Binding of Monobactams to Penicillin-Binding Proteins of Escherichia coli and Staphylococcus aureus: Relation to Antibacterial Activity

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Received 25 June 1982/Accepted 15 October 1982

A series of novel monocyclic \( \beta \)-lactam antibiotics having side chains related to penicillin, piperacillin, azlocillin, and cefotaxime were examined with respect to binding to essential penicillin-binding proteins (PBPs) in Escherichia coli and Staphylococcus aureus. In the penicillin series, there was poor binding to all essential PBPs of E. coli (>100 \( \mu \)g/ml) but good binding to PBPs 1, 2, and 3 of S. aureus (~1 \( \mu \)g/ml). In the piperacillin and azlocillin series, there was good binding to PBP 3 of E. coli (0.1 \( \mu \)g/ml) and PBPs 1, 2, and 3 of S. aureus (~1 \( \mu \)g/ml). In the cefotaxime series, there was generally good binding to PBP 3 of E. coli (0.1 \( \mu \)g/ml) but poor binding to PBPs 1, 2, and 3 of S. aureus (~100 \( \mu \)g/ml). With a few exceptions in the cefotaxime series, antibacterial activity paralleled essential PBP binding. Binding studies with radioactively labeled compounds revealed no additional essential monobactam-binding proteins in the two organisms. The studies suggest that monobactams are intrinsically active against both gram-positive and gram-negative bacteria; the activity spectrum of a given monobactam is determined by the binding to essential PBPs, which in turn is determined by the nature of the substituents on the \( \beta \)-lactam nucleus.

Monobactams are monocyclic \( \beta \)-lactam antibiotics characterized by the 2-oxazetidine-1-sulfonic acid moiety (Table 1) recently isolated from gram-negative bacteria (14, 26). The naturally occurring compounds have generally weak antibacterial activity, but synthetic derivatives are potent antibiotics (3), with stability to \( \beta \)-lactamases equal to or better than that of third-generation cephalosporins (6). A synthetic monobactam, aztreonam, is currently being developed for clinical use (25).

Penicillins and cephalosporins are believed to kill bacteria by binding covalently to specific membrane proteins involved in peptidoglycan biosynthesis (1, 23). These penicillin-binding proteins (PBPs) have been extensively studied in Escherichia coli, for which essential PBPs have been identified and their functions elucidated (22, 24). E. coli PBPs appear to be representative of enterobacteria and pseudomonads. Other bacteria have different PBP patterns (amounts, molecular weights, and \( \beta \)-lactam-binding profiles) and different essential PBPs. In Staphylococcus aureus, for example, four PBPs have been detected, PBPs 2 (molecular weight, 80,000 [80K]), 3 (75K), and possibly 1 (87K) being essential (9, 13).

The present study was undertaken to assess the effect of side chain variation in the monobactams on intrinsic activity. Binding to PBPs of E. coli and S. aureus was examined, as was inhibition of E. coli peptidoglycan transpeptidase and Streptomyces sp. strain R61 DD-carboxypeptidase. Intrinsic activity of monobactams was subsequently compared to that of penicillins and cephalosporins with similar side chains. Direct binding of two radioactively labeled monobactams to proteins of E. coli and S. aureus was also examined.

