JOURNAL OF BACTERIOLOGY, Jan. 1979, p. 474-479
0021-9193/79/01-0474/06$02.00/0

Vol. 137, No. 1

Penicillin-Binding Proteins in *Proteus* Species

SATOSHI OHYA,[1]* MITSUO YAMAZAKI,[1] SHINICHI SUGAWARA,[1] AND MICHIO MATSUHASHI[2]

Central Research Laboratories, Sankyo Co., Ltd., Shinagawa-ku, Tokyo 140,[1] and The Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113,[2] Japan

Received for publication 30 October 1978

Penicillin-binding proteins in three species of *Proteus*, *Proteus mirabilis*, *P. morganii*, and *P. rettgeri*, were investigated by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. Penicillin-binding proteins in these *Proteus* species were compared with those in *Escherichia coli* K-12. An approximate correlation between penicillin-binding proteins in *E. coli* and those in *Proteus* species was shown by several criteria: electrophoretic mobilities; affinities of several β-lactam antibiotics which show characteristic patterns of binding to penicillin-binding proteins in *E. coli*; relation between affinities of antibiotics to the proteins and effects on morphological changes in *Proteus* species; location of β-lactamase activity among penicillin-binding proteins; and thermostability. The electrophoretic mobilities and several other characteristics of penicillin-binding proteins among the *Proteus* species examined were found to be similar from species to species and differed only slightly from those of *E. coli*.

Recent developments in isolation and separation of penicillin-binding proteins (PBPs) have provided much useful information for studying the mechanisms of cell wall peptidoglycan synthesis in growth and division of the cells (8, 13, 15, 16, 18, 19). Seven major PBPs have been found in *Escherichia coli* (14, 18, 19). Isolation of *E. coli* mutants defective in one of the PBPs allowed the study on functions of the PBPs: PBP-1Bs are related to an important enzyme reaction involved in peptidoglycan cross-linking of cell wall (4, 19); PBP-1A (and/or PBP-2) may function in compensating for lack of PBP-1Bs (19); PBP-2 is related to a mechanism for formation of rod-shaped cells (13, 16); PBP-3 is related to formation of septum (13); and PBP-4 and PBP-5, which are identical to D-alanine carboxypeptidases IB (3, 9) and IA (10), respectively, have apparently no essential function in normal cell growth (3, 9). Moreover, β-lactam antibiotics bind to each PBP with different affinities and cause different morphological changes in *E. coli* cells (11, 13, 16).

Investigation, however, of PBPs from other genera of gram-negative bacteria is also required. On the one hand, PBPs are supposed to perform essential roles in determination of shape and size of the cell typical of each genus or species. Therefore, PBPs may provide important information for the field of bacterial taxonomy. On the other hand, many β-lactam antibiotics are not very effective against gram-negative bacteria such as *Pseudomonas*, *Proteus*, *Enterobacter*, and *Serratia*, and the development of antibiotics that are more effective against these bacteria is urgently needed.

In this communication the characteristics of PBPs in three species of *Proteus*, *Proteus mirabilis*, *P. morganii*, and *P. rettgeri*, are reported. A preliminary account of this subject has already appeared (S. Ohya, M. Yamazaki, S. Tamaki, and M. Matsuhashi, Abstr. 2nd Tokyo Symp. Microb. Drug-Resist., p. III-3, 1977).

MATERIALS AND METHODS

Chemicals. ^{14}C-labeled penicillin G (potassium 6-phenyl [1-^{14}C]acetamidopenicillanate, 40 to 60 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, England. CS-1170 {7β-[[(cyanomethyl)thio]-acetamido]-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl-thio]methyl]-3-cephem-4-carboxylic acid}, R-45656 (7α-H analog of CS-1170), and [7α-methoxy-^{14}C]CS-1170 were products of Sankyo Co., Ltd., Tokyo. Mecillinam was obtained from Leo Co., Copenhagen, Denmark. Cephaloridine, cephaloglycine, cephalexin, and cephalothin were obtained from Shionogi Co., Ltd., Osaka; cefoxitin was from Daiichi Seiyaku Chemical Co., Ltd., Tokyo; ampicillin and cloxacillin were from Meiji Seika Co., Ltd., Tokyo; and penicillin G from Takeda Industries, Ltd., Osaka. Monoacrylamide, *N,N'*-methylenebisacrylamide and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were obtained from Wako Pure Chemical Co., Ltd., Osaka.

