PENICILLIN-BINDING PROTEINS IN *BACTEROIDES FRAGILIS*

NAFSIKA H. GEORGOPAPADAKOU, SANDRA A. SMITH and RICHARD B. SYKES

The Squibb Institute for Medical Research
P.O. Box 4000, Princeton, New Jersey 08540, U.S.A.

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The penicillin binding proteins (PBSs) of *Bacteroides fragilis*, a clinically important Gram-negative rod, were studied. Four PBPs were detected by polyacrylamide gel electrophoresis/fluorography, PBP 4 (molecular weight, 35,000) being a minor PBP. The PBP pattern was thus different from that of the Enterobacteria and Pseudomonads. Antibacterial activity of β-lactam antibiotics was associated with binding to PBP 1 (molecular weight, 100,000), 2 (molecular weight, 86,000) and 3 (molecular weight, 68,000). Binding to PBP 2 was associated with filamentation while binding to PBP 1 resulted in cell lysis.

Penicillin and other β-lactam antibiotics kill growing bacteria by binding covalently to specific membrane proteins involved in cell wall biosynthesis[1,2]. These penicillin-binding proteins (PBPs) have been extensively studied in *Escherichia coli*, where essential PBPs have been identified and their functions elucidated[3,4]. They are all peptidoglycan transpeptidases involved in cell elongation, shape and septation[5~8]. *E. coli* PBPs are representative of enterobacterial and pseudomonal PBPs. Other aerobic bacteria have different PBP patterns (amounts, molecular weights, β-lactam binding profiles) and different essential PBPs[9]. Studies with anaerobic bacteria, such as Clostridia, have only recently appeared in the literature[10,11].

Bacteroides fragilis is an anaerobic Gram-negative organism implicated in a wide range of infections especially following abdominal trauma or surgery[12]. It is generally resistant to β-lactam antibiotics, resistance being usually attributed to the presence of β-lactamases[13]. Cefoxitin and the oxacephalosporin moxalactam (latamoxef) are the only currently available β-lactams effective against this organism.

In the present study the PBP patterns of eight *B. fragilis* strains were examined in order to establish the PBP pattern for that species. Subsequently, the PBP binding patterns of several structurally diverse β-lactam antibiotics were correlated with antibacterial activity in order to determine essential PBPs.

Materials and Methods

Organisms and Culture Conditions

The *B. fragilis* strains used in the present study were clinical isolates from the Squibb Culture Collection. They were grown anaerobically in chopped meat carbohydrate broth (Scott Laboratories, Fiskeville, R. I.) at 37°C for 16 hours in static culture. Cells were harvested by centrifugation, sonicated for 3 minutes and membranes were solubilized (2% Triton X-100) as previously described[9].

PBP Assay

Solubilized membranes (150 mg protein, determined according to LOWRY *et al.*[14]) were incubated with the appropriate β-lactam antibiotic at 30°C for 10 minutes in a total volume of 50 μl. Then 10 n-mol of [8-^{14}C]benzylpenicillin (Amersham-Searle, Arlington Heights, Ill.; specific activity, 51 μCi/μmol) was added and the incubation was continued for 10 minutes. PBPs were visualized after sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis[15] (running gel, 10% acrylamide - 0.2% Bis) and fluorography[16].

Morphological Response

For morphology studies, the organism was grown (0.1% inoculum from a 16-hour culture) in chopped meat carbohydrate broth with the appropriate antibiotic at 37°C for 3 hours. Cells were stained with 1% methylene blue diluted 1: 25 with water and were examined under light microscope.

Results and Discussion

In all eight *B. fragilis* strains three PBPs of molecular weights 100,000, 86,000, and 65,000 daltons were detectable (Table 1). In three strains a 32,000 dalton PBP with weak penicillinase activity (half-life of release of bound benzylpenicillin 10 minutes) was also detectable. This PBP is analogous to the low molecular weight PBP (range: 35,000~45,000 daltons) with DD-carboxypeptidase and weak penicillinase activity found in most bacteria[17]. Three strains had a very minor PBP of molecular weight 78,000 daltons.