MATERIALS AND METHODS

Materials. Culture media were obtained from Difco Laboratories, Detroit, Mich.; \(^{125}\)I-labeled Bolton-Hunter reagent (2 Ci/\( \mu \)mol) was obtained from New England Nuclear Corp., Boston, Mass.; phenyl\( (1-^{14}\)C)acetic acid (50 \( \mu \)Ci/\( \mu \)mol), [methyl\( -^{14}\)C]iodide (58 \( \mu \)Ci/\( \mu \)mol), [\( 8-^{14}\)C]penicillin G (51 \( \mu \)Ci/\( \mu \)mol), and UDP-N-acetyl-b-D(\( L-^{14}\)C)glucosamine (213 \( \mu \)Ci/\( \mu \)mol) were obtained from Amersham Corp., Arlington Heights, Ill.; trichloroacetic acid, glycine, bromophenol blue, and 2,5-diphenyloxazole were obtained from Fisher Scientific Co., Pittsburgh, Pa.; Bacillus cereus penicillinase was obtained from Calbiochem, La Jolla, Calif.; DNase, Tris base, and Triton X-100 were obtained from Sigma Chemical Co., St. Louis, Mo.; reagents for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis were obtained from Bio-Rad Laboratories, Richmond, Calif.; and X-ray film XR-5 was obtained from Eastman-Kodak, Rochester, N.Y. UDP-N-acetylmuramyl pentapeptide was
isolated from vancomycin-treated B. cereus as previously described (12). [14C]diacetyl-l-Lys-D-Ala-D-Ala and [14C]SO 26,324 were synthesized as previously described (11). [123I]ampicillin derivative was synthesized according to a published procedure (21). [14C]SO 81,377 was synthesized as follows. An excess of syn-
ethyl-α-(methoxyimino)-2-[[(triphenylmethyl)amino]-thiazol-4-ylacetate was alkylated (4) with approximately 10 mCi of [methyl-14C]iodide (K2CO3; dimethylforma-
ide) and subsequently saponified to give 52 mg of syn-
α-(methoxyimino)-2-[(triphenylmethyl)amino]-thiazol-4-
ylacetic acid. Coupling with (S)-3-amino-2-oxoazetidine-
1-sulfonic acid, tetrabutylammonium salt (18) gave 59
mg (85%) of potassium (S)-3-α-(methoxyimino)-2-[(tri-
phenylmethyl)amino]thiazol-4-ylacetyl amine)-2-oxo-
azetidine-1-sulfonate. A solution of this material in 4 ml of
water, cooled to 0°C, and filtered. The filtrate was
concentrated, and applied to a 20-ml HP-20 AG column.
Elution with water, followed by evaporation in vacuo,
gave 10 to 11 mg of pure [14C]SO 81,377, as judged by
analytical silica gel thin-layer chromatography in 1-
butanol-acetic acid-water (3:1:1 by vol) or electrophore-
sis at pH 7.2, followed by visualization with UV light,
Rydon reagent, or autoradiography. The overall yield
was 20%, based on [methyl-14C]iodide.

Bacteria and culturing conditions. E. coli SC 8294 and S. aureus SC 2399 were from the Squibb Culture Collection; E. coli DC2 and Streptomyces sp. strain R61 were, respectively, gifts from M. Richmond, University of Bristol, and J. M. Ghysen, University of Liege. All organisms were grown as previously described (10).

PBP binding assay. Solubilized (2% Triton X-100)
membranes of sonicated E. coli and S. aureus were incubated (~100 μg of protein) at 30°C with the appropriate β-lactam for 10 min in a total volume of 50 μl. Then 10 nmol of [14C]penicillin G was added, and the incubation was continued for 10 min. PBPs were visualized after SDS-polyacrylamide gel electrophoresis and fluorography (10).

**β-Lactam-binding protein assay.** Solubilized membranes (~100 μg of protein) were incubated with the appropriate β-lactam for 10 min as described above, except that the β-lactam was radiolabeled ([125I]azlocillin derivative, [14C]SQ 26,324, and [14C]SQ 81,377). β-Lactam-binding proteins were detected as described above.

**Release of bound β-lactams.** Solubilized membranes (~100 μg of protein) were incubated at 30°C with 10 nmol of [14C]penicillin G or [14C]SQ 26,324 for 10 min in a total volume of 50 μl. Penicillinase (4,000 U) was added to destroy the unbound β-lactam, and the incubation was continued for 10, 20, or 50 min. Hydroxylamine-induced release was examined by adding the appropriate amount of neutral hydroxylamine (final concentration, 0.2, 0.4, and 0.8 M) after 20 min of incubation in the penicillinase and incubating the mixture for 30 min. Residual [14C]-β-lactam binding was detected after SDS-polyacrylamide gel electrophoresis and fluorography.