Culture of cells and preparation of membrane fractions. The method used for cell culture and membrane fraction preparation was practically the same as described previously for *E. coli* (9, 19). Cells of *P. mirabilis* ATCC 15290, *P. morganii* IFO 3045, and *P. rettgeri* ATCC 14505 were each cultured overnight in

80 ml of Difco Penassay broth with shaking at 37°C. The cultures were each transferred to 2 liters of the same broth, and after 3.5 h of shaking at 37°C cells were harvested by centrifugation at 3,000 × *g* for 10 min at 4°C. Cell pellets from the 2 liter cultures were suspended in 85 ml of 10 mM sodium phosphate buffer (pH 7.0) and sonically treated for 5 min under ice cooling in a sonic oscillator (9 kHz, Tōyō Co., Ltd., Tokyo). Cell debris was removed by centrifugation at 3,000 × *g* for 10 min at 4°C, and the supernatants were centrifuged at 100,000 × *g* for 30 min at 4°C. The pellets were washed once with the same buffer, and the washed pellets were suspended in 50 mM sodium phosphate buffer (pH 7.0) containing 10 mM $MgCl_2$ to make a solution containing 20 mg of protein per ml, which was stored at −20°C until use. A membrane fraction was also prepared from *E. coli* K-12 strain JE1011 (19) by a similar method.

Binding of [^{14}C]penicillin G and competition with various β-lactam antibiotics. The methods used were essentially the same as those described previously (12, 16). Thirty microliter samples of membrane preparations were mixed with 3 μl of a 0.91 mM solution of [^{14}C]penicillin G. The mixture was incubated for 10 min at 30°C. In the competition experiment, one of the various unlabeled β-lactam antibiotics was added to the mixture simultaneously. Then, 2 μl of a solution containing 15% (wt/vol) Sarkosyl and 45 mg of unlabeled penicillin G per ml was added, and after 20 min of incubation at 20°C the insoluble outer membrane fraction was removed by centrifugation at 10,000 × *g* for 30 min at 20°C. The supernatants were mixed with 5 μl of 2-mercaptoethanol (Eastman Kodak), and 15 μl of sodium dodecyl sulfate (SDS) buffer, containing 0.2 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 6.8), 3% (wt/vol) SDS, 30% (wt/vol) glycerol, and 0.02% (wt/vol) bromophenol blue, and was heated in a boiling-water bath for 2 min. The total solution was subjected to slab gel electrophoresis.

SDS-polyacrylamide slab gel electrophoresis. The procedure for SDS-polyacrylamide slab gel electrophoresis was that of Laemmli and Favre (5), with slight modifications (19). A running gel (2 mm thick, 15 by 15 cm) was composed of 7.5% (wt/vol) monoacrylamide–0.16% (wt/vol) *N,N'*-methylenebisacrylamide in 0.375 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.7) and was solidified with 0.1% (vol/vol) TEMED and 0.1% (wt/vol) ammonium persulfate. A stacking gel was composed of 3.75% (wt/vol) monoacrylamide–0.08% (wt/vol) *N,N'*-methylenebisacrylamide in 0.125 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 6.8) solidified with 0.1% (vol/vol) TEMED and 0.12% (wt/vol) ammonium persulfate (16).

