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Penicillin-Binding Protein 2 Is Essential for the Integrity of Growing Cells of *Escherichia coli ponB* Strains

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Analysis of *Escherichia coli pbpA*(Ts) or *rodA*(Ts) strains defective for penicillin-binding protein (PBP) 1A or PBP 1B indicated that the activity of PBP 2 is essential to prevent cell lysis in PBP 1B⁻ strains and suggested that PBP 2 is active or activatable in *rodA*(Ts) mutants under restrictive conditions.

In *Escherichia coli* seven genetically defined penicillin-binding proteins (PBPs) have been identified. The three smaller proteins (PBPs 4, 5, and 6) are apparently dispensable. In contrast, high-M_r PBPs (PBPs 1A, 1B, 2, and 3) play essential roles (9). Presumably all four high-M_r PBPs are bifunctional enzymes with transglycosylase and DD-transpeptidase activities (2, 4, 11, 13). However, each one plays specific functions, and they differ greatly in the ways their inhibition causes cell death (2, 9). PBPs 1A and 1B are able to functionally complement for each other, but cell lysis is triggered when both are simultaneously inactivated. Inhibition of PBP 2 leads to the development of spherical morphology, impairment of cell division, and loss of viability, whereas inhibition of PBP 3 results in blocked septation and the formation of mutinucleated filaments, which eventually die (9).

It has been shown that PBP 1B⁻ strains are hypersensitive to mecillinam, a β-lactam with high selectivity of binding to PBP 2 (1). In the presence of mecillinam, wild-type and *ponA* strains (PBP 1A⁻) develop spherical morphology but cell integrity is preserved. However, *ponB* mutants (PBP 1B⁻) lyse when treated similarly. This suggests that PBP 1A requires the concerted action of PBP 2 to compensate for the lack of activity of PBP 1B and to allow cell enlargement without lysis. Even though mecillinam is highly selective for binding to PBP 2 in *Escherichia coli* (1, 8, 9), the partial inhibition of other PBPs or unknown side effects cannot be ruled out.

To study the functional interrelations among PBPs 1 and 2, double mutants were constructed by transducing defective alleles of *ponA* or *ponB* into strains harboring thermosensitive mutations in *pbpA* or *rodA*, which code for PBP 2 and RodA (a protein required for activity of PBP 2 [3]), respectively.

Cells of *E. coli* SP4500 [K-12 F⁻ *his pro purB thi mtl xyl galK lacY rpsL pbpA45*(Ts)] and SP5211 [K-12 F⁻ *his pro purB thi mtl xyl galK lacY rpsL rodA52*(Ts)] (10) were infected with $P1_{virA}$ phages grown on *E. coli* SP1026 (K-12 F⁻ *his lac tsx supF rpsL ponB*::Spc^r) and SP1027 (K-12 F⁻ *his lac tsx supF rpsL ponA*::Kan^r) (14) as described previously (6). Transductants were selected by their ability to grow on L-broth (5) plates containing kanamycin (30 μg/ml) or spectinomycin (40 μg/ml) for the PBP 1A⁻ or PBP 1B⁻ mutants, respectively. Selected transductants were checked for lack of the corresponding PBP as described previously (8). Resistance to mecillinam was checked on freshly made L-agar plates containing mecillinam at 10 μg/ml.

Expression of *pbpA*(Ts) alleles results in a thermosensitive form of PBP 2 in which the ability to bind β-lactams is impaired at 42°C. Mutant cells are viable at both 30 and 42°C,

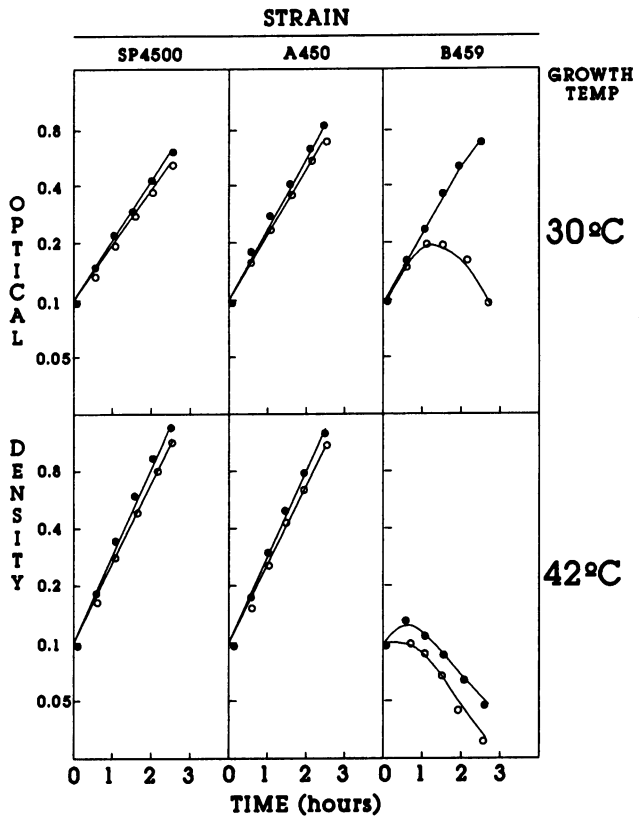

FIG. 1. Effect of temperature and mecillinam on the growth of *E. coli* SP4500, A450, and B459. Aliquots from cultures of *E. coli* SP4500 [*pbpA*(Ts)], A450 [*ponA pbpA*(Ts)], and B459 [*ponB pbpA* (Ts)] exponentially growing in LB at 30°C were diluted 1:8 in L broth prewarmed at 30 or 42°C and supplemented (○) or not supplemented (●) with mecillinam (10 μg/ml for strains SP4500 and A450; 2 μg/ml for strain B459). Cultures were further incubated at the corresponding temperatures, and samples were periodically withdrawn to measure the optical density at 550 nm and to assess cell morphology. The MIC of mecillinam for strain SP4500 at 30°C was about 1 μg/ml, as determined by using serial dilutions on L-agar plates.

