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New Antipseudomonal Penicillin, PC-904: Affinity to Penicillin-Binding Proteins and Inhibition of the Enzyme Cross-Linking Peptidoglycan

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The mechanism of action of a new antipseudomonal penicillin, PC-904, was studied with respect to its binding affinities to penicillin-binding proteins (PBPs) and its inhibitory activities on cross-linking enzymes of peptidoglycan synthesis in vitro. PC-904 showed especially high affinity (compared with that of penicillin G) to *Escherichia coli* PBP-3. It also had high affinities to PBP-2 and -1Bs and low affinities to PBP-1A, -4, -5, and -6. Similar results were obtained with *Pseudomonas aeruginosa*, in which this antibiotic showed very high affinity (compared with that of penicillin G) to PBP-3, -1A (presumably corresponding to *E. coli* PBP-1Bs), and -2; there was especially high affinity to PBP-3 and much less affinity to PBP-1B (presumably corresponding to *E. coli* PBP-1A). These results are compatible with morphological observations that at concentrations near its minimal inhibitory concentration or less, this antibiotic induced the formation of filamentous cells of *E. coli* and *P. aeruginosa*. At higher concentrations or after prolonged incubation, it induced lysis of the cells. The remarkably high affinity of PC-904 to pseudomonal PBP-3, -1A, and -2 may partly explain the potent antipseudomonal activity of this antibiotic. In *E. coli*, the concentration of PC-904 required to inhibit the cross-linking reaction in enzymatic peptidoglycan synthesis, presumably carried out by PBP-1Bs, was as low as the inhibitory concentrations of penicillin G, ampicillin, and carbenicillin.

An increasing number of gram-negative bacteria, especially *Escherichia coli* and *Pseudomonas aeruginosa*, have been isolated recently as clinical pathogens (1, 2, 11). Most strains of *E. coli* isolated clinically are moderately susceptible to various chemotherapeutic agents. In contrast, the clinical isolates of *P. aeruginosa* are insusceptible or resistant to these agents (11). Therefore, *P. aeruginosa* has become notorious as a drug-resistant pathogen and is a major object of chemotherapeutic research.

PC-904, sodium 6-[D(−)-α-(hydroxy-1,5-naphthyridine-3-carboxamido)phenylacetamido]-penicillanate, is a new antipseudomonal penicillin with a broad and potent antibacterial spectrum against gram-negative bacteria, including *Pseudomonas* species (8, 9).

In studies on the mechanism of its antibacterial action, we investigated its affinities to penicillin-binding proteins (PBPs) of *E. coli* and *P. aeruginosa* and its abilities in vitro to inhibit cross-linking enzymes involved in peptidoglycan synthesis in these bacteria.

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MATERIALS AND METHODS

Antibiotics. PC-904 (8) was a product of Sumitomo Chemical Co., Osaka, Japan. Penicillin G potassium salt (Takeda Pharmaceutical Co., Osaka), ampicillin (Toyama Chemical Co., Tokyo), and carbenicillin (Fujisawa Pharmaceutical Co., Osaka) were commercial products. Radioactive [^{14}C]penicillin G potassium salt (54 mCi/mmol) was from the Radiochemical Centre, Amersham, England. Radioactive PC-904 labeled with ^{14}C in the naphthyridine moiety (5.4 mCi/mmol) was prepared by Sumitomo Chemical Co.

Bacterial strains used. *E. coli* NIHJC-2 and *P. aeruginosa* PAO2142 (kindly provided by E. Matsumoto, Shinshu University) were used in morphological experiments. *E. coli* K-12 strain JE1011 (7) and *P. aeruginosa* NCTC 10490 were used in experiments on PBPs. *E. coli* strain JE10012 (*dacB* [6]) and strain JE11191 (*dacA* [5]), a *dacA dacB* double mutant (5), were used in enzyme assays.

Assay of PBPs in *E. coli* and *P. aeruginosa*. Membrane fractions were prepared from cells growing exponentially in Penassay broth (Difco) at 37°C, as previously described (15, 17). PBPs were separated by sodium dodecyl sulfate-acrylamide slab gel electropho-

resis and located by fluorography (17). For assay of the binding affinities of PC-904 to proteins, competition experiments were performed by adding various concentrations of nonradioactive PC-904 to reaction mixture containing 30 μg of [^{14}C]penicillin G per ml. Binding of [^{14}C]PC-904 (0.15 μCi/reaction) to proteins was assayed in the same manner as binding of [^{14}C]-penicillin G.