**dd-Carboxypeptidase assay.** Partially purified Streptomyces sp. strain R61 dd-carboxypeptidase (8) was incubated at 30°C with the appropriate β-lactam for 10 min in a total volume of 20 μl. [14C]diacetyl-L-Lys-D-Ala-d-Ala (2 nmol) was added, and the incubation was continued for 30 min. The hydrolysis product, [14C]diacetyl-L-Lys-D-Ala, was separated by high-voltage paper electrophoresis and quantitated by liquid scintillation counting (27).

**Peptidoglycan transpeptidase assay.** Ether-treated E. coli cells were incubated with the appropriate cofactors and β-lactam for 10 min in a total volume of 40 μl (12). Then 0.25 nmol of UDP-N-[14C]acetylglucosamine and 6 nmol of UDP-N-acetylmuramyl pentapeptide were added, and the incubation was continued for 20 min. The SDS-insoluble peptidoglycan was collected and quantitated as previously described (12).

### RESULTS

The monobactams studied possess side chains analogous to those found in penicillins and cephalosporins. On that basis, they can be conveniently divided into four groups (Table 1): penicillin-cephalothin, piperacillin-cefoperazone, azlocillin-mezlocillin, and cefotaxime-ceftazidine. Monobactams in the latter three groups are generally active against both E. coli and S. aureus, whereas those in the penicillin group are active mainly against S. aureus.

**Binding to PBPs.** Active monobactams bound to the essential PBP 3 of E. coli (Table 2) and to PBPs 1, 2, and 3 of S. aureus (Table 3). In the penicillin series, 3α-methoxylation increased binding to PBP 3 of E. coli but decreased binding

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**TABLE 2. Binding of monobactams to PBPs of E. coli SC 8294**

| Monobactam Series | SQ No. | Amt (μg/ml) of compound to completely (≥90%) inhibit penicillin G binding | MIC (μg/ml) | 10^6 CFU^a |
|-------------------|-------|---------------------------------------------------------------|-------------|-------------|
|                   |       | PBP 1a | PBP 1b | PBP 2 | PBP 3 | PBP 4 | PBP 5/6 |            |
| Penicillin        |       |        |        |        |        |        |        |            |
| 26,324            | 2.0   | >100   | >100   | >100  | 10    | >100  | 50     | (100)      |
| 26,522            | 2.0   | >100   | >100   | >100  | 0.5   | 2.0   | 50     | (50)       |
| 26,591            | 10    | >100   | >100   | >100  | 10    | >100  | 100    | (50)       |
| 81,387            | 2.0   | >100   | >100   | >100  | 10    | >100  | 25     | (25)       |
| 26,559            | 0.5   | >100   | >100   | 30    | 0.5   | 2.0   | 25     | (25)       |
| Piperacillin      |       |        |        |        |        |        |        |            |
| 81,641            | 10    | >100   | >100   | 10    | 10    | >100  | 3.1    | (0.4)      |
| 81,427            | 0.5   | >100   | >100   | 10    | 30    | >100  | 0.8    | (<0.05)    |
| 26,630            | 2.0   | >100   | >100   | 20    | 10    | 10    | 12.5   | (0.8)      |
| 81,612            | 10    | ≥100   | ≥100   | 10    | 10    | >100  | 1.6    | (<0.05)    |
| Azlocillin        |       |        |        |        |        |        |        |            |
| 81,518            | 10    | >100   | >100   | 0.5   | 100   | ≥100  | 6.3    | (0.8)      |
| 81,536            | 10    | >100   | >100   | 0.5   | 100   | ≥100  | 12.5   | (0.4)      |
| 81,396            | 2.0   | ≥100   | ≥100   | 0.1   | ≥100  | ≥100  | 0.4    | (<0.05)    |
| 81,699            | 0.5   | >100   | >100   | 0.1   | ≥100  | ≥100  | 0.8    | (0.05)     |
| 81,746            | >100  | >100   | >100   | 0.1   | >100  | >100  | 6.3    | (0.1)      |
| Cefotaxime        |       |        |        |        |        |        |        |            |
| 81,377            | 2.0   | >100   | >100   | 100   | 10    | >100  | 3.1    | (3.1)      |
| 81,388            | 10    | 100    | >100   | 0.1   | 10    | >100  | 0.1    | (<0.05)    |
| 26,690            | 30    | >100   | >100   | 0.1   | >100  | >100  | 0.2    | (<0.05)    |
| 81,389            | 2.0   | >100   | >100   | 10    | 10    | ≥100  | 0.4    | (0.8)      |
| 81,402            | 30    | >100   | >100   | 0.1   | 30    | >100  | 0.4    | (0.4)      |
| 26,776            | 10    | ≥100   | ≥100   | 0.1   | 100   | 100   | 0.4    | (0.1)      |
| 26,917            | 30    | >100   | >100   | 0.1   | 100   | 100   | 0.4    | (0.1)      |

