X-ray free electron laser studies reveal dioxygen binding to isopenicillin N synthase induces correlated motions during catalysis

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A frontier challenge in structural biology is the characterisation of time-resolved structures to high resolution from intermediates within catalytic reactions of biological systems at physiological