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Biochemical Properties of β-Lactamase Produced by *Legionella gormanii*

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β-Lactamase was purified from a strain of *Legionella gormanii*. The molecular weight of the purified enzyme was 25,000, and its isoelectric point was 10.5. The enzyme hydrolyzed oxyiminocephalosporins, cephamycins, penicillins, and imipenem. The enzyme activity was inhibited by EDTA, Hg^{2+}, and Cu^{2+}, but not by clavulanic acid, sulbactam, or imipenem.

Legionella pneumophilia and other *Legionella* species have been isolated with increasing frequency from clinical specimens (1, 6). β-Lactamases produced by gram-negative and gram-positive bacteria play a significant role in resistance against β-lactam antibiotics (8, 10). Several investigations have already described β-lactamases from *Legionella* species (4, 9). However, there has been no report on the purification and biochemical properties of the β-lactamase from *Legionella gormanii*. This paper deals with the purification and some properties of β-lactamase from *L. gormanii* ATCC 33297.

Ampicillin, carbenicillin, cloxacillin, cefazolin, cephalexin, cephaloridine, cephalothin, and penicillin G were commercially available materials. The following compounds were received as gifts from their manufacturers: aztreonam, cefmenoxime, cefoperazone, cefotaxime, cefoxitin, ceftizoxime, cefuroxime, clavulanic acid, imipenem, moxalactam, and sulbactam.

MICs were determined by the agar dilution method. BCYE agar (Oxoid Ltd.) plates containing serial twofold dilutions of a drug were indicated with one loopful (ca. 0.05 ml) of 10^8 CFU/ml of a culture in BCYE broth. The MICs were scored after 48 h of incubation at 37°C.

Procedures for extraction of the enzyme were as follows. Bacterial cells on BCYE agar plates were suspended in 50 mM phosphate buffer (pH 7.0) and harvested by centrifugation. The cells were suspended in the buffer and disrupted sonically. The supernatant after centrifugation at 45,000 × *g* for 30 min at 4°C was used as the crude enzyme. Further purification was by the method of Saino et al. (11, 12). The crude enzyme was purified by chromatography on a carboxymethyl-Sepharose column, by gel filtration on a Toyopearl HW-55F (Toyo Soda Manufacturing Co., Ltd.) column, and by rechromatography on carboxymethyl-Sepharose.

Enzyme activity was determined by measuring the change in maximum absorption for the β-lactam ring in a temperature-controlled spectrophotometer (Beckman model 24) at 30°C (2, 7). Michaelis constants (K_m) and maximum rates of hydrolysis (relative V_{max}) were expressed as micromoles of substrate hydrolyzed per minute per milligram of protein and were determined from Lineweaver-Burk plots. Dissociation constants for enzyme-inhibitor complexes (K_i) for various compounds were determined by both Lineweaver-Burk and Dixon plots with cephaloridine as a substrate. Inhibition of enzymatic activity was estimated spectrophotometrically after preincubation with inhibitors for 5 min at 30°C with 0.1 mM cephaloridine as a substrate.

MICs for *L. gormanii* ATCC 33297 of ampicillin, carbenicillin, cephalothin, and cefoxitin were 1.56, 0.19, 0.39, and 0.19 μg/ml, respectively.

β-Lactamase from *L. gormanii* ATCC 33297 was purified about 90-fold and appeared homogeneous according to polyacrylamide gel electrophoresis. Physicochemical properties of the purified enzyme were as follows. The molecular weight of the enzyme, estimated from electrophoretic mobility in a sodium dodecyl sulfate-acrylamide gel (3), was about 25,000. The isoelectric point (pI) of the enzyme, determined from polyacrylamide gel electrophoresis, was 10.5. In a previous study the β-lactamase produced by

TABLE 1. Hydrolysis of various β-lactam antibiotics by β-lactamase from *L. gormanii* ATCC 33297