The PBP binding of β-lactam antibiotics in two strains, SC 11,318 relatively sensitive to β-lactam antibiotics and SC 11,085 relatively resistant, was next examined. Fig. 1 represents a typical experiment. PBP 1 had the lowest affinity for β-lactam antibiotics, binding primarily to thienamycin (Table 2). PBP 2 had the highest affinity, and binding correlated with minimum inhibitory concentrations (MICs). It is noted that MICs were determined at the level of 10^2 colony-forming units (CFU) to minimize β-

Table 1. PBP profiles in *B. fragilis*.

Strain SC No.	Molecular weight (×1,000)				
	PBP 1	PBP 2	PBP 2′	PBP 3	PBP 4
11,033	100	86	78	65	ND
11,034	100	86	78	65	ND
11,085	100	86	ND[a]	65	32
11,086	113	100	ND	65	ND
11,317	100	86	ND	65	ND
11,318	100	86	ND	65	ND
12,664	110	86	ND	65	32
12,678	110	86	78	65	32

[a] ND; Not detected.

Table 2. Binding of β-lactam antibiotics to PBPs of *B. fragilis* SC 11,318 and SC 11,085.

Compound	To completely inhibit [^{14}C]benzylpenicillin binding (μg/ml)								MIC (μg/ml) 10^2 CFU	
	PBP 1-100,000		PBP 2-86,000		PBP 3-65,000		PBP 4-32,000			
	11,318	11,085	11,318	11,085	11,318	11,085	11,318	11,085	11,318	11,085
Clavulanic acid	>100	>100	>100	>100	30	30	ND[a]	0.1	12	6
Mecillinam	>100	>100	>100	>100	>100	>100	ND	>100	>100	>100
Piperacillin	30	30	2.0	30	10	10	ND	>100	2	12
Cefoperazone	>100	100	10	>100	30	100	ND	>100	6	100
Cefotaxime	>100	>100	2.0	30	>100	>100	ND	>100	2	50
Ceftazidime	>100	>100	10	>100	>100	>100	ND	>100	6	>100
Cefoxitin	>100	>100	10	10	30	30	ND	30	3	3
Moxalactam	30	>100	0.5	0.5	30	30	ND	30	3	6
Azthreonam	>100	>100	100	100	>100	>100	ND	>100	50	>100
N-Formimidoyl-thienamycin	10	10	0.5	10	2.0	2.0	ND	0.1	<0.05	0.1

[a] ND; Not detected.

Fig. 1. Binding of clavulanic acid to *B. fragilis* PBPs.
Concentrations of clavulanic acid (μg/ml) are indicated. C denotes control.

