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Hydrolysis of Cefotaxime by a Beta-Lactamase from *Bacteroides fragilis*

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A β-lactamase isolated from a strain of *Bacteroides fragilis* subsp. *fragilis* possessed hydrolytic activity toward cefotaxime. This antibiotic was degraded to a lower extent than was cephalothin, cephaloridine, and cefamandole, whereas cefoxitin remained unaffected by the enzyme. Kinetic parameters V_{max} and K_m for cefotaxime were calculated at 0.172 μmol/min and 1.1×10^{-2} mM, respectively.

Cefotaxime is a new semisynthetic cephalosporin, which has been introduced mainly because of its potent activity against gram-negative bacilli (3, 5, 12, 13, 15). The activity appeared particularly remarkable against β-lactamase-producing species of *Enterobacter*, *Serratia*, and *Pseudomonas* (4). This new cephalosporin shows real promise of being a significant improvement in the treatment of severe gram-negative infections notably in pelvic or abdominal sepsis where *Bacteroides fragilis* can also be involved. With regard to *B. fragilis*, cefotaxime has been claimed to be active against a majority of isolates. Using 36 strains, Hamilton-Miller et al. (5) did not find any resistance; all their isolates were inhibited by 16 μg of cefotaxime per ml. In another study (15), a cefotaxime concentration of 2 μg/ml inhibited 90% of the 33 tested strains of *B. fragilis*. In contrast, Drasar et al. (3) observed some resistant strains and mentioned the role of β-lactamases as a resistance factor in these bacteria.

This paper reports on the hydrolytic spectrum of a β-lactamase from *B. fragilis* on cefotaxime and several other cephalosporins.

MATERIALS AND METHODS

Cefotaxime was a gift of Roussel Hoescht Laboratories. The other β-lactam antibiotics were obtained from their respective manufacturers.

B. fragilis subsp. *fragilis* MULB-1008 is a clinical isolate which produces a β-lactamase with an isoelectric point of 4.9, similar to that derived by Leung and Williams (8). The strain was grown anaerobically at 37°C for 12 h in Brucella broth (Difco) supplemented with 5.0 μg of hemin and 0.5 μg of menadione per ml. After 2 h of incubation, 100 μg of cephalothin per ml was added as an inducer. The bacteria were harvested by centrifugation, washed twice in phosphate buffer (0.1 M; pH 7.0), and suspended in the same buffer to a cell density of 10% (wet wt/vol). Bacterial cells were sonicated in a Sonic 300 dismembrator (Artek Systems Corp.) for two periods of 3 min at 0°C. Remaining cell debris were removed by centrifugation at 40,000 × *g* in a model J2-21 Beckman refrigerated centrifuge for 1 h at 2°C. Clear supernatant fluid was used as a source of β-lactamase.

β-Lactamase activity was determined spectrophotometrically by measuring the change in absorbancy at 260 nm for cefotaxime, cephalothin, cephaloridine, cefamandole, and cefoxitin in a Cary 219 recording spectrophotometer. Reaction mixtures were maintained at 37°C by means of a circulating water bath, and the reaction was started by the addition of the enzyme. For all assays, controls were performed by omitting from the reaction mixture the specific substrate or the cell-free extract. Kinetic parameters were estimated from a least-squares fit of Lineweaver-Burk plots with a substrate concentration ranging from 0.02 to 0.12 mM. In a series of experiments involving comparative hydrolysis of cephalosporins, 0.1 IU of enzyme was allowed to react with 0.5 μmol of substrate at 37°C. One unit is defined as the amount of β-lactamase which hydrolyzes 1.0 μmol of substrate per min at 37°C and pH 7.0. Hydrolysis of the substrate was estimated from the absorbancy decrease at 260 nm and was assumed to be complete after a reaction for 3 h.

RESULTS

The ultraviolet absorption spectrum of cefotaxime before and after degradation by β-lactamase of *B. fragilis* is presented in Fig. 1. The absorbancy decrease at 260 nm was used to observe the enzyme substrate reaction. Since no literature data were available, the molar absorbancy value for cefotaxime was calculated at 260 nm to be 19,231 and then decreased to 11,017 for the hydrolysis product.

V_{max} and K_m calculated for cefotaxime at 0.172 μmol/min and 1.1×10^{-2} mM, respectively, were determined from data appearing in Fig. 2. By using the same cell-free extract and cephalothin as the substrate (molar absorbancy of 8,700 at 260 nm for cephalothin, decreasing to 1,700 for hydrolysis product), we calculated V_{max} and K_m at 23.2 μmol/min and 1.63×10^{-1} mM, respectively. The smaller K_m value obtained with cefotaxime indicated a greater affinity, and the lower V_{max} value indicated a slower activity of *B. fragilis* β-lactamase toward this antibiotic than toward cephalothin.

These results were confirmed in an examination of the rate of degradation of different cephalosporins by the β-lactamase (Fig. 3). Over 85% of cephalothin and cephaloridine were destroyed in 10 min, whereas 50% of cefotaxime was hydrolyzed during the same period. Cefamandole hydrolysis followed cephalothin and cephaloridine degradation but to a lesser extent (60%). Cefoxitin remained completely unaffected by *B. fragilis* β-lactamase. The percent hydrolysis refers to the percent total absorbancy change at 260 nm.