^a Numbers in parentheses indicate minimum inhibitory concentrations (MICs) against DC2, a permeability mutant of E. coli (20). CFU, Colony-forming units.
to PBPs 2 and 3 of *S. aureus* (SQ 26,522 versus SQ 26,324 and SQ 26,559 versus SQ 81,387). 3α-Methoxylation also increased binding to the nonessential PBPs 4 and 5/6 of *E. coli* and PBP 4 of *S. aureus*, an effect similar to that observed with cephalosporins (7). 4-Methylation did not affect binding to PBP 3 of *E. coli* but decreased binding to PBPs 1, 2, and 3 of *S. aureus* (SQ 26,591 versus SQ 26,324). In the piperacillin series, 3α-methoxylation decreased binding to essential PBPs in both *E. coli* and *S. aureus* but increased binding to the nonessential PBPs 4 and 5/6 of *E. coli* and PBP 4 of *S. aureus* (SQ 26,630 versus SQ 81,427). 4-Methylation did not affect binding to PBP 3 of *E. coli* but decreased binding to PBPs 1, 2, and 3 of *S. aureus* (SQ 81,612 versus SQ 81,427). In the azlocillin series, 4-phenoxylolation did not affect binding to PBP 3 of *E. coli* (although it did affect permeability) but decreased binding to PBPs 1, 2, and 3 of *S. aureus* (SQ 81,746 versus SQ 81,427). Extending the side chain did not affect binding to PBPs in either organism (SQ 81,758 and SQ 81,536 versus SQ 81,396). In the cefotaxime series, 4-phenoxylolation did not affect binding to PBP 3 of *E. coli* (although, again, it affected permeability) but decreased binding to PBP 2 of *S. aureus* (SQ 81,402 versus SQ 81,755). 4-Methylation increased binding to PBP 3 of *E. coli* in one case (SQ 26,668 and SQ 26,690 versus SQ 81,377) but not in another (SQ 26,776 and SQ 26,917 versus SQ 81,402), whereas it decreased binding to PBPs 1, 2, and 3 of *S. aureus*. Ionic substituents on the side chain increased binding to PBP 3 of *E. coli* but decreased binding to PBPs 1, 2, and 3 of *S. aureus*. Ionic substituents on the side chain decreased binding to PBP 3 of *E. coli* but decreased binding to PBPs 1, 2, and 3 of *S. aureus* (SQ 81,402 versus SQ 81,389). Note that SQ 81,377 and SQ 81,389 bound to PBP 3 of *E. coli* only moderately, even though they are potent antibiotics. SQ 81,377 (and SQ 26,776) did not bind, up to 100 μg/ml, to PBP 1c. This is a 76K β-lactam-binding protein detected with an [125I]ampicillin derivative according to published procedures (21). It has also been detected with [125I]mezlocillin and has been implicated in seption (2), being thus an attractive target for monobactams.