Assay of enzyme inhibition. In studies on *E. coli*, D-alanine carboxypeptidase IA was measured by release of terminal D-alanine from ^{14}C-labeled UDP-MurNAc-pentapeptide (L-Ala-D-Glu-*m*-A_2pm-D-[^{14}C]-Ala-D-[^{14}C]Ala) (21 μCi/μmol) (4, 6) with an enzyme preparation from the *dacB* mutant strain (6), which is defective in D-alanine carboxypeptidase IB activity, and D-alanine carboxypeptidase IB was measured in a similar way with an enzyme preparation from the *dacA* mutant (5), which is defective in D-alanine carboxypeptidase IA activity. The activity of cross-linking peptidoglycan (5) and concomitant release of D-alanine (4) were assayed as described in the literature, using an enzyme preparation from the *dacA dacB* double mutant (5), which is defective in D-carboxypeptidase IA and IB activities. For assay of cross-linkage formation, ^{14}C-labeled UDP-MurNAc-pentapeptide (L-Ala-D-Glu-*m*-[^{14}C]A_2pm-D-Ala-D-Ala) (62 μCi/μmol) (5, 17) and unlabeled UDP-GlcNAc were used as substrates, and release of D-alanine concomitant with the formation of cross-linked peptidoglycan was measured by using ^{14}C-labeled UDP-MurNAc-pentapeptide (L-Ala-D-Glu-*m*-A_2pm-D-[^{14}C]Ala-D-[^{14}C]Ala) and unlabeled UDP-GlcNAc as substrates. Reaction mixtures for assay of D-alanine carboxypeptidases contained (in a final volume of 33 μl): 2 μmol of tris-(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.6), 1 μmol of $MgCl_2$, 0.44 nmol of UDP-MurNAc-pentapeptide labeled at D-[^{14}C]Ala-D-[^{14}C]-Ala, 50 nmol of 2-mercaptoethanol, 0.1% (wt/vol, final concentration) Triton X-100, and 100 μg of appropriate enzyme (as protein). The reaction mixture for assay of cross-linked formation contained (in a final volume of 33 μl): 2 μmol of tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.6), 1 μmol of $MgCl_2$, 0.35 nmol of UDP-MurNAc-pentapeptide labeled at *m*-[^{14}C]A_2pm, 10 nmol of UDP-GlcNAc, 50 μmol of 2-mercaptoethanol, and 100 μg of enzyme (as protein). The reaction mixture for assay of the release of D-alanine concomitant to the formation of cross-linked peptidoglycan was similar to D-alanine carboxypeptidase assay, except 10 nmol of UDP-GlcNAc was present and 0.1% (wt/vol, final concentration) Triton X-100 was omitted. All reactions were performed at 30°C for 60 min. More details of the methods will be published elsewhere (K. Kunugita and M. Matsuhashi, manuscript in preparation).

Abbreviations. The abbreviations used were as follows: *m*-A_2pm, *meso*-diaminopimelic acid; GlcNAc, *N*-acetylglucosamine; MurNAc, *N*-acetylamuramic acid.

RESULTS

Morphological changes of *E. coli* and *P. aeruginosa* induced by PC-904. PC-904 induced the formation of filamentous cells from normal rod-shaped cells of *E. coli* and *P. aeruginosa* at its minimal inhibitory concentration (0.8 μg/ml for *E. coli* or 1.6 μg/ml for *P. aeruginosa*) (Fig. 1A and B). Similar morphological changes could be observed at concentrations of about 1/10 to more than 10 times its minimal inhibitory concentration. These filamentous cells seemed to have no septa as judged by phase-contrast microscopy, suggesting that at low concentrations this antibiotic inhibited the formation of septa. Sometimes a large bulge was seen on a filamentous cell. However, after prolonged incubation (more than 5 h at 37°C), the cells appeared less dense under a phase-contrast microscope (Fig. 1C and D) and seemed to have been lysed by the antibiotic. These results suggest that under these conditions, inhibition of septum formation and then lysis of the cells occur.

