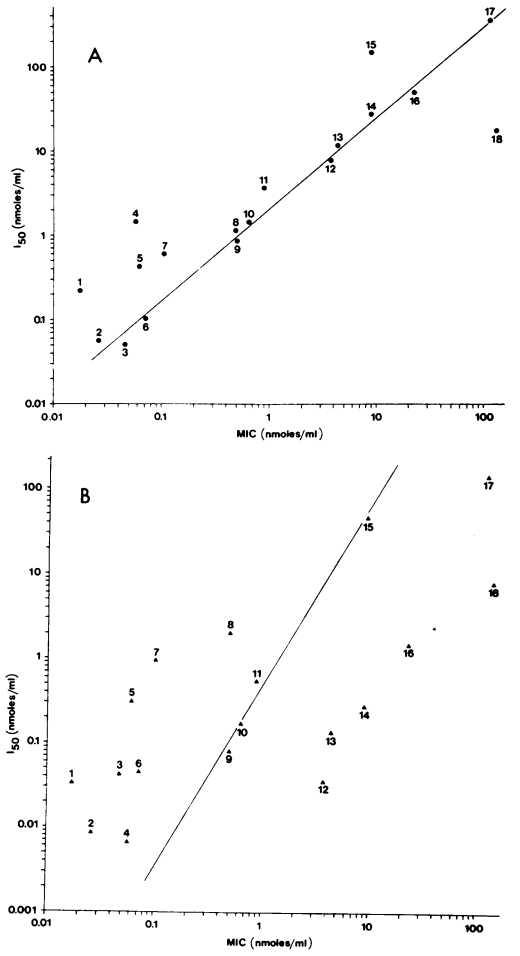

FIG. 4. *Correlation between MICs of the antibiotics tested and their affinities (I_{50} values) for (A) PBP2b and (B) PBP3. The data were obtained directly from Table 1, and the line for PBP3 was plotted after linear regression analysis.*

of a β-lactam antibiotic and the specific inhibitory activity of these compounds in assays of either model transpeptidase activity (14, 17, 18) or synthesis of peptidoglycan using whole organisms (12). Several authors have similarly attempted to establish a quantitative correlation between the MICs of β-lactam antibiotics and their affinity for the various PBPs of *E. coli*, assayed as the ability of each antibiotic to inhibit the subsequent binding of radioactive benzylpenicillin by 50% (I_{50} value) in isolated membranes (2, 4, 15, 23). Using four cephalosporins, Nozaki et al. (15) obtained a straight line plotting the logarithm of the MIC of each antibiotic tested against the logarithm of the respective I_{50} value for PBP1b and 3 of *E. coli*; similar relationships were not obtained from data for the other PBPs. However, such a relationship was not so evident in data from the later and more extensive studies of Curtis et al. (2, 4). In the present studies a similar evaluation of the affinities and the MICs the 18 antibiotics indicated that only one of the PBPs (2b) exhibited proportionality between these values for the majority of the β-lactams tested.

In an extension of the notion that the PBP with the highest in vitro (membrane) affinity for β-lactams would be the likely candidate for the physiologically important target (22), Reynolds et al. (19) studied the affinities of PBPs for benzylpenicillin in growing *Bacillus megaterium*. Only one (PBP1) of the five PBPs was labeled during exposure of the organisms to the MIC of the antibiotic for 5 min. On the basis of this concept, PBP3 of *S. pneumoniae* would fulfill this role, as 9 of the 18 antibiotics had their highest affinities for this PBP. However, the affinities in molar terms for this PBP and the corresponding MICs did not show the expected correlation (Fig. 4) since most of the β-lactams inhibited this PBP at sub-MIC values. It is also relevant that PBP 2b had the lowest affinity for 10 of the antibiotics, and the affinities for each antibiotic except cephalosporin C were all above the MIC values. PBP1a was also found to have relatively low affinity for most of the antibiotics, PBP2a was generally high, and PBP1b was intermediate. Furthermore, a completely different approach for the identification of physiologically important PBPs was against PBP3 being this target. This approach involved the identification of the changes in PBPs that accompanied acquisition of different levels of intrinsic penicillin resistance in genetic transformants of pneumococci (29, 30) and also in various clinical isolates of pneumococci (5). The analysis of the affinities for benzylpenicillin of the PBPs in the transformants of various resistance levels clearly implicated each PBP except PBP3 as physiologically important targets at specific levels of resistance. This is in clear contrast with the behavior of PBPs in the benzylpenicillin-susceptible bacteria, in which only the β-lactam affinity of PBP2b showed a quantitative relationship with the MIC of the particular antibiotic. Thus, these findings apparently contradict the notion that the most drug-sensitive PBP should be the antimicrobial target and are also inconsistent with the approach as applied in Fig. 4, correlating MIC and I_{50} values.