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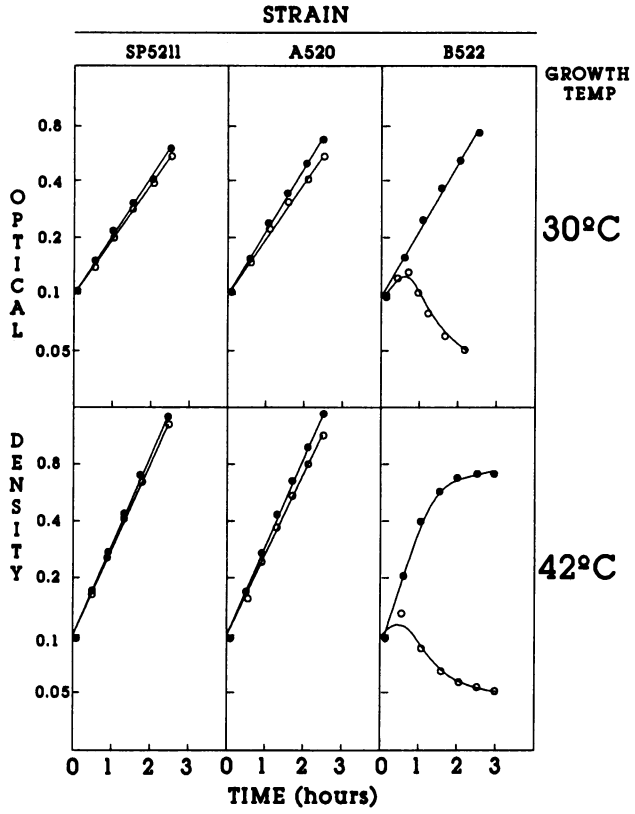

FIG. 2. Effect of temperature and mecillinam on the growth of *E. coli* SP5211, A520, and B522. Aliquots from cultures of *E. coli* SP5211 [*rodA*(Ts)], A520 [*ponA rodA*(Ts)], and B522 [*ponB rodA* (Ts)] exponentially growing in LB at 30°C were diluted 1:8 in L broth prewarmed at 30 or 42°C and supplemented (○) or not supplemented (●) with mecillinam (10 μg/ml for strains SP5211 and A520; 2 μg/ml for strain B522). Cultures were further incubated at the corresponding temperatures, and samples were periodically withdrawn to measure the optical density at 550 nm and to assess cell morphology. The MIC of mecillinam for strain SP5211 at 30°C was 1.2 to 1.5 μg/ml, as determined by using serial dilutions on L-agar plates.

but at 42°C the cells develop spherical morphology and resistance to mecillinam (10, 12). However, deletion of *pbpA* is lethal (7).

Thermosensitive *rodA* mutants share the phenotype of *pbpA*(Ts) mutants in all respects, except that PBP 2 retains its ability to bind β-lactams (10, 12). A direct PBP 2-RodA interaction seems to be necessary for the activity of PBP 2 (3).

The behavior of *pbpA*(Ts) strains defective for PBP 1A (strain A450) or 1B (strain B459) is shown in Fig. 1. At the permissive temperature (30°C), both double mutants and SP4500, the parental *pbpA*(Ts) strain, grew and divided normally. The addition of mecillinam to cultures of SP4500 [*pbpA*(Ts)] and A450 [*ponA pbpA*(Ts)] elicited a normal response to the antibiotic, leading to formation of large spheroid cells. In contrast, cultures of B459 [*ponB pbpA*(Ts)] lysed when treated with mecillinam, as expected (1).

The lack of PBP 1A had no obvious detrimental consequences for *pbpA*(Ts) strains at the restrictive temperature (42°C). In contrast, B459 [*ponB pbpA*(Ts)] lysed immediately after being transferred to 42°C, revealing that inactivation of PBP 2 was lethal to *ponB* strains.

These observations support the hypothesis that mecillinam-induced lysis of *ponB* mutants is due to the inhibition of PBP 2 and suggest that PBP 1A needs the concerted action of PBP 2 to permit the enlargement of *ponB* cells.

The results of similar experiments performed with *ponA* and *ponB* derivatives of SP5211 [*rodA*(Ts)] (strains A520 and B522, respectively) are displayed in Fig. 2. The behaviors of both strains were very similar to that of the equivalent *pbpA*(Ts) derivatives, with the exception of B522 [*ponB rodA*(Ts)] at 42°C. This strain was able to grow in mass and divide for about 2 h (roughly two doubling times) after the temperature shift-up. However, the addition of mecillinam triggered cell lysis, in spite of the resistance to this β-lactam normally conferred by *rodA*(Ts) mutations at 42°C.

The differences observed in the response of B459 [*ponB pbpA*(Ts)] and B522 [*ponB rodA*(Ts)] suggest that PBP 2 might be active in *rodA*(Ts) mutants at the restrictive temperature. Since the *rodA*(Ts) allele present in SP5211 confers resistance to mecillinam, this assumption implies that in *rodA*(Ts) strains at the restrictive temperature either PBP 2 is active but dispensable (except in a *ponB* background) or PBP 2 can be forced into activity when PBP 1B is impaired.

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