Substrate	V_{max} (μmol/min)	Relative V_{max}[a]	K_m (μM)
Cephaloridine	0.94	100	31.0
Cephalothin	1.49	158	157.9
Cefazolin	1.12	119	28.3
Cephalexn	0.05	5	29.2
Cefuroxime	0.85	90	162.0
Cefotaxime	0.42	45	55.0
Ceftizoxime	0.10	11	17.3
Cefmenoxime	0.26	28	31.2
Cefoperazone	0.26	28	38.9
Moxalactam	0.85	90	333.3
Cefoxitin	0.10	11	18.9
Penicillin G	0.07	7	13.0
Ampicillin	0.65	69	34.5
Carbenicillin	0.18	19	34.5
Cloxacillin	0.14	17	16.7
Imipenem	0.05	5	23.7
Aztreonam	$<9 \times 10^{-3}$	<1	
Clavulanic acid	$<9 \times 10^{-3}$	<1	—[b]
Sulbactam	$<9 \times 10^{-3}$	<1	—[b]

[a] Rates of hydrolysis are expressed relative to cephaloridine as 100.
[b] K_i values, determined using cephaloridine as substrate, were >100 μM.

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TABLE 2. Effect of inhibitors on the activity of β-lactamase from *L. gormanii* ATCC 33297

Inhibitor[a]	Concn (mM)	Inhibition (%)
Clavulanic acid	0.1	0
Sulbactam	0.1	0
Imipenem	0.01	0
Imipenem	0.1	5
EDTA	5	20
EDTA	10	55
Iodine	0.01	100
Hg^{2+}	0.1	100
Cu^{2+}	0.1	100
Fe^{2+}	0.1	0
Co^{2+}	0.1	0
*p*CMB[b]	0.1	86

[a] The enzyme was preincubated in 50 mM phosphate buffer (pH 7.0) for 5 min at 30°C with each inhibitor at the indicated concentration, and the remaining activity was assayed with 0.1 mM cephaloridine as substrate.
[b] *p*CMB, *p*-Chloromercuribenzoate.

another strain (LS-13) of *L. gormanii* (4) was found to have a pI between 7.6 and 8.0 and a molecular weight between 18,000 and 22,000. Whether the enzyme from *L. gormanii* ATCC 33297 differs from that produced by strain LS-13 will require further study of both β-lactamases under identical conditions.

According to kinetic parameters (V_{max}, relative V_{max}, K_m, K_i), the enzyme showed higher hydrolyzing activity against cephalosporins than against penicillins (Table 1). The enzyme showed a broad substrate profile and was capable of hydrolyzing oxyiminocephalosporins such as cefotaxime and cefmenoxime, cephamycins, and penicillins. Furthermore, the enzyme hydrolyzed imipenem. However, aztreonam was resistant to hydrolysis by the enzyme. Clavulanic acid and sulbactam had low affinities for the enzyme, as indicated by their high K_is in competitive inhibitions.

The effects of inhibitors on enzyme activity are shown in Table 2. The enzyme activity was inhibited by the EDTA, Hg^{2+}, and Cu^{2+}, but not by clavulanic acid, sulbactam, or imipenem.

Our previous studies established that oxyiminocephalosporins were hydrolyzed at high rates by β-lactamases from the *Bacteroides fragilis* group (14), *Proteus vulgaris* GN7919 (5), *Pseudomonas cepacia* GN11164 (2), and *Flavobacterium odoratum* GN14053 (13) and by L-1 and L-2 β-lactamases from *Pseudomonas maltophilia* GN12873 (11, 12). These enzymes can be devided into two subgroups, type I (*B. fragilis*, *P. vulgaris*, *P. cepacia*, and L-2 from *P. maltophilia*) and type II (*F. odoratum* and L-1 from *P. maltophilia*), by substrate and inhibitor profiles (S. Mitsuhashi, Program Abstr. 14th Int. Congr. Chemother., abstr. no. SL-2, 1985).

According to this classification, the *L. gormanii* ATCC 33297 β-lactamase belongs to type II.

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