Affinity of PC-904 to PBPs of *E. coli* and *P. aeruginosa*. At least seven PBPs, numbered 1A, 1B, 2, 3, 4, 5, and 6, have been separated from *E. coli* by sodium dodecyl sulfate-acrylamide slab gel electrophoresis (14, 17). A similar number of PBPs have been separated from *P. aeruginosa* (10; H. Noguchi, M. Matsuhashi, T. Nikaido, J. Itoh, N. Matsubara, M. Takaoka, and S. Mitsuhashi, *In* S. Mitsuhashi, ed., *Microbial Drug Resistance, vol. 2. Microbial Drug Resistance and Related Plasmids*, in press). PBP-5 and -6 in *E. coli* could be separated into two distinct bands, but in *P. aeruginosa* only one band could be detected in the position of PBP-5 and -6, and no evidence has yet been obtained that *P. aeruginosa* has two proteins, 5 and 6. Moreover, PBP-1A and -1B of *P. aeruginosa*, which were numbered in the order of bands from the top of electrophoresis, seemed to correlate with PBP-1Bs and -1A of *E. coli*, respectively, as judged from the specific patterns of affinities of several β-lactam antibiotics.

The affinities of PC-904 to PBPs were estimated in two different ways: by measuring the competition of unlabeled PC-904 with [^{14}C]penicillin G for binding to PBPs and by measuring the direct binding of [^{14}C]PC-904 to PBPs. Figure 2 quantitatively shows the pattern of competition of unlabeled PC-904 with [^{14}C]penicillin G for binding to PBPs in *E. coli* (Fig. 2A) and in *P. aeruginosa* (Fig. 2B). In this figure, the magnitude of the competition of PC-904 with penicillin G is expressed as the ratio of the remaining radioactivity of [^{14}C]penicillin G caused by competition with unlabeled penicillin G (homologous competition system) to that by competition with unlabeled PC-904 (PC-904 competition system) at various concentrations of antibiotics. Among the PBPs, PC-904 has the higher affinity (compared with that of penicillin G) to PBP-3, followed by PBP-2 and PBP-1Bs (*E. coli*) or

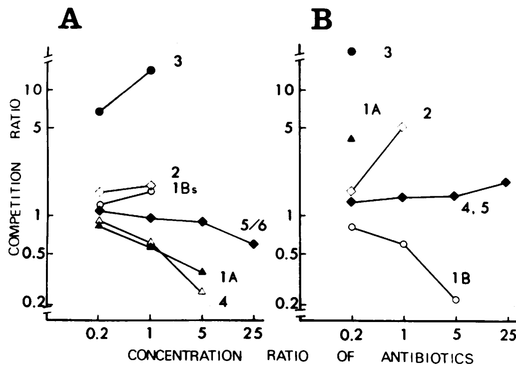


FIG. 2. *Competitive ratios of PC-904 to penicillin G for binding of [^{14}C]penicillin G to proteins of E. coli and P. aeruginosa. (Ordinate) Ratio of competition (expressed as the amounts of remaining [^{14}C]-penicillin G bound to proteins on competition with different concentrations of unlabeled penicillin G to that on competition with the same concentrations of PC-904). A ratio of 1.0 indicates that at the given concentration the two antibiotics have the same affinities, and a ratio of 10 indicates that PC-904 has an affinity 10 times higher than penicillin G to the protein. (Abscissa) Molar concentration ratios of competing antibiotics to [^{14}C]penicillin G. A 600-μg amount of protein of a membrane preparation was incubated with [^{14}C]penicillin G (final concentration, 30 μg/ml) and various concentrations of PC-904 for 10 min at 30°C. The reaction mixture was subjected to sodium dodecyl sulfate-acrylamide slab gel electrophoresis and followed by fluorography. X-ray film was scanned in a densitometer. (A) E. coli JE1011; (B) P. aeruginosa NCTC 10490. Numbers indicate PBP. The numbering of pseudomonal PBPs is tentative and should be rearranged after the functions of each PBP have been confirmed.*

PBP-1A (*P. aeruginosa*). This antibiotic showed low affinities to PBP-4 (*E. coli*) and PBP-1A (*E. coli*) or PBP-1B (*P. aeruginosa*). PBP-3 of *E. coli* has been demonstrated to be essential for septum formation (12, 13), and PBP-2 has been suggested to be involved in some mechanism that maintains the rod-shaped cell morphology (12, 15). The results of these competition experiments on the binding of PC-904 to PBPs are in good agreement with the observations described above that, over a wide range of concentrations, PC-904 induced the formation of filamentous cells of *E. coli* and *P. aeruginosa*. Many other penicillins, such as penicillin G, induce the formation of filamentous cells, but their effective concentration ranges are much narrower than that of PC-904. From our findings we conclude that the primary target of this antibiotic is PBP-3 and that binding of PC-904 to this protein may cause the formation of filamentous cells and then the lysis of the cells.