**Binding to proteins other than PBPs.** Binding of [14C]SQ 26,324 and [14C]SQ 81,377 to solubilized membranes of *E. coli* and *S. aureus* was limited to PBPs. Two additional proteins (65K and 55K) were detected with [14C]SQ 81,377 but are not physiologically important, since the antibiotic concentration used (100 and 300 μg/ml, respectively) was two orders of magnitude higher than the minimum inhibitory concentration.

**Release of bound monobactams.** The release of bound [14C]SQ 26,324 was next compared to that of the homologous classical β-lactam, peni-
cillin G (Table 4). SQ 26,324 was not released spontaneously by PBP 5/6 of E. coli, a \( \text{DD-carboxypeptidase with penicillin} \) activity (27), although it was released in the presence of 0.2 M hydroxylamine. The release of SQ 26,324 from the other E. coli PBPs was similar to that of penicillin G. SQ 26,324 was released from PBPs 1, 2, and 3 of S. aureus at lower concentrations of hydroxylamine than penicillin G. Unfortunately, PBP 4 of S. aureus, also a \( \text{DD-carboxy} \) peptidase with penicillinase activity (16), did not bind SQ 26,324, and thus its release could not be determined.

**Effect on DD-carboxypeptidase and peptidoglycan transpeptidase.** Monobactams inhibited *Streptomyces* sp. strain R61 \( \text{DD-carboxypeptidase} \), with amounts causing 50% inhibition ranging from \( 10^{-6} \) M to greater than \( 10^{-3} \) M (Table 5). Inhibition paralleled binding to PBP 4 of E. coli, a partially soluble \( \text{DD-carboxypeptidase with model transpeptidase activity} \) (27). 3α-Methylation increased inhibitory activity by an order of magnitude (SQ 26,522 versus SQ 26,324), an effect previously observed with cephalosporins. 4-Methylation decreased activity (SQ 26,690 and SQ 26,668 versus SQ 81,377) and so did ionic substituents on the 3β-side chain (SQ 81,402 versus SQ 81,377). None of the compounds showed any significant activity against E. coli peptidoglycan transpeptidase (amount causing 50% inhibition, \( \geq 5 \) mM), consistent with their poor binding to PBP 1b.

**DISCUSSION**

Monobactams are novel monocyclic \( \beta \)-lactam antibiotics with activity against both gram-negative and gram-positive bacteria. Those active against E. coli and related bacteria (enterobacteria and pseudomonads) bind specifically to PBP 3 (a peptidoglycan transpeptidase involved in septation [15]) and induce filamentation (22). Those active against S. aureus bind to PBPs 1, 2, and 3.

Binding to PBP 3 of E. coli is generally less affected by structural changes on the \( \beta \)-lactam nucleus than is binding to the other PBPs (or the \( \beta \)-lactamases). For example, an amoxicillin derivative did not bind to PBPs 1b and 2 and was not hydrolyzed by \( \beta \)-lactamases (19) but retained the ability of the parent compounds to bind to PBP 3. (N. H. Georgopapadakou and F. Y. Liu, unpublished results). Thus, monobactams, by virtue of their high affinity for PBP 3 (a PBP relatively tolerant to structural changes), permit optimization of structure for both resistance to \( \beta \)-lactamase and outer membrane permeability. The latter involves predictable parameters, such as hydrophobicity and charge (17), whereas the former is mostly empirical. It should be noted that the tradeoff for \( \beta \)-lactamase stability might be loss of ability to bind to essential PBPs of S. aureus (SQ 26,776 versus SQ 81,377).