The examination of degrees of inhibition of the various PBPs in growing bacteria after exposure to the antibiotics at their MICs (Table 2) indicated that there was no obvious correlation to a given amount of inhibition. This approach was used to eliminate potential problems that

might have arisen from the interpretation of similar experiments performed in vitro with membrane preparations due to possible differences in accessibility to the PBPs. The great differences in MICs (6,700-fold) of the antibiotics tested are most likely to reflect differences in the affinities and inhibitory power of the compounds for the PBPs of pneumococci since preliminary experiments have indicated no apparent selective permeability barrier in this organism.

None of the varied approaches discussed above yielded a consistent, satisfactory interpretation of the quantitative relationship of the MIC and PBP pattern of a given β-lactam. One major factor may have contributed to the difficulty in establishing this correlation using studies of the type used here and in other bacterial systems. The problem is illustrated in Fig. 2, which demonstrates that the saturation curves of cefoxitin and cefsulodin for the individual PBPs have distinctly different shapes. Thus, although the plot of I_{50} against MIC of some antibiotics for PBP2b gave a good correlation, the more important relationship would seem to be the degree of saturation of PBP2b by these same antibiotics added to growing bacteria at their MICs. The data in Table 2 indicated that in fact under this condition, the different antibiotics with a good I_{50}-MIC correlation for PBP2b (Fig. 4) gave from 11 to 48% inhibition. However, nafcillin, which did not have a good I_{50}-MIC correlation (Fig. 4), gave 44% inhibition.

The data reported in these studies indicate that affinities of the β-lactam antibiotics for the pneumococcal PBPs are greatly modified by the chemical structure and site of modification of the substituents. Further examination of the interactions of other β-lactam analogs with the approach described in this report could yield an establishment of important structure-function relationships.

ACKNOWLEDGMENTS

These investigations have been supported by the National Institute of Health (Public Health Service grant AI-16170) and the National Science Foundation (grant PCM 7812770). Additional support has been provided by the Deutsche Forschungsgemeinschaft (to R.H.) and a Merck Research Fellowship (to R.W.). R.W. was also the recipient of a Travelling Fellowship from the Wellcome Trust.

We also thank D. Phillips of this University for the scanning electron micrographs.

LITERATURE CITED

1. **Chase, H. A., and P. E. Reynolds.** 1980. The interaction of β-lactam antibiotics with penicillin-binding proteins studied *in vivo*. Soc. Gen. Microbiol. Q. **7**:78.
2. **Curtis, N. A. C., C. Brown, M. Boxall, and M. G. Boulton.** 1979. Inhibition of *Escherichia coli* K-12 by β-lactam antibiotics with poor antibacterial activity: interaction of permeability and intrinsic activity against penicillin-binding proteins. Antimicrob. Agents Chemother. **15**:332–336.
3. **Curtis, N. A. C., D. Orr, G. W. Ross, and M. G. Boulton.** 1979. Competition of β-lactam antibiotics for the penicillin-binding proteins of *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella aerogenes*, *Proteus rettgeri*, and *Escherichia coli*: comparison with antibacterial activity and effects upon bacterial morphology. Antimicrob. Agents Chemother. **16**:325–328.
4. **Curtis, N. A. C., D. Orr, G. W. Ross, and M. G. Boulton.** 1979. Affinities of penicillins and cephalosporins for the penicillin-binding proteins of *Escherichia coli* K-12 and their antibacterial activity. Antimicrob. Agents Chemother. **16**:533–539.
5. **Hakenbeck, R., M. Tarpay, and A. Tomasz.** 1980. Multiple changes of penicillin-binding proteins in penicillin-resistant clinical isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **17**:364–371.
6. **Iida, K., S. Hirata, S. Nakamuta, and M. Koike.** 1978. Inhibition of cell division of *Escherichia coli* by a new synthetic penicillin, piperacillin. Antimicrob. Agents Chemother. **14**:257–266.
7. **Iwaya, M., and J. L. Strominger.** 1977. Simultaneous deletion of D-alanine carboxypeptidase 1B-C and penicillin-binding component IV in a mutant of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **74**:2980–2984.
8. **Kitano, K., and A. Tomasz.** 1979. Triggering of autolytic cell wall degradation in *Escherichia coli* by β-lactam antibiotics. Antimicrob. Agents Chemother. **16**:838–848.
9. **Lacks, S., and R. D. Hotchkiss.** 1960. A study of the genetic material determining an enzyme activity in Pneumococcus. Biochim. Biophys. Acta **39**:508–517.
10. **Matsuhashi, M., I. N. Maruyama, Y. Takagaki, S. Tamaki, Y. Nishimura, and Y. Hirota.** 1978. Isolation of a mutant of *Escherichia coli* lacking penicillin-sensitive D-alanine carboxypeptidase IA. Proc. Natl. Acad. Sci. U.S.A. **75**:2631–2635.
11. **Matsuhashi, M., Y. Takagaki, N. Maruyama, S. Tamaki, Y. Nishimura, H. Suzuki, U. Ogino, and Y. Hirota.** 1977. Mutants of *Escherichia coli* lacking in highly penicillin-sensitive D-alanine carboxypeptidase activity. Proc. Natl. Acad. Sci. U.S.A. **74**:2976–2979.
12. **Moore, B. A., S. Jevons, and K. W. Brammar.** 1979. Peptidoglycan transpeptidase inhibition in *Pseudomonas aeruginosa* and *Escherichia coli* by penicillins and cephalosporins. Antimicrob. Agents Chemother. **15**: 513–517.
13. **Noguchi, H., M. Matsuhashi, and S. Mitsuhashi.** 1979. Comparative studies of penicillin-binding proteins in *Pseudomonas aeruginosa* and *Escherichia coli*. Eur. J. Biochem. **100**:41–49.
14. **Noguchi, H., M. Matsuhashi, M. Takaoka, and S. Mitsuhashi.** 1978. New antipseudomonal penicillin, PC-904: affinity to penicillin-binding proteins and inhibition of the enzyme cross-linking peptidoglycan. Antimicrob. Agents Chemother. **14**:617–624.
15. **Nozaki, Y., A. Imada, and M. Yoneda.** 1979. SCE-963, a new potent cephalosporin with high affinity for penicillin-binding proteins 1 and 3 of *Escherichia coli*. Antimicrob. Agents Chemother. **15**:20–27.
16. **Ohya, S., M. Yamazaki, S. Sugawara, and M. Matsuhashi.** 1979. Penicillin-binding proteins in *Proteus* species. J. Bacteriol. **137**:474–479.
17. **Ohya, S., M. Yamazaki, S. Sugawara, S. Tamaki, and M. Matsuhashi.** 1978. New cephamycin antibiotic, CS-1170: binding affinity to penicillin-binding proteins and inhibition of peptidoglycan cross-linking reactions in *Escherichia coli*. Antimicrob. Agents Chemother. **14**: 780–785.
18. **Oka, T., and H. Fujita.** 1978. Effect of β-lactam antibiotics on in vivo peptidoglycan cross-linking by a particulate fraction from *Escherichia coli* K-12 and *Bacillus*