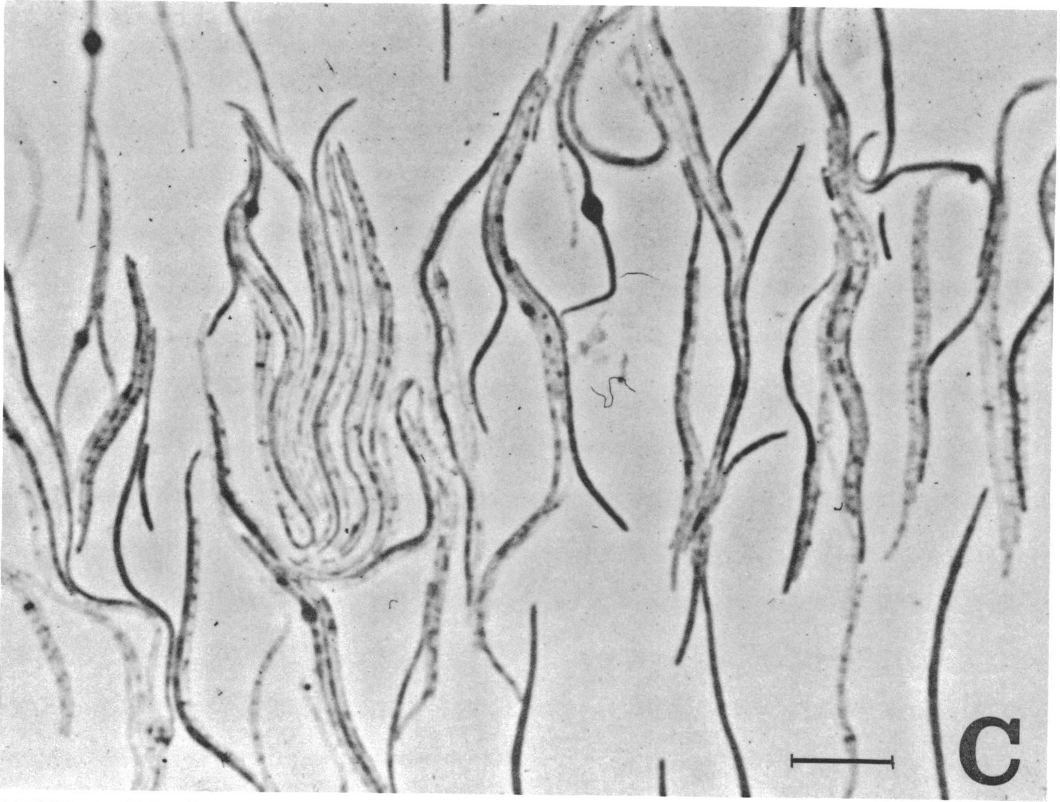
The correlation between the PBPs of *E. coli* and *P. aeruginosa* has been estimated on the basis of several criteria (H. Noguchi, M. Matsuhashi, and S. Mitsuhashi, manuscript in preparation). The function of PBP-2 is still unknown. It has been reported that a mutation in PBP-2 in *E. coli* can cause thermosensitivity of cell growth (12), although other experiments suggested that a defect of PBP-2 alone did not always cause thermosensitivity of cell growth (S. Nakajima and M. Matsuhashi, manuscript in preparation). PBP-1Bs of *E. coli* is reported to be involved in the cross-linking reaction of cell wall peptidoglycan (17), and PBP-1A of *P. aeruginosa* presumably corresponds to PBP-1Bs of *E. coli* (Noguchi et al., manuscript in preparation). PC-904 and penicillin G showed similar affinities to PBP-5 (and -6; *E. coli* and *P. aeruginosa*), whereas PC-904 showed much less affinity than did penicillin G to PBP-1A and -4 (*E. coli*) or PBP-1B (*P. aeruginosa*, presumably corresponding to PBP-1A of *E. coli*; Noguchi et al., manuscript in preparation).