Monobactams resemble cephalosporins in their biological properties, such as interaction with *Streptomyces* sp. strain R61 \( \text{DD-carboxy} \) peptidase (11), \( \beta \)-lactamases (5), and to some extent PBPs. The effect of 3α-methylation on binding to E. coli PBPs (increased binding to PBPs 4 and 5/6 and variable effect on binding to other PBPs) is very similar to that of 7α-methylation in cephalosporins (7). Nevertheless, structure-activity relationships based on the cephalosporin 7β-side chain are not transferrable to the monobactam 3β-side chain. In E. coli, for example, cefotaxime binds, in addition to PBP 3 (0.1 \( \mu \)g/ml), to PBP 1b (2.0 \( \mu \)g/ml) and PBP 2 (10 \( \mu \)g/ml); SQ 81,377 does not. Similar differences can be observed with SQ 81,427 (ceftobiprole) and SQ 26,559 (cefotaxime).

The low affinity of PBP 3 of E. coli for SQ 81,377 is puzzling. The compound induces filamentation at near-minimum inhibitory concentrations (12) and, in the radiolabeled form, does not bind to any other protein. Neither does it bind to PBP 1c, a very minor protein (estimated

**TABLE 4.** Release of bound \([^{14}C]SQ 26,324\) and \([^{14}C]\)penicillin G from membranes of E. coli and S. aureus

| Organism | PBP | Spontaneous (min required) | Hydroxylamine induced (M required) |
|----------|-----|----------------------------|-----------------------------------|
|          |     | SQ 26,324                  | Penicillin G                      |
| E. coli  | 1a  | >50                        | >50                               |
|          | 1b  | >50                        | >50                               |
|          | 4   | >50                        | >50                               |
|          | 5/6 | >50                        | 20                                |
| S. aureus| 1   | >50                        | >50                               |
|          | 2   | >50                        | >50                               |
|          | 3   | >50                        | >50                               |
|          | 4   | ND*                        | 20                                |

* ND, Not determined (SQ 26,324 does not bind to PBP 4 up to 100 \( \mu \)g/ml).
TABLE 5. Effect of monobactams on *E. coli* peptidoglycan transpeptidase and *Streptomyces* sp. strain R61 DD-carboxypeptidase

| Monobactam series | SQ no. | Approximate $I_50^a$ (M) | Transpeptidase | Carboxypeptidase |
|-------------------|-------|--------------------------|----------------|-----------------|
| Penicillin        | 26,324| $>5 \times 10^{-4}$       | $10^{-5}$      |                 |
|                   | 26,522| ND                       | $10^{-6}$      |                 |
|                   | 26,591| $5 \times 10^{-4}$       | ND             | $10^{-5}$       |
|                   | 26,559| ND$^b$                   |                 |                 |
| Piperacillin      | 81,612| $10^{-4}$                | ND             |                 |
| Azlocillin        | 81,396| $10^{-3}$                |                 |                 |
| Cefotaxime        | 81,377| $>5 \times 10^{-4}$      | $10^{-4}$      |                 |
|                   | 26,668| ND                       | $>10^{-3}$     |                 |
|                   | 26,690| ND                       | $10^{-3}$      |                 |
|                   | 81,389| ND                       | $10^{-3}$      |                 |
|                   | 81,402| $>5 \times 10^{-4}$      | $>10^{-3}$     |                 |
|                   | 26,776| $>5 \times 10^{-4}$      | ND             |                 |

$^a$ $I_50$, Amount causing 50% inhibition.

$^b$ ND, Not determined.

to be present in five copies per cell) which is possibly involved in septation (2).

Monobactams also bind to the nonessential PBPs 1a and 4 of *E. coli*, which are generally sensitive to β-lactam antibiotics. However, binding to PBP 3 occurs at still lower monobactam concentrations and occasionally in the absence of binding to either nonessential PBP, as with SQ 81,755. Thus, monobactams appear to bind to PBP 3 of *E. coli* far more specifically than the bicyclic β-lactam antibiotics.

Monobactams bind poorly to PBP 5/6 of *E. coli* and PBP 4 of *S. aureus*, the 3α-methoxylated compounds being exceptions. Monobactams do not bind to PBP 1b of *E. coli* and accordingly do not induce lysis.