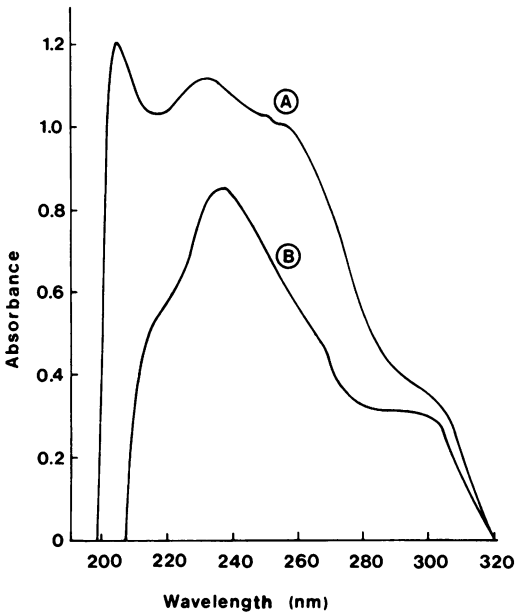

FIG. 1. *Ultraviolet spectra of 1.0 mM cefotaxime in 0.1 M phosphate buffer (pH 7.0) (A) and hydrolysis product (B) after degradation by crude extract of B. fragilis* MULB-1008.

DISCUSSION

Although other mechanisms can be involved in the resistance pattern of *B. fragilis* against β-lactam antibiotics, such as a permeability barrier (3, 9), the production of β-lactamase (2, 9, 14) seems to be the most important factor. One-third of *B. fragilis* isolates show considerable cephalosporinase activity (2); furthermore, Olson et al. (10) found that more than 90% of their strains elaborate a β-lactamase. Among several types of β-lactamases produced by *B. fragilis* subspecies (8, 11), the enzyme having an isoelectric point of 4.9 is the most commonly found. However, all the enzymes share some general properties with respect to substrate specificity: hydrolysis of cephalosporins is good, whereas activity against penicillins is low, and cefotoxin is completely unaffected. *B. fragilis* β-lactamases are inhibited by cloxacillin, p-CMB, iodine, and clavulanic acid.

Surprisingly, in some studies (5, 15) the resistance of *B. fragilis* to cefotaxime was scarcely observed. The discrepancy may depend on the

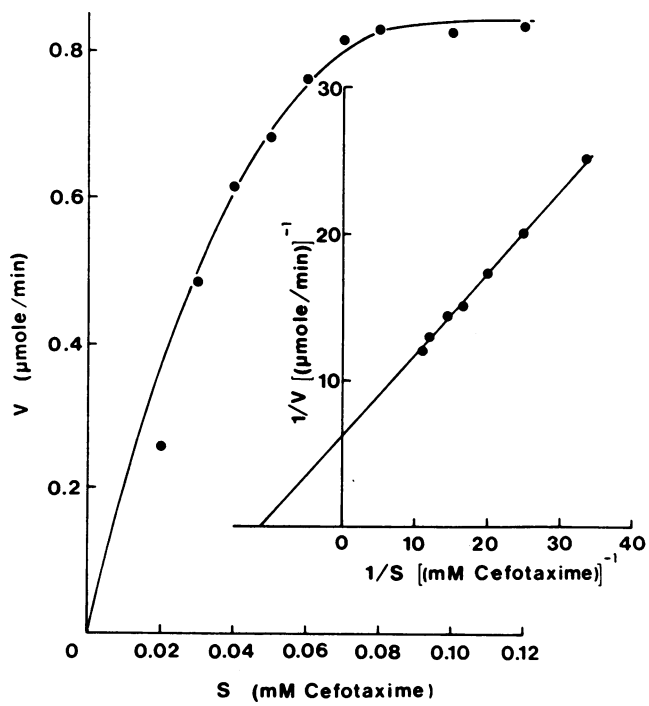

FIG. 2. *Kinetic of cefotaxime hydrolysis by crude extract of B. fragilis* MULB-1008.

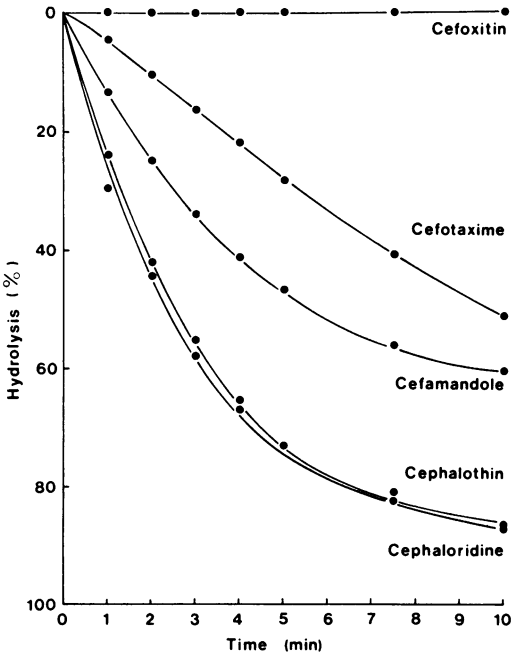

FIG. 3. *Comparative hydrolysis of different cephalosporins by B. fragilis MULB-1008 β-lactamase as a function of time.*

origin of the strains. It may also depend on a technicality in the determination of minimum inhibitory concentrations; according to some authors (2, 11), β-lactamase synthesis is dependent on culture age, and if one waits for the stationary phase to harvest bacteria, very low β-lactamase activity is obtained.

The antibacterial activity of cefotaxime toward facultative or anaerobic gram-negative bacteria is therefore well correlated with its stability to β-lactamase. In the case of *B. fragilis* strains, care must be exercised because of the eventual production of a β-lactamase with hydrolytic activity against cefotaxime.

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