Bacillus cereus metallo-β-lactamase uses a branched mechanism to hydrolyze different antibiotics

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Metallo-β-lactamases (MBLs) are Zn(II)-containing hydrolytic enzymes that degrade β-lactam antibiotics rendering them ineffective. The most thoroughly characterized MBLs are dinuclear, but in some cases the mono Zn(II) enzymes are almost fully active. The metallo-β-lactamase from *Bacillus cereus* (BcII) shows a weak affinity for the second Zn(II) and is active with one metal ion per enzyme although binding of a second Zn(II) increases its catalytic efficiency. While the global fold is conserved among MBLs, the aminoacid sequences are quite variable. One might hypothesize that this sequence diversity is reflected in the catalytic mechanism followed by these enzymes, as well as in their Zn(II) requirements.

We used non-steady state kinetics to examine the reaction of BcII with two different substrates: benzylpenicillin (BP) and imipenem (IMI). For this purpose, the enzyme was substituted with Co(II), since this metal has extensively been shown to be a successful spectroscopic probe for Zn(II). The Co(II)-substituted enzyme exhibits a characteristic electronic spectrum with features associated with each metal binding site.

We followed the hydrolysis of IMI and BP with BcII using a stopped flow/diode array system. With both substrates, we could detect transient species with distinctive spectral features. We analyzed the reactions of the enzyme substituted with one and two Co(II) equivalents and although the overall rate of reaction was lower in the mono-substituted form, we found identical intermediate spectra in both cases. This suggests that the catalytic species involved in the reaction could be the same whether the enzyme is in its mono- or di-metallated form. In each case, single wavelength traces could be fit to a branched model with two intermediates as proposed originally by Bicknell *et al*. (*Biochemistry* **25**, 7208-15, 1986). While the reactions with both substrates proceed through similar minimal mechanisms, there are some differences in the spectral features of the intermediate species. This suggests that the geometry of the metal-binding site during turnover is flexible enough to accommodate different substrates. This behaviour contrasts with the one recently reported for the MBL L1 from *Stenotrophomonas maltophilia* (Garrity *et al. Biochemistry* **44**, 1078-87, 2005).