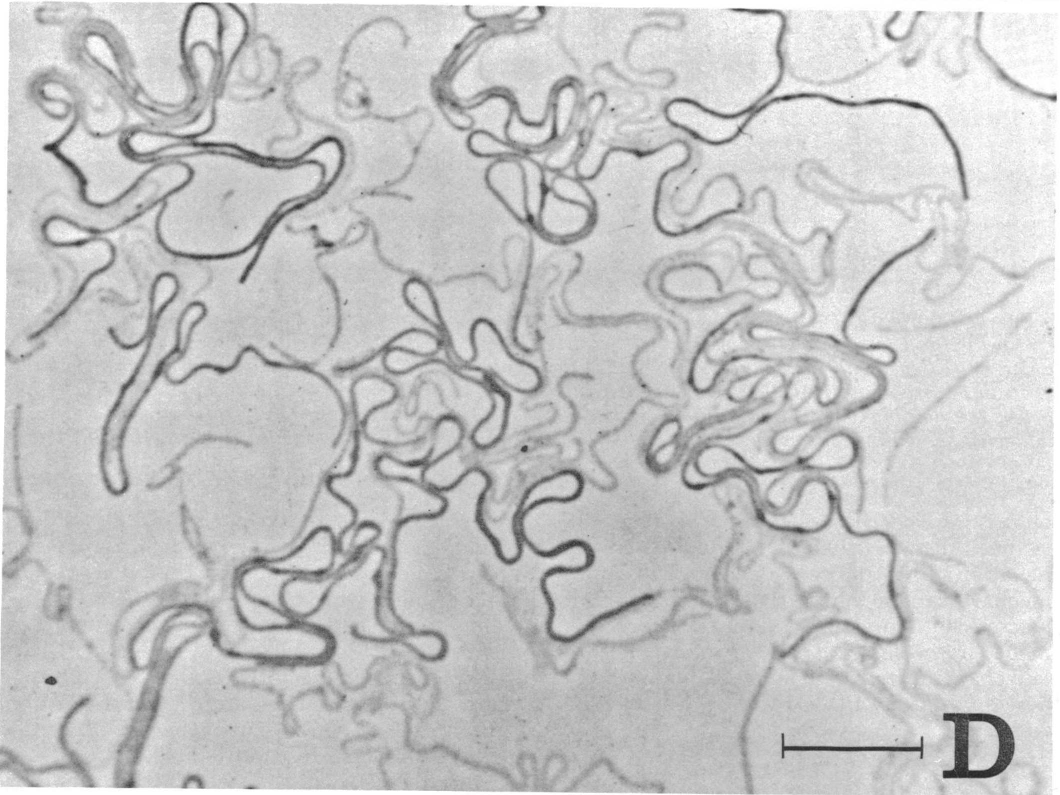
As shown in Fig. 3, direct binding experiments with [^{14}C]PC-904 indicated that this antibiotic binds to all PBPs from 1A to 6 in *E. coli* as well as in *P. aeruginosa*, the highest radioactivity appearing in PBP-5 (and -6). The radioactivity in the position of each PBP on a slab gel could be eliminated by competition with excess unlabeled PC-904. Control experiments with [^{14}C]-penicillin G indicated that PC-904 binds to almost all of the proteins that bind to penicillin G.

Assay of inhibition of enzymes involved in cross-linking of peptidoglycan in vitro. The inhibitory effects of PC-904 on peptidoglycan cross-linking and D-alanine carboxypeptidase reactions in *E. coli* are shown in Table 1. Penicillin G, ampicillin, and carbenicillin were used as controls in this experiment. PC-904, like the other penicillins listed in Table 1, inhibited the cross-linking reaction of peptidoglycan in vitro at concentrations of 3 to 12 μg/ml. Similar concentrations of these antibiotics inhibited D-alanine release concomitant with the formation of cross-linked peptidoglycan. PC-904 also inhibited D-alanine carboxypeptidase IA (corresponding to PBP-5/6 in *E. coli*) as strongly as did penicillin G, but it was slightly less inhibitory than penicillin G on D-alanine carboxypeptidase IB (corresponding to PBP-4 in *E. coli*). These results are compatible with those obtained in experiments on binding to PBP, which indicated that PC-904 had lower affinity to PBP-4 of *E. coli* than did penicillin G (Fig. 2).