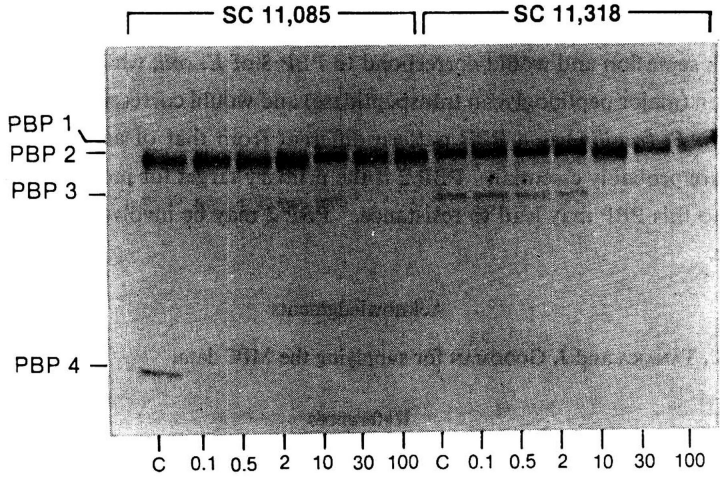
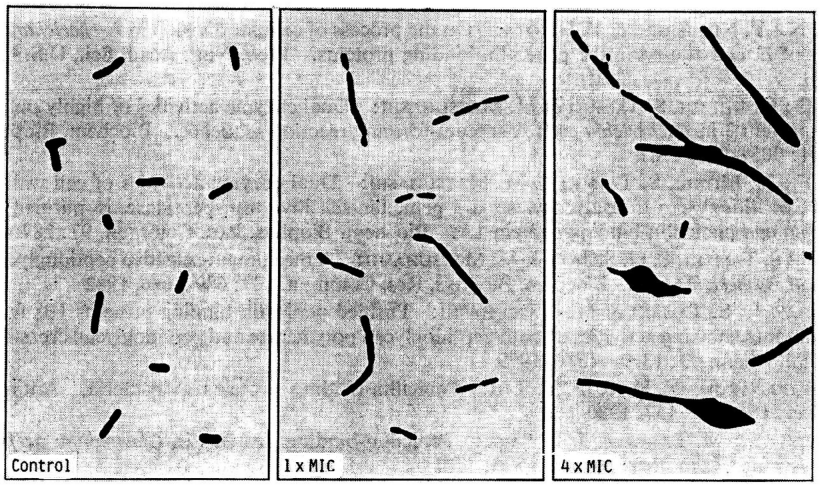


Fig. 2. Effect of *N*-formimidoylthienamycin on *B. fragilis* SC 11,085 morphology.
Concentrations are indicated as multiples of MIC.



lactamase interference, which is relevant to all β-lactam antibiotics examined except for moxalactam, cefoxitin and *N*-formimidoylthienamycin[18]. In the resistant strain, SC 11,085, PBP 2 had reduced affinity for piperacillin, cefoperazone, cefotaxime, ceftazidime and thienamycin. Binding to PBP 3 was not similarly affected in this strain, indicating that the decreased binding to PBP 2 was not the result of β-lactamase activity. PBP 3 had intermediate affinity for β-lactam antibiotics. PBP 4, detectable only in SC 11,085, bound mainly to clavulanic acid and thienamycin. None of the PBPs has affinity for mecillinam. MICs correlated with binding to PBP 1 (thienamycin *vs.* moxalactam), PBP 2 (moxalactam *vs.* cefotaxime) and PBP 3 (clavulanic acid *vs.* ceftazidime). Therefore, these PBPs may be essential. However, PBP 2 appears to be the primary target for β-lactam antibiotics by virtue of its sensitivity. *N*-Formimidoylthienamycin, cefotaxime, and moxalactam were further studied for effects on cell morphology of *B. fragilis* SC 11,085. *N*-Formimidoylthienamycin caused filaments at 0.1 μg/ml and bulges at 0.4 μg/ml (Fig. 2). Cefotaxime and moxalactam caused filaments at 2.0 μg/ml and 0.5 μg/ml, respec-

tively, and no additional morphological effects up to 20 μg/ml. It is noted that cefoxitin, which has a PBP binding pattern identical to that of moxalactam, also produces filaments[10]. Thus, filamentation appears to correlate with binding to PBP 2 while bulging with binding to PBP 1. PBP 2 may therefore be associated with septation and would correspond to PBP 3 of *E. coli*, while PBP 1 may be associated with cell elongation (major peptidoglycan transpeptidase) and would correspond PBP 1 of *E. coli*.

In conclusion, *B. fragilis* has a PBP pattern different from that of aerobic Gram-negative rods. PBPs 1, 2 and 3 are probably essential. PBP 2 is the primary target for most β-lactam antibiotics and reduced binding to this PBP may lead to resistance. PBP 2 may be involved in septation while PBP 1 in cell elongation.

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