The Renal Membrane Dipeptidase (Dehydropeptidase I) Inhibitor, Cilastatin, Inhibits the Bacterial Metallo-
β-Lactamase Enzyme CphA

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The Aeromonas hydrophila AE036 chromosome contains a cphA gene encoding a metallo-β-lactamase which is highly active against carbapenem antibiotics such as imipenem. Here we show that the cphA gene product shares inhibitory similarities with a mammalian zinc peptidase, membrane dipeptidase (MDP; dehydropeptidase I). Both enzymes are able to hydrolyze imipenem and are inhibited by cilastatin. The active site similarities of these enzymes are not reflected in any significant primary sequence similarity.

Thienamycin (11) and related carbapenem antibiotics are rapidly hydrolyzed and inactivated in vivo in humans by renal or membrane dipeptidase (MDP; EC 3.4.13.19), also commonly referred to as dehydropeptidase, I, which is a zinc metalloenzyme located in the microvilli of kidney proximal tubular cells (2, 10, 13). MDP exhibits versatile substrate specificity, hydrolyzing dipeptides and dehydropeptides, as well as β-lactam antibiotics of the trans-conformation, such as imipenem (2). Cilastatin (MK0791; [Z-S-[6-carboxy-6-(2,2-dimethyl-(S)-cyclopropyl) carboxy]-amino-3-hexenyl]-l-cysteine) was developed as a reversible, competitive inhibitor of MDP (50% inhibitory concentration = 0.1 mM) on the basis of the structural similarities between the scissile bonds in imipenem and dehydropeptides (10) (Fig. 1).

Bacterial zinc metallo-β-lactamases are typified by the β-lactamase II of Bacillus cereus (9, 14), and the cphA gene product of Bacteroides fragilis (20) and are capable of hydrolysing different classes of β-lactam compounds, including oximinocephalosporins, cephamycins, penicillins, and carbapenems (1, 7, 18). Some of these metalloenzymes pose a considerable threat to antibiotic therapy, particularly since carbapenems show an exceptionally wide spectrum of antibacterial activity. The recently described cphA gene product of Aeromonas hydrophila (16) shares a lower degree of sequence similarity with the B. cereus, B. fragilis, and Xanthomonas maltophilia (16, 21) metallo-β-lactamases and appears to fall into a different molecular subclass within the class B metalloenzymes. It can hydrolyze carbapenems efficiently, but it is unable to cleave with very high efficiency the cis-conformation of the β-lactam ring seen in the classical penicillins and cephalosporins (5, 6, 17). Since MDP is also a Zn2+-dependent carbapenem-specific β-lactamase, we have examined possible similarities in structural features and catalytic mechanisms between the mammalian and bacterial enzymes and the possibility that the A. hydrophila enzyme may be inhibited by cilastatin.

MDP was purified to homogeneity from porcine kidney cortex by affinity chromatography on cilastatin-Sepharose (15), and the bacterial CphA enzyme was purified from an Esche-
cubation with CphA enzyme (10 μg of protein, 16 h) failed to result in peptide hydrolysis (Fig. 3c and f).

Thus, mammalian MDP and the bacterial CphA enzyme are both zinc metalloenzymes which share the ability to hydrolyze imipenem, and both are inhibited by cilastatin, although with differing sensitivities. Imipenem was more efficiently hydrolyzed by CphA enzyme than MDP (specific activities, 26 and 0.2 μmol/min/mg of protein for the purified, homogeneous enzymes, respectively). However, the CphA enzyme is unable to hydrolyze dipeptides or dehydropeptides that are efficient substrates of MDP. Comparison of the predicted amino acid sequences of MDP and cphA enzyme by using the GCG program (4) fails to reveal any significant sequence similarity. Crystallographic structure analysis of the β-lactamase II (Blm) from B. cereus (19) has revealed that the zinc ion is coordinated to three histidine residues and a cysteine with the motif -HXH-(79 residues)-C-(41 residues)-H-. This motif is conserved in the B. fragilis CifA enzyme (20). However, in the CphA enzyme the first histidine of the motif is replaced by asparagine (16) and in the X. maltophilia L1 enzyme (21) the three histidines are conserved but the cysteine is substituted by a serine. Directed mutagenesis is required to confirm the essential residues in all these enzymes. In MDP a critical histidine residue (His-219) has been pinpointed by site-directed mutagenesis (12), but other zinc ligands have not been identified.

Comparative structural studies of MDP and CphA enzyme may provide insight into mechanistic aspects of this class of β-lactamase enzymes and could be helpful in the development of novel and specific inhibitors of bacterial metallo-β-lactamas.

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