In the in vitro method for assay of the formation of cross-linked peptidoglycan (see above), only enzyme activity which seems to be due to PBP-1Bs (17) can be measured. No enzyme activity responsible for the formation of

FIG. 1. *Morphological changes caused by PC-904 in E. coli and P. aeruginosa. Cells were grown in Trypticase soy broth (BBL) at 37°C to 10^8 cells per ml, and then one loopful of culture was transferred onto a thin film of heart infusion agar (BBL) containing the minimal inhibitory concentration of PC-904. Incubation*





was performed for 3 to 9.5 h at 37°C. Cell morphology was observed under a phase-contrast microscope. Bars = 10 μm. (A and C) E. coli NIHJC-2 (0.8 μg of PC-904 per ml); (B and D) P. aeruginosa PAO2142 (1.6 μg of PC-904 per ml). Incubation time: 3 h for (A) and (B), 6 h for (C), and 9.5 h for (D).

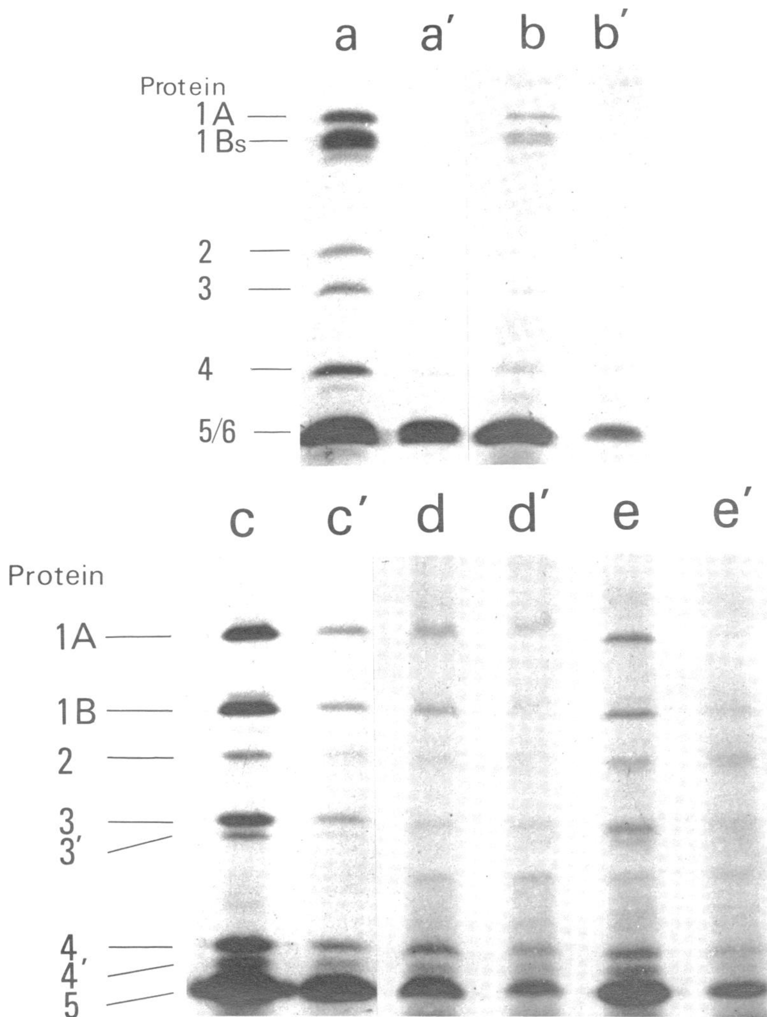


FIG. 3. *Binding of [^{14}C]penicillin G or [^{14}C]PC-904 to PBPs in E. coli and P. aeruginosa. (a and a') [^{14}C]penicillin G bound to PBPs in E. coli JE1011; (b and b') [^{14}C]PC-904 bound to PBPs in E. coli JE1011; (c and c') [^{14}C]penicillin G bound to PBPs in P. aeruginosa NCTC 10490; (d and d') [^{14}C]PC-904 bound to PBPs in P. aeruginosa PAO2142; (e and e') [^{14}C]PC-904 bound to PBPs in P. aeruginosa NCTC 10490. In (a') to (e'), the bindings of ^{14}C-labeled penicillins occurred in the presence of a five fold excess of the same unlabeled penicillins. The concentration of [^{14}C]PC-904 was about 10 times that of [^{14}C]penicillin G, because the specific activity of [^{14}C]PC-904 was lower.*

septa, in which PBP-3 seems to be involved (12), has been demonstrated. A method for assay of the latter activity must be developed to confirm the characteristic inhibitory activity of this new β-lactam antibiotic.