In conclusion, monobactam activity against gram-negative or -positive bacteria or both is most likely a function of binding to essential PBPs. In the case of *E. coli* and *S. aureus*, activity is due to binding to PBP 3 and PBPs 1, 2, and 3, respectively. The essential PBP profile of a given monobactam is in turn determined by the nature of the 3α-side chain, as well as other substituents on the β-lactam nucleus.

ACKNOWLEDGMENTS

We thank D. P. Bonner for supplying the minimum inhibitory concentration data, H. M. Tsay for synthesizing the [125I]ampicillin derivative, and F. Y. Liu for her expert technical assistance during the initial stages of this work.

LITERATURE CITED

1. Blumberg, P. M., and J. L. Strominger. 1974. Interaction of penicillin with the bacterial cell: penicillin-binding proteins and penicillin-sensitive enzymes. Bacteriol. Rev. 38:291–335.

2. Botta, G. A., and J. T. Park. 1981. Evidence for involvement of penicillin-binding protein 3 in murein synthesis during septation but not during cell elongation. J. Bacteriol. 145:333–340.

3. Breuer, H., C. M. Cimmarusti, D. M. Floyd, W. H. Koster, W. C. Liu, P. A. Principe, M. L. Rathnam, and W. A. Slussarchyk. 1981. Monobactams—structure-activity relationships leading to SQ 26,776. J. Antimicrob. Chemother. 8(Suppl. E):21–28.

4. Bucourt, R., R. Heymes, A. Lutz, L. Pénasse, and J. Perronnet. 1977. Céphalosporines a châines amino-2-thiazoly-4 acétyles. Influence de la présence et de la configuration d’un groupe oxymino sur l’activité antibactérienne. Tetrahedron 34:2233–2243.

5. Bush, K., J. S. Freudenberger, and R. B. Sykes. 1982. Interaction of aztreonam and related monobactams with β-lactamases from gram-negative bacteria. Antimicrob. Agents Chemother. 22:414–420.

6. Cimmarusti, C. M., R. B. Sykes, H. A. Applegate, D. P. Bonner, H. Breuer, H. W. Chang, Th. Denzel, D. M. Floyd, A. W. Fritz, W. H. Koster, W. C. Liu, W. L. Parker, M. L. Rathnam, W. A. Slussarchyk, U. D. Treuner, and M. G. Young. 1982. Monobactams (monocyclic β-lactam antibiotics derived from bacteria). The derivation of SQ 26,776, p. 35–43. In H. C. Neu (ed.), New beta-lactam antibiotics: a review from chemistry to clinical efficacy of the new cephalosporins. College of Physicians of Philadelphia, Philadelphia.

7. Curtis, N. A. C., G. W. Ross, and M. G. Boulton. 1979. Effect of 7α-methoxy substitution of cephalosporins upon their affinity for the penicillin-binding proteins of *E. coli* K12. Comparison with antibacterial activity and inhibition of membrane bound model transpeptidase activity. J. Antimicrob. Chemother. 5:391–398.

8. Frère, J. M., J. M. Ghuyens, H. R. Perkins, and M. Nieto. 1973. Molecular weight and amino acid composition of the exocellular DD-carboxypeptidase-transpeptidase of *Streptomyces* R61. Biochem. J. 135:463–468.

9. Georgopapadakou, N. H., and F. Y. Liu. 1980. Binding of β-lactam antibiotics to penicillin-binding proteins of *Staphylococcus aureus* and *Streptococcus faecalis*: relation to antibacterial activity. Antimicrob. Agents Chemother. 18:834–836.

10. Georgopapadakou, N. H., and F. Y. Liu. 1980. Penicillin-binding proteins in bacteria. Antimicrob. Agents Chemother. 18:148–157.

11. Georgopapadakou, N. H., S. A. Smith, and C. M. Cimmarusti. 1982. Interaction between monobactams and *Streptomyces* R61 DD-carboxypeptidase. Eur. J. Biochem. 124:507–512.