Electrophoresis was carried out at a constant current of 25 mA for 5 to 6 h. After electrophoresis the gel was fixed in 300 ml of 50% (vol/vol) methanol–7% (vol/vol) acetic acid for 30 min at room temperature and was washed three times, 30 to 60 min each time, with 300 ml of washing solution containing 5% methanol–7% acetic acid. Then the gel was soaked in 300 ml of dimethyl sulfoxide (Me_2SO) for 30 min. This treatment was repeated two times, using the same volume of fresh Me_2SO each time. The gel was impregnated with 2,5-diphenyloxazole (Wako Pure Chemical Co., Osaka, Japan) by soaking the gel in Me_2SO containing 22.2% (wt/vol) 2,5-diphenyloxazole for 3 h at room temperature, and then Me_2SO was removed by washing the gel in running water for 1 h. The 2,5-diphenyloxazole-impregnated gel was dried in vacuo on Whatman no. 3MM filter paper. A fluorograph was prepared by contacting the dried gel with Fuji Rx-s X-ray film at −80°C for 3 to 10 days. The film was illuminated slightly beforehand to sensitize it for fluorography (6). Quantitation of the levels of [^{14}C]penicillin G bound to the PBP was carried out by microdensitometry (Sakura PDM-2 microdensitometer).

Antisera against β-lactamases from *P. morganii* and *P. rettgeri* and inhibition experiment of penicillin-binding ability of PBPs by the antisera. Rabbit antisera against β-lactamases from *P. morganii* and *P. rettgeri* were prepared as described previously (2). The antisera have strict specificities against β-lactamases from the homologous species of *Proteus* (2). Membrane preparations described above (30 μl, 600 μg of protein) were mixed with either of the antisera (3 μl, 290 μg of protein) and incubated at 30°C for 10 min. Then [^{14}C]penicillin G was added, and further binding procedures were carried out as described above. Control experiments with normal rabbit serum and membrane preparations were carried out simultaneously.

RESULTS

Gel electrophoretic pattern of [^{14}C]penicillin G-binding proteins. PBPs in three species of *Proteus* showed similar electrophoretic patterns on an SDS-polyacrylamide gel (Fig. 1A, B, and C, lane b) but slight differences from those of PBPs in *E. coli* (Fig. 1A, B, and C, lane a).

Seven major proteins were detected in each species of *Proteus*. We have tentatively designated PBPs of *Proteus* species, in order of increasing mobility (or from top to bottom in Fig. 1), as PBP-1A, -1B(s), -2, -3, -4, -5(s), and -6. We assume that *P. mirabilis* has a PBP-2 and a PBP-3, but they are not resolved by the electrophoresis conditions used (Fig. 1A, lane b). In *P. rettgeri* (Fig. 1C, lane b), two bands in the region of PBP-5 were observed.

To correlate the PBPs of *Proteus* species with those of *E. coli*, affinities of various β-lactam antibiotics for each PBP were determined by a competition experiment. Figure 1 shows the binding of [^{14}C]penicillin G to PBPs in *Proteus* species in the presence of a fivefold excess of mecillinam (lane c) or cephaloridine (lane d). Mecillinam, which induced formation of ovoid cells in normal rod-shaped cells of all three *Proteus* species tested, specifically competed with [^{14}C]penicillin G for PBP-2 in *Proteus*. Since this antibiotic exclusively binds to PBP-2 in *E. coli* (13, 16) and causes similar morphological