The enzymes in *P. aeruginosa* have not been well characterized yet, and the inhibitions of these enzymes by PC-904 will be described in a later report.

DISCUSSION

PC-904, a new antipseudomonal penicillin, induced the formation of filamentous cells from rod-shaped cells of *E. coli* and *P. aeruginosa* and then the lysis of the cells. *P. aeruginosa* forms much longer filaments than does *E. coli* on treatment with PC-904. Experiments on the competition of PC-904 with [^{14}C]penicillin G in binding to PBPs in vitro indicated that PC-904 had the highest affinity to PBP-3, a protein supposed to be concerned in septum formation (12), and that it also had higher affinity than penicillin G to PBP-2 and -1Bs (*E. coli*) or -1A (*P. aeruginosa*). PBP-1Bs of *E. coli*, which is thought to correspond to PBP-1A of *P. aeruginosa,* is supposed to be related to cross-link

TABLE 1. *Inhibition of peptidoglycan cross-linking and D-alanine carboxypeptidase reaction in vitro*

Enzyme source	Antibiotic	MIC[a] (μg/ml)	Concn[b] (μg/ml)			
			Cross-link formation	D-Alanine release concomitant with cross-link formation	D-Alanine carboxypeptidase IA	D-Alanine carboxypeptidase IB
E. coli	PC-904	0.8	3	3	0.4	0.9
	Carbenicillin	3.1	12	9	100	0.3
	Ampicillin	6.2	10	9	14	<0.01
	Penicillin G	50	3	3	1	0.01

[a] Minimal inhibitory concentration measured by twofold agar dilution method with *E. coli* JE1011 at an inoculum size of 10^6 cells per ml.
[b] Concentration required for 50% inhibition of enzyme reactions (see text for assay).

formation in peptidoglycan synthesis with concomitant release of D-alanine (17). PBP-2 is supposed to be involved in maintaining the cell shape (12, 15). However, the assay technique available at present can only measure the cross-linking enzyme reaction of peptidoglycan related to PBP-1Bs (*E. coli*) and not that related to PBP-1A (*E. coli*), -2, or -3.

PC-904 has stronger affinity than penicillin G to PBP-3, -2, and -1A of *P. aeruginosa* (among these PBPs, this antibiotic shows especially high affinity to PBP-3). These findings are compatible with the physiological changes of cells induced by this antibiotic and may also partly explain why PC-904 has stronger antipseudomonal activity than penicillin G. It should be emphasized that the affinities of PC-904 to these proteins were much higher than those of other penicillins and cephalosporins (Noguchi et al., manuscript in preparation).

PBP-4 (*E. coli*) is identical to D-alanine carboxypeptidase IB (3, 6), and PBP-5/6 (*E. coli*) corresponds to D-alanine carboxypeptidase IA (5, 16). The affinities of PC-904 to PBP-5 and -6 in *E. coli* and the inhibitory activity of PC-904 on D-alanine carboxypeptidase IA are similar to those of penicillin G. The significantly low affinity of PC-904 to PBP-4 of *E. coli* compared with that of penicillin G is compatible with the weak inhibitory activity of PC-904 against D-alanine carboxypeptidase IB of *E. coli*. However, these enzymes have been shown not to be required in normal peptidoglycan synthesis in *E. coli* (5, 6).

The lack of PBP-1A alone in *E. coli* does not affect cell growth, because this protein is supposed to be a "detour" enzyme which functions in compensating for the lack of PBP-1Bs (17). Because PC-904 has lower affinity than penicillin G to PBP-1A in *E. coli* or PBP-1B in *P. aeruginosa*, improvement of its binding to both PBP-1A and -1B or use of PC-904 in combination with an antibiotic that binds to PBP-1A in *E. coli* or to PBP-1B in *P. aeruginosa* should enhance the effectiveness of this antibiotic.

The potent antipseudomonal activity of PC-904 can be explained partly by the abilities of this compound to bind to pseudomonal PBPs that are essential for cell growth, cell division, and maintenance of cell shape. However, its strong antipseudomonal activity must be due considerably to other factors, such as its penetration through the cell envelope to a penicillin target(s) or its resistance to intracellular inactivation by β-lactamase (10).

LITERATURE CITED

1. **Altemeier, W. A., R. P. Hummel, E. O. Hill, and S. Lewis.** 1973. Changing pattern in surgical infections. Ann. Surg. **178**:436–445.
2. **Finland, M.** 1970. Changing ecology of bacteria infections as related to antibacterial therapy. J. Infect. Dis. **122**:419–431.
3. **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.
4. **Izaki, K., M. Matsuhashi, and J. L. Strominger.** 1966. Glycopeptide transpeptidase and D-alanine carboxypeptidase: penicillin-sensitive enzymatic reactions. Proc. Natl. Acad. Sci. U.S.A. **55**:656–663.
5. **Matsuhashi, M., I. N. Maruyama, Y. Takagaki, S. Tamaki, Y. Nishimura, and Y. Hirota.** 1978. Isolation of a mutant of *Escherichia coli* lacking in the activity of a penicillin-sensitive D-alanine carboxypeptidase IA. Proc. Natl. Acad. Sci. U.S.A. **75**:2631–2635.
6. **Matsuhashi, M., Y. Takagaki, I. 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.
7. **Miyakawa, T., H. Matsuzawa, M. Matsuhashi, and Y. Sugino.** 1972. Cell wall peptidoglycan mutants of *Escherichia coli* K-12: existence of two clusters of genes, *mra* and *mrb*, for cell wall peptidoglycan biosynthesis. J. Bacteriol. **112**:950–958.
8. **Noguchi, H., Y. Eda, H. Tobiki, T. Nakagome, and T. Komatsu.** 1976. PC-904, a novel broad-spectrum semisynthetic penicillin with marked antipseudomonal ac-

tivity: microbiological evaluation. Antimicrob. Agents Chemother. **9**:262–273.

9. **Noguchi, H., M. Kubo, S. Kurashige, and S. Mitsuhashi.** 1978. Antibacterial activity of apalcillin (PC-904) against gram-negative bacilli, especially ampicillin-, carbenicillin-, and gentamicin-resistant clinical isolates. Antimicrob. Agents Chemother. **13**:745–752.
10. **Noguchi, H., and S. Mitsuhashi.** 1978. Bacteriological and biochemical approaches to anti-*Pseudomonas* activity of PC-904 (apalcillin sodium), p. 635–638. *In* W. Siegenthaler and R. Lüthy (ed.), Current chemotherapy, Proceedings of the 10th International Congress of Chemotherapy, vol. 1, Zurich, Switzerland, 18–23 September 1977. American Society for Microbiology, Washington, D.C.
11. **Ohkoshi, M.** 1970. Drug resistance patterns of bacterial isolates from clinical sources in Japan, p. 717–722. *In* International Society of Chemotherapy (ed.), Progress in antimicrobial and anticancer chemotherapy, vol. 2. University of Tokyo Press, Tokyo.
12. **Spratt, B. G.** 1975. Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K 12. Proc. Natl. Acad. Sci. U.S.A. **72**:2999–3003.
13. **Spratt, B. G.** 1977. Properties of the penicillin-binding proteins of *Escherichia coli* K 12. Eur. J. Biochem. **72**:341–352.
14. **Spratt, B. G., and V. Jobanputra.** 1977. Mutants of *Escherichia coli* which lack a component of penicillin-binding protein 1 are viable. FEBS Lett. **79**:374–378.
15. **Spratt, B. G., and A. B. Pardee.** 1975. Penicillin-binding proteins and cell shape in *E. coli.* Nature (London) **254**:516–517.
16. **Spratt, B. G., and J. L. Strominger.** 1976. Identification of the major penicillin-binding proteins of *Escherichia coli* as D-alanine carboxypeptidase IA. J. Bacteriol. **127**:660–663.
17. **Tamaki, S., S. Nakajima, and M. Matsuhashi.** 1977. Thermosensitive mutation in *Escherichia coli* simultaneously causing defects in penicillin-binding protein-1Bs and in enzyme activity for peptidoglycan synthesis *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **74**:5472–5476.