12. Georgopapadakou, N. H., S. A. Smith, and R. B. Sykes. 1982. Mode of action of aztreonam. Antimicrob. Agents Chemother. 21:950–956.

13. Hayes, M. V., N. A. C. Curtis, A. W. Wyke, and J. B. Ward. 1981. Decreased affinity of a penicillin-binding protein for β-lactam antibiotics in a clinical isolate of *Staphylococcus aureus* resistant to methicillin. FEMS Microbiol. Lett. 10:119–122.

14. Imada, A., K. Kitanou, K. Kintaka, M. Muroi, and M. Asai. 1981. Sulfazecin and isosulfazecin, novel β-lactam antibiotics of bacterial origin. Nature (London) 288:590–591.

15. Ishino, F., and M. Matsuhashi. 1981. Peptidoglycan synthetase enzyme activities of highly purified penicillin-binding protein 3 in *Escherichia coli*: a septum-focusing reaction sequence. Biochem. Biophys. Res. Commun. 101:905–911.

16. Kazarich, J. W., and J. L. Strominger. 1978. A membrane enzyme from *Staphylococcus aureus* which catalyzes transpeptidase, carboxypeptidase and penicillinase activities. J. Biol. Chem. 253:1272–1278.

17. Nikaido, H., and T. Nakae. 1979. The outer membrane of gram-negative bacteria. Adv. Microb. Physiol. 20:163–250.

18. Parker, W. L., and M. L. Rathnam. 1982. EM 5400, a family of monobactam antibiotics produced by *Agrobacterium radiobacter*. II. Isolation and structure determina-
tion. J. Antibiot. 35:300–305.
19. Presslitz, J. E. 1980. Mode of action of a structurally novel beta-lactam. Antimicrob. Agents Chemother. 14:144–150.
20. Richmond, M. H., D. C. Clark, and S. Wotton. 1976. Indirect method of assessing the penetration of beta-lactamase non-susceptible penicillins and cephalosporins in Escherichia coli strains. Antimicrob. Agents Chemother. 10:215–218.
21. Schwarz, U., K. Seeger, F. Wengenmayer, and H. Strecker. 1981. Penicillin-binding proteins of Escherichia coli identified with a 125I-derivative of ampicillin. FEMS Microbiol. Lett. 10:107–109.
22. Spratt, B. G. 1975. Distinct penicillin-binding proteins involved in the division, elongation and shape of Escherichia coli K12. Proc. Natl. Acad. Sci. U.S.A. 72:2999–3003.
23. Spratt, B. G. 1978. Mechanism of action of penicillin. Sci. Progr. Oxford 65:101–128.
24. Suzuki, H., Y. Nishimura, and Y. Hirota. 1978. On the process of cellular division in Escherichia coli: a series of mutants of E. coli altered in the penicillin-binding proteins. Proc. Natl. Acad. Sci. U.S.A. 75:664–668.
25. Sykes, R. B., D. P. Bonner, K. Bush, and N. H. Georgopapadakou. 1982. Azthreonam (SQ 26,776), a synthetic monobactam specifically active against aerobic gram-negative bacteria. Antimicrob. Agents Chemother. 21:85–92.
26. Sykes, R. B., C. M. Cimmarusti, D. P. Bonner, K. Bush, D. M. Floyd, N. H. Georgopapadakou, W. H. Koster, W. C. Liu, W. L. Parker, P. A. Principe, M. L. Rathnum, W. A. Slusarchyk, W. H. Trejo, and J. S. Wells. 1981. Monocyclic beta-lactam antibiotics produced by bacteria. Nature (London) 291:489–491.
27. Tamura, T., Y. Imae, and J. L. Strominger. 1976. Purification to homogeneity and properties of two D-alanine carboxypeptidases from Escherichia coli. J. Biol. Chem. 251:414–423.