megaterium KM. Antimicrob. Agents Chemother. **14:** 625-627.

19. **Reynolds, P. E., S. T. Shepherd, and H. A. Chase.** 1978. Identification of the binding protein which may be the target of penicillin action in *Bacillus megaterium*. Nature (London) **271:**568-570.
20. **Rodriguez, W. J., and A. K. Saz.** 1978. Differential binding of penicillin by membrane fractions from penicillin-susceptible and -resistant gonococci. Antimicrob. Agents Chemother. **13:**589-597.
21. **Shepherd, S. T., H. A. Chase, and P. E. Reynolds.** 1977. The separation and properties of two penicillin-binding proteins from *Salmonella typhimurium*. Eur. J. Biochem. **78:**521-532.
22. **Spratt, B. G.** 1975. Distinct penicillin-binding proteins involved in the division, elongation, and shape of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72:**2999-3003.
23. **Spratt, B. G.** 1977. Properties of the penicillin-binding proteins of *Escherichia coli*. Eur. J. Biochem. **72:**341-352.
24. **Spratt, B. G., and A. Pardee.** 1975. Penicillin-binding proteins and cell shape in *Escherichia coli*. Nature (London) **254:**516-517.
25. **Suzuki, H., Y. Nishimura, and Y. Hirota.** 1978. On the process of cell division in *Escherichia coli*: a series of mutants of *Escherichia coli* altered in the penicillin-binding proteins. Proc. Natl. Acad. Sci. U.S.A. **75:**664-668.
26. **Tamaki, S., S. Nakajima, and M. Matsuhashi.** 1977. Thermosensitive mutation in *Escherichia coli* simultaneously causing defects in penicillin-binding proteins 1B's and in enzyme activity for peptidoglycan synthesis *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **74:**5472-5476.
27. **Tomasz, A., and S. Waks.** 1975. Mechanism of action of penicillin: triggering of the pneumococcal autolytic enzyme by inhibitors of cell wall synthesis. Proc. Natl. Acad. Sci. U.S.A. **72:**4162-4166.
28. **Williamson, R., R. Hakenbeck, and A. Tomasz.** 1980. The penicillin-binding proteins of *Streptococcus pneumoniae* grown under lysis-permissive and lysis-protective (tolerant) conditions. FEMS Microbiol. Lett. **7:** 127-131.
29. **Zighelboim, S., and A. Tomasz.** 1979. Stepwise acquisition of resistance to oxacillin in *Streptococcus pneumoniae*, p. 290-292. *In* D. Schlessinger (ed.), Microbiology—1979. American Society for Microbiology, Washington, D.C.
30. **Zighelboim, S., and A. Tomasz.** 1980. Penicillin-binding proteins of multiply antibiotic-resistant South African strains of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **17:**434-442.