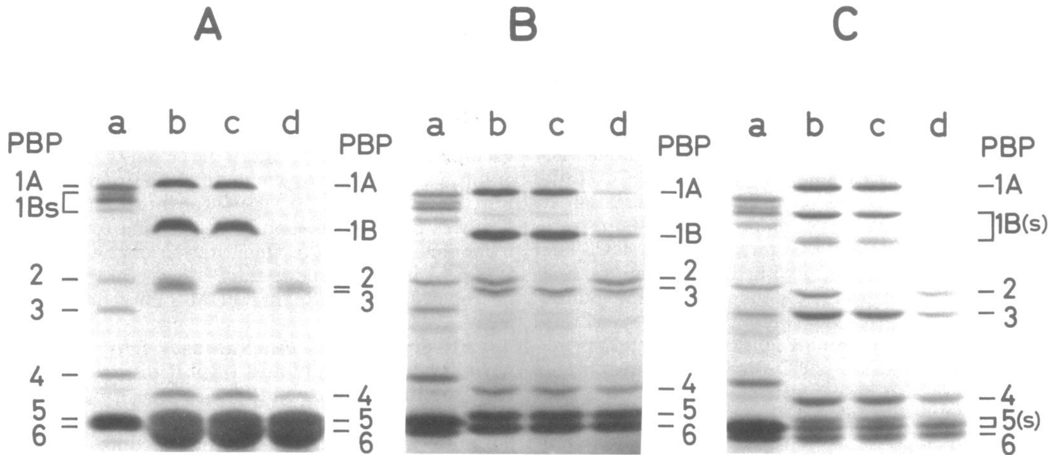

FIG. 1. *Gel electrophoretic patterns of [14C]penicillin G-binding proteins in Proteus species and in E. coli. Membrane preparation from P. mirabilis (A), P. morganii (B), or P. rettgeri (C) was mixed with [14C]penicillin G and distilled water (b), mecillinam (c), or cephaloridine (d) and incubated at 30°C for 10 min. The PBPs of an E. coli K-12 strain (JE1011) are included for comparison (a). The reaction was terminated, the samples were loaded on an SDS-polyacrylamide slab gel, and a fluorograph was prepared by exposure of the gel to X-ray film as described in the text. A fivefold excess amount (molar ratio) of the antibiotics added to [14C]-penicillin G was used.*

changes in *E. coli* (7), PBP-2 in each *Proteus* should correspond to PBP-2 in *E. coli*.

Somewhat more complicated patterns were obtained with a group of proteins with lower electrophoretic mobilities. Two major proteins, PBP-1A and -1B, were detected in *P. mirabilis* and *P. morganii*, and three major proteins, PBP-1A and PBP-1B(s), were detected in *P. rettgeri*. Moreover, a minor band was detected between the two bands in *P. mirabilis* and *P. morganii*. Since cephaloridine, which predominantly binds to PBP-1A and -1Bs in *E. coli* and inhibits cell elongation (13), also bound to both PBP-1A and -1B(s) in *Proteus*, these *Proteus* PBPs may be involved in cell elongation also. In the case of *E. coli* PBPs, PBP-1A and -1Bs were easily distinguished from each other by specific binding of β-lactam antibiotics. Cephalosporins such as cephalothin, cephalexin, cephaloglycine, cefazolin, and cephalosporin C showed a higher affinity for PBP-1A than for PBP-1Bs in *E. coli*. On the contrary, penicillins such as ampicillin, amoxicillin, sulbenicillin, and carbenicillin showed much higher affinities for PBP-1Bs than for PBP-1A in *E. coli* (11). In *Proteus*, specific bindings of these antibiotics to PBP-1A and -1B(s) could not be clearly reproduced. Therefore, it is difficult to correlate PBP-1A and -1B(s) in *Proteus* species to PBP-1A and -1Bs in *E. coli* by specific binding of the antibiotics. Recently, however, it was found that PBP-1A and -1Bs in *E. coli* have different thermostabilities (J. Nakagawa, H. Matsuzawa and M. Matsuhashi, submitted for publication). PBP-1A was thermolabile and PBP-1Bs were very thermostable with respect to penicillin-binding activities. Thus, the thermostabilities of *Proteus* PBPs were determined (Fig. 2). PBP-1A in *P. morganii* and *P. rettgeri* completely lost its activity for [14C]penicillin G binding when it was incubated at 48°C for 10 min. On the contrary, the activity of PBP-1B(s) in *Proteus* for binding of [14C]penicillin G was not affected by incubating even at 55°C for 10 min. These results suggest that PBP-1A in *Proteus* species corresponds to PBP-1A in *E. coli*, and PBP-1B(s) in *Proteus* species corresponds to PBP-1Bs in *E. coli*. In *E. coli*, using a mutant lacking PBP-1Bs, evidence was provided indicating that PBPs that disappear upon heating do not chase into PBP-1Bs that are more heat stable. Lack of a similar mutant in *Proteus* prevented us from obtaining similar evidence.

