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NOTES

Purification and Some Properties of a Cephalosporinase from *Proteus vulgaris*

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The purified cephalosporinase from *Proteus vulgaris* hydrolyzed a variety of cephalosporins, including cefuroxime, at a high level; its activity was inhibited by clavulanic acid.

Most *Proteus vulgaris* strains are resistant to β-lactam antibiotics, and, like other species of resistant strains, they produce β-lactamase. Many studies on cephalosporinases from gram-negative bacteria have been reported (2, 4, 8, 11, 15, 20, 21). We purified the cephalosporinase from *P. vulgaris* and investigated its properties, using various β-lactam compounds as substrates (3, 5, 10, 17).

P. vulgaris GN7919 was a clinical isolate and was used for cephalosporinase preparation because of its hyper-resistance to β-lactam antibiotics. Cefsulodin (SCE-129; Takeda Pharmaceutical, Osaka), cefamandole (Shionogi Pharmaceutical, Osaka), cefoperazone (T-1551; Toyama Chemical, Tokyo), cefoxitin (Daiichi Pharmaceutical, Tokyo), and cefuroxime (Shinnihon Jitsugyo, Tokyo) were kindly provided by the respective companies. The other β-lactam antibiotics were commercial products.

The cephalosporinase extraction and purification procedures were as described previously (4) (Table 1). The Michaelis constant (K_m) and the maximum rate of hydrolysis (V_{max}) were determined from Lineweaver-Burk plots. Dissociation constants of the enzyme-inhibitor complex (K_i) values for β-lactam antibiotics were determined from both Lineweaver-Burk and Dixon plots, using cephalothin as a substrate. Cephalosporinase inhibition was determined spectrophotometrically after 10 min of preincubation with an inhibitor at 30°C, using 0.1 mM cephalothin as a substrate (13, 18).

The cephalosporinase from *P. vulgaris* GN7919 was purified about 70-fold by column chromatography and was found to be homogeneous by polyacrylamide gel electrophoretic analysis (7). The molecular weight of the *P. vulgaris* GN7919 cephalosporinase was about 30,000 ± 2,000 on Sephadex G-200 gel filtration, using bovine serum albumin, ovalbumin, chymotrypsinogen A, and cytochrome *c* as the molecular weight markers. The molecular weight of cephalosporinase was also estimated from electrophoretic mobility in sodium dodecyl sulfate-acrylamide gels to be about 29,000 (19). Focusing column chromatography (6) revealed the isoelectric point of the enzyme to be 8.8.

The optimum pH was approximately 7.0, and the enzyme showed the highest β-lactamase activity at 45°C.

The K_m, K_i, and V_{max} values are shown in Table 2. This enzyme had broad substrate specificity and hydrolyzed cefazolin, cephalothin, cephalexin, cefotaxime, cefamandole, and cefuroxime at a high rate. Although cefuroxime has been reported to be resistant to β-lactamases from gram-positive and gram-negative bacteria (9), it was readily hydrolyzed by *P. vulgaris* cephalosporinase and showed a high K_m value for the enzyme. However, cefsulodin and cefoperazone were stable to the enzyme. Cloxacillin, methicillin, and clavulanic acid were found to be good competitive inhibitors with low K_i values from Lineweaver-Burk and Dixon plots. Although cefoxitin was extremely resistant to hydrolysis by the enzyme, it inhibited the enzyme activity at a higher concentration than it did many other cephalosporinases (12).

We also examined the effects of various inhibitors on enzyme activity. Magnesium ion (0.1 to 1.0 mM) did not inhibit, but zinc ion (1.0 mM), mercury ion (0.1 mM), iodine (0.1 mM), and *p*-chloromercuribenzoic acid (1.0 mM) completely inhibited enzyme activity.

The cephalosporinase from *P. vulgaris* had broad substrate specificity and easily hydrolyzed most of the substrates tested, including cefuroxime. However, cefoperazone, cefsulodin, and penicillins were rather stable to the enzyme. The

TABLE 1. *Purification of cephalosporinase from P. vulgaris GN7919*

Step no.	Procedure	Protein (mg)	Total activity (U)	Sp act (U/mg)	Yield[a] (%)
1	Ultrasonic disintegration	2,264	7,314	3.23	100
2	Diethylaminoethyl-cellulose	742	6,854	9.24	93.7
3	Carboxymethyl-Sephadex C-50	24.8	3,305	133	45.2
4	Sephadex G-200	14.3	3,208	225	43.9

[a] Overall yield of the enzyme from sonic extract.

TABLE 2. *Kinetics of hydrolysis of cephalosporins and penicillins by cephalosporinase from P. vulgaris GN7919*

Substrate	K_m (μM)	K_i (μM)[a]	V_{max}[b]
Cefaclor	60.6		100
Cefazolin	54.1		387
Cephalothin	56.2		173
Cephalexin	357		274
Cefsulodin	222		9
Cefotaxime	222		351
Cefamandole	278		381
Cefuroxime	769		1,140
Cefoperazone	27.5		15
Cefoxitin		17.8	
Cloxacillin		1.31	
Methicillin		2.52	
Clavulanic acid		1.07	

[a] The K_i values were determined by using cephalothin (100 μM) as a substrate. The inhibitors were added in 100 μM of cephalothin, and the enzyme activity was assayed.

[b] Relative rates of hydrolysis of the substrates are expressed in percent of hydrolysis of cefaclor.

enzyme activity was inhibited by clavulanic acid, a good inhibitor of type I, II, III, and IV penicillinases mediated by R-plasmids and chromosomal penicillinases, but, unlike the typical cephalosporinases, it was not strongly inhibited by cefoxitin, indicating that this cephalosporinase possesses unique properties like those of penicillinases.

The molecular weight of the enzyme was smaller than that of the typical cephalosporinase and was somewhat larger than those of type I, II, and IV penicillinases (1, 15, 16). With respect to behavior against inhibitors and molecular weight, the *P. vulgaris* cephalosporinase was very similar to those of *Pseudomonas cepacia* and *Bacteroides fragilis*, which belong to a cefuroxime-hydrolyzing β-lactamase group (4, 14).

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