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Fighting Antibiotic Resistance

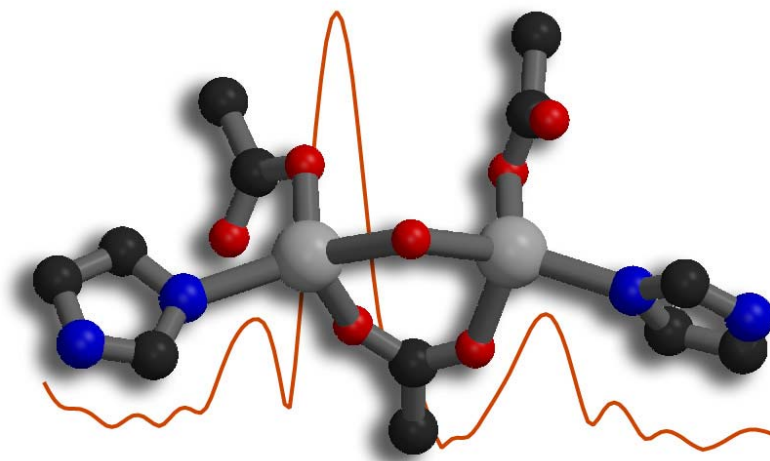
The *dapE*-encoded N-succinyl-L,L-Diaminopimelic Acid Desuccinylase from *Haemophilus influenzae* is a Dinuclear Metallohydrolase

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Bacterial infections, such as tuberculosis, have been identified as a world-wide problem leading to the deaths of millions of people each year. The importance of developing new drugs to fight infectious disease caused by these pathogenic organisms is underscored by the emergence of several bacterial strains that are resistant to all currently available antibiotics.¹⁻⁴ Antibiotics, such as β -lactams, succeed by targeting vital cellular functions either killing the organism or hindering their multiplication. However, through evolution, bacteria will most likely develop resistance to these drugs rendering them useless. Targeting new bacterial-specific pathways is a valid approach to combating this problem.

The *meso*-diaminopimelate (mDAP)/lysine biosynthetic pathway offers several potential anti-bacterial targets that have yet to be explored.⁵⁻⁷ Since both products of this pathway, mDAP and lysine, are essential components for the synthesis of the peptidoglycan cell wall in Gram-negative and some Gram-positive bacteria, inhibitors of enzymes within this pathway may provide a new class of antibiotics.³ The fact that there are no similar pathways in mammals suggests that inhibitors of enzymes in the mDAP/lysine pathway will provide selective toxicity against bacteria and have little or no effect on humans.

One of the enzymes in this pathway,⁸ the *dapE*-encoded N-succinyl-L,L-diaminopimelic acid desuccinylase (DapE), catalyzes the hydrolysis of N-succinyl-L,L-diaminopimelate to L,L-

diaminopimelate and succinate.⁹ It has been shown that deletion of the gene encoding DapE is lethal to *Helicobacter pylori* and *Mycobacterium smegmatis*.^{10,11} Therefore, DapEs are essential for cell growth and proliferation making them potential molecular targets for a new class of antibiotics.

Rational design of inhibitors for DapE relies on an understanding of the active site structure and mechanism. DapE is known to be a metallohydrolase utilizing Zn at its active site, but no crystallographic information is available. We have used Zn K-edge Extended X-ray Absorption Fine Structure (EXAFS) data, of DapE from *Haemophilus influenzae* in the presence of one or two equivalents of Zn(II) (*i.e.* [Zn_(DapE)] and [ZnZn(DapE)]), to provide structural information about the active site.¹² The Fourier transforms of the Zn EXAFS are dominated by a peak at *ca.* 2.0 Å, which can be fit for both [Zn_(DapE)] and [ZnZn(DapE)] assuming *ca.* 5 (N,O) scatterers at 1.96 and 1.98 Å, respectively. A second-shell feature at *ca.* 3.34 Å appears only in the [ZnZn(DapE)] FT, demonstrating that DapE contains a dinuclear Zn(II) active site.

In addition, Zn EXAFS data for DapE incubated with two competitive inhibitors, 2-carboxyethylphosphonic acid (CEPA) and 5-mercaptopentanoic acid, establish the binding modes of phosphonate- and thiolate-containing inhibitors. The structural data obtained for CEPA bound to [ZnZn(DapE)] also provides an initial understanding of the transition state for the hydrolysis reaction catalyzed by DapE. Since most pharmaceuticals target the transition state of enzymatic reactions, the structural aspects of [ZnZn(DapE)]-CEPA are particularly important for the rational design of new potent inhibitors of DapE enzymes.

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