Cloxacillin, which induces filamentous cells from normal rod-shaped cells of all three *Proteus* species tested, showed a strong competition for PBP-3 in *Proteus* (Table 1). Other antibiotics which cause filament formation, such as apalcillin and piperacillin, also showed high affinity for PBP-3 in *Proteus*. This PBP may therefore correspond to PBP-3 in *E. coli*, which has been shown to be the target to which β-lactams bind to cause filamentation (13).

CS-1170 and cefoxitin, both cephamycin antibiotics, which have a methoxyl group at the 7α-position of a cephalosporin skeleton, competed for all the *Proteus* PBPs, with the exception of PBP-2 (Table 1). The most prominent

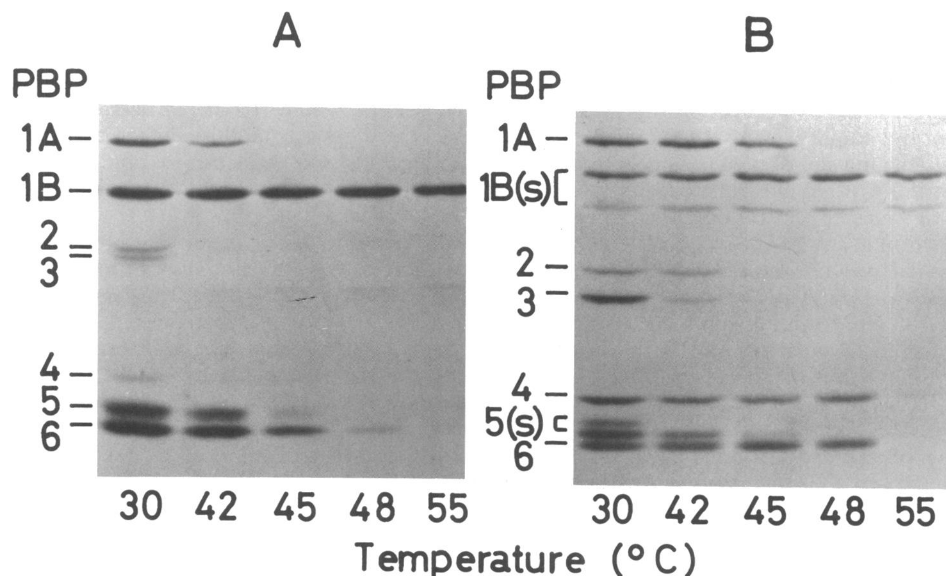

Fig. 2. *Thermostability of the PBPs in P. morganii (A) and P. rettgeri (B). Membranes from P. morganii and P. rettgeri were pretreated at 30, 42, 45, 48, or 55°C for 10 min, and the [^{14}C]penicillin G-binding experiment was performed as described in the text.*

Table 1. *Competition of antibiotics for [^{14}C]penicillin G binding to membrane from P. mirabilis, P. morganii, or P. rettgeri*

Membrane prep from:	Antibiotic[a]	Residual binding of [^{14}C]penicillin G (%) to PBP:							
		1A	1B(s)		2	3	4	5	6
P. mirabilis[b]	Cloxacillin	32	46		35		27	36	16
	CS-1170	8	1		38		10	19	11
	R-45656	9	12		44		51	79	83
P. morganii	Cloxacillin	25	19		40	0	21	16	8
	CS-1170	18	11		105	22	10	10	4
	R-45656	14	9		85	28	79	86	95
P. rettgeri[c]	Cloxacillin	39	70	75	89	5	43	42	27
	CS-1170	18	21	19	102	20	15	12	16

[a] A fivefold excess amount (molar ratio) added to [^{14}C]penicillin G was used.

