CEFUROXIME INHIBITION OF CEPHALOTHIN HYDROLYSIS BY THE CONSTITUTIVE β-LACTAMASE FROM *E. CLOACAE* P99

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Generally the cephalosporin antibiotics substituted at the 7-position with an α-hydroxyimino phenylacetic acid are excellent non-competitive inhibitors of the Type I β-lactamase from *Enterobacter cloacae* P99, irrespective of the substituents at the 3-position. In addition, cephalosporins such as cefonicid and cefamandole, where the 7 substituent is a mandelic acid, also non-competitively inhibit cephalothin hydrolysis by this enzyme, but at much higher inhibitor concentrations[1]. Three other cephalosporin antibiotics, cefotaxime, cefoxitin and cefuroxime have been reported to inhibit cephaloridine hydrolysis by enzymes isolated from *Citrobacter* 2732 and *Proteus morganii* 771[2]. These investigators however, did not report kinetic data with these antibiotics.

In this paper, we report on the kinetics of inhibition of the *E. cloacae* P99 type I β-lactamase by cefuroxime with cephalothin as the substrate.

Enterobacter cloacae P99 was grown at 28°C in a simple defined medium[3] with 0.4% w/v glycerol as the carbon source. Cells were harvested during mid-log phase, washed with phosphate buffered saline (10 mM phosphate, 154 mM sodium chloride, pH 7.0), resuspended at a ratio of 1 : 3 cells to buffer and broken in liquid nitrogen. The soluble enzyme was extracted and concentrated by differential centrifugation followed by ultrafiltration. The enzyme preparations (10 mg:ml^{-1} protein) were stored at −80°C until needed. Assays were run at 30°C and pH 7.0 in 100 mM phosphate buffer. The decrease in absorbance at 260 nm was followed with 50~200 μM cephalothin as the substrate and suitable levels of cefuroxime as the inhibitor. The assays were optimized to enable initial velocities to be measured and the data were analyzed by least-squares analysis of LINEWEAVER-BURK[4] and DIXON[5] plots. Confirmation of the type of β-lactamase inhibition observed was performed by a replot of the slopes of the DIXON plots *versus* the reciprocal of the substrate concentration[6].

Similar inhibitor levels, IC_{50} values, were observed with cefuroxime and the α-iminohydroxy derivatives of cefonicid[1]. Although the apparent affinity of the cefonicid series and cefuroxime for the enzyme were similar, the kinetic constants were dramatically different. The cefonicid series were all non-competitive inhibitors whereas cefuroxime was a competitive inhibitor (Fig. 1), with a Ki_{app} of 40 nM. Thus, cefuroxime and the cefonicid series, although both inhibitors of β-lactamase, cannot be considered in the same structure-activity relationship.

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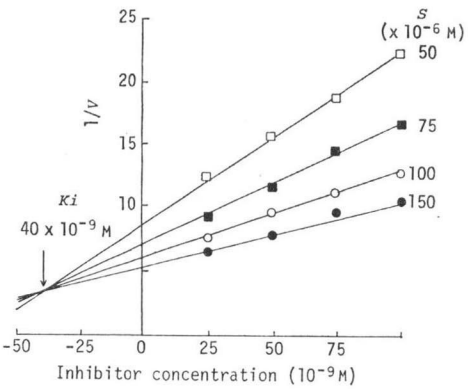
Fig. 1. DIXON plot of cefuroxime inhibition of cephalothin hydrolysis by the β-lactamase from *E. cloacae* P99.



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