[b] PBP-2 and -3 in *P. mirabilis* could not be separated from each other on SDS-polyacrylamide gel under the conditions used, and the data are expressed as combined binding of [^{14}C]penicillin G to PBP-2 and -3.

[c] In *P. rettgeri*, two major bands were identified as PBP-1B(s), and the bindings were measured separately.

nature of these antibiotics is their high affinity for the low-molecular-weight PBPs, PBP-4, -5, and -6. Among the penicillins and cephalosporins tested (penicillin G, cloxacillin, ampicillin, methicillin, mecillinam, cephaloridine, cephalothin, cefazolin, and cephaloglycine), only penicillin G and cloxacillin showed affinity for the low-molecular-weight PBPs, but most other antibiotics had exceedingly low affinities for these proteins. The high affinities of CS-1170 for PBP-4, -5, and -6 disappeared when the 7α-methoxyl group was substituted by a hydrogen atom (R-45656) (Table 1). These relationships were completely in agreement with results obtained with *E. coli* (12) and suggest a correspondence of the low-molecular-weight PBPs among *Proteus* species and those of *E. coli*.

Release of [^{14}C]penicillin G from PBP-5 and -6 in *Proteus*. Spratt observed that [^{14}C]-penicillin G that had bound to PBP-5 or -6 of *E. coli* was readily released from the proteins by further incubation, and the release from PBP-5 was much faster than that from PBP-6 (14). Practically no release of [^{14}C]penicillin G from other *E. coli* PBPs could be observed (14). In *E. coli* PBP-5 has been identified as D-alanine carboxypeptidase IA by Matsuhashi et al. (10). It is established that release of [^{14}C]penicillin G from penicilloyl-PBP-5 and -6 is due to a weak β-lactamase reaction catalyzed by the proteins

(20). Observations by Curtis and Strominger (1) suggest that release of [^{14}C]penicillin G from the penicilloyl-PBP occurs in the same manner as the release of the *N*-acetylmuramyl(MurNac)-tetrapeptidyl moiety from the MurNAc-tetrapeptidyl-enzyme complex to the acceptor amino group in the peptidoglycan transpeptidase reaction or to water in the D-alanine carboxypeptidase reaction (17).

Figure 3 shows the release of [^{14}C]penicillin G from PBP-4, -5, and -6 in three species of *Proteus*. PBP-5 and -6 in all three species released [^{14}C]penicillin G, whereas PBP-4 released very little [^{14}C]penicillin G. Therefore, it may be concluded that *Proteus* PBP-5 and -6 correspond to *E. coli* PBP-5 and -6.

Effect of rabbit antisera against β-lactamases from *P. morganii* and *P. rettgeri* on binding of [^{14}C]penicillin G to PBPs. As described above, the reaction mechanism of β-lactamases is similar to that of penicillin-sensitive transpeptidases or carboxypeptidases involved in the peptidoglycan cross-linking reaction (4). PBPs, at least some of them, seem to be identical to enzymes carrying out transpeptidation or carboxypeptidation in *Proteus* also.

It is therefore expected that β-lactamases and some of the PBPs from homologous bacteria may have a common part in protein molecules. If this is the case, β-lactamases and certain PBPs might originate from a common origin.

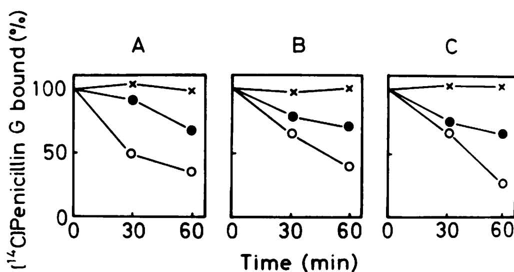

FIG. 3. *Release of bound [^{14}C]penicillin G from PBP-4, -5, or -6 in P. mirabilis (A), P. morganii (B), and P. rettgeri (C). Thirty microliters of a 50-μCi/ml amount of [^{14}C]penicillin G and 50 μl of distilled water were added to 20 μl of a preparation of membranes, and the mixture was incubated for 10 min at 30°C. Nonradioactive penicillin G (8.9 mg) was added, and 30-μl samples were removed immediately and at intervals during continued incubation at 30°C. Three microliters of 20% (wt/vol) Sarkosyl was added to each sample to prevent further release of bound [^{14}C]penicillin G, the Sarkosyl-soluble proteins were fractionated on an SDS-polyacrylamide slab gel, and a fluorograph was prepared. The level of [^{14}C]penicillin G remaining bound to each PBP was measured by microdensitometry of the X-ray film. Symbols:* ×, *PBP-4;* ○, *PBP-5;* ●, *PBP-6.*

Effects of antisera against β-lactamases from *P. morganii* and *P. rettgeri* on [^{14}C]penicillin G binding to homologous as well as heterologous PBPs were tested. If the antisera cross-react with a PBP, this should be detected by the loss of the ability of the PBP to bind radioactive penicillin. A broad band of [^{14}C]penicillin G-protein complex appeared between the positions of PBP-1Bs and -2 (*E. coli*) or those of PBP-1B(s) and -2 (*Proteus* species) by addition of normal rabbit serum or one of the anti-β-lactamase sera. However, detectable differences in binding patterns of PBPs could not be observed either between the "with antiserum" and "without antiserum" runs or between the "with normal serum" and "with antiserum" runs (data not shown).

DISCUSSION

All three *Proteus* species tested, *P. mirabilis*, *P. morganii*, and *P. rettgeri*, showed patterns of PBPs on electrophoresis in SDS-polyacrylamide slab gel fundamentally similar to those shown by *E. coli* PBPs. From the slowest moving protein they were numbered as *Proteus* PBP-1A, -1B(s), -2, -3, -4, -5(s), and -6. By measuring the affinities of various cephalosporins and penicillins and the thermostabilities of PBPs, PBP-1A and -1B(s) in *Proteus* seemed to correspond to PBP-1A and -1Bs in *E. coli*, respectively. However, to establish correlation between PBPs of *Proteus* and *E. coli*, genetic work is required which provides correlation between defect of a PBP and change of the phenotype of the cell. In earlier experiments with *E. coli*, PBP-1A and -1Bs could not be separated by SDS-polyacrylamide slab gel electrophoresis under the conditions originally used by Spratt and Pardee (16), and sometimes their order of mobilities was even reversed. Therefore, it is difficult to relate the electrophoretic mobilities of the appropriate proteins to their molecular weights.

Correlation of PBP-2 and -3 of *Proteus* to those of *E. coli* was estimated from specific binding affinities of appropriate β-lactam antibiotics which cause specific morphological changes in both bacteria.

PBP-5(s) and -6 of all three *Proteus* species were shown to have in vitro ability to release [^{14}C]penicillin G presumably by their β-lactamase-like activities (20).

Schilf and Martin demonstrated two D-alanine carboxypeptidase activities in membranes of *P. mirabilis*, one with high and another with low sensitivity toward penicillin G. These enzymes decomposed penicillin G that had bound to the enzymes. The half-lives of the enzyme-penicillin G complexes at 30°C were determined as 5 min

for the enzyme of lower penicillin sensitivity and 500 min for the enzyme of higher penicillin sensitivity (W. Schilf and H. H. Martin, Abstr. Tübingen Symp. Function Microb. Membr., 1977). It is probable from our experiments on [^{14}C]penicillin G release from PBPs of *Proteus* that the enzyme with low penicillin sensitivity may correspond to PBP-5, but more work is necessary to confirm this.

The nature of PBP-4 in *Proteus* is not precisely determined yet. Similar to *E. coli*, [^{14}C]CS-1170, a cephamycin antibiotic, bound strongly to this protein, but affinity of its 7α-H analog (R-45656) to this protein was far lower. From observations using these and other antibiotics, it may be inferred that *Proteus* PBP-4 correlates to PBP-4 in *E. coli*, which is highly penicillin-sensitive D-alanine carboxypeptidase IB, but correlation of the enzyme activity with PBP-4 is needed.

A relation between β-lactamases and PBPs is suggested by the resemblance of the reaction mechanisms of β-lactamases and penicillin-sensitive transpeptidases or D-alanine carboxypeptidases (17). It is probable that some of PBPs and β-lactamases originate from a common gene. Tests for binding of anti-β-lactamase antisera to PBPs so far, however, have not shown any evidence for remarkable binding of the antisera to any of the PBPs tested.

LITERATURE CITED

1. **Curtis, S. J., and J. L. Strominger.** 1978. Effects of sulfhydryl reagents on the binding and release of penicillin G by D-alanine carboxypeptidase IA of *Escherichia coli*. J. Biol. Chem. **253**:2584-2588.
2. **Fujii-Kuriyama, Y., M. Yamamoto, and S. Sugawara.** 1977. Purification and properties of beta-lactamase from *Proteus morganii*. J. Bacteriol. **131**:726-734.
3. **Iwaya, M., and J. L. Strominger.** 1977. Simultaneous deletion of D-alanine carboxypeptidase IB-C and penicillin-binding component IV in a mutant of *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S.A. **74**:2980-2984.
4. **Izaki, K., M. Matsuhashi, and J. L. Strominger.** 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. XIII. Peptidoglycan transpeptidase and D-alanine carboxypeptidase: penicillin-sensitive enzymatic reaction in strains of *Escherichia coli*. J. Biol. Chem. **243**: 3180-3192.
5. **Laemmli, U. K., and M. Favre.** 1973. Maturation of the head of bacteriophage T4. I. DNA packaging events. J. Mol. Biol. **80**:575-599.
6. **Laskey, R. A., and A. D. Mills.** 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56**:335-341.
7. **Lund, F., and L. Tybring.** 1972. 6β-Amidinopenicillanic acids—a new group of antibiotics. Nature (London) New Biol. **236**:135-137.
8. **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.
9. **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.
10. **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.
11. **Matsuhashi, M., S. Tamaki, S. J. Curtis, and J. L. Strominger.** 1979. Mutational evidence for identity of penicillin-binding protein 5 in *Escherichia coli* with the major D-alanine carboxypeptidase IA activity. J. Bacteriol. **137**:644-647.
12. **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.
13. **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.
14. **Spratt, B. G.** 1977. Properties of the penicillin-binding proteins of *Escherichia coli* K12. Eur. J. Biochem. **72**: 341-352.
15. **Spratt, B. G., V. Jobanputra, and U. Schwarz.** 1977. Mutants of *Escherichia coli* which lack a component of penicillin-binding protein 1 are viable. FEBS Lett. **79**: 374-378.
16. **Spratt, B. G., and A. B. Pardee.** 1975. Penicillin-binding proteins and cell shape in *E. coli*. Nature (London) **254**: 516-517.
17. **Strominger, J. L., K. Izaki, M. Matsuhashi, and D. J. Tipper.** 1967. Peptidoglycan transpeptidase and D-alanine carboxypeptidase: penicillin-sensitive enzymatic reactions. Fed. Proc. **26**:9-22.
18. **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.
19. **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.
20. **Tamura, T., Y. Imae, and J. L. Strominger.** 1976. Purification to homogeneity and properties of two D-alanine carboxypeptidases I from *Escherichia coli*. J. Biol. Chem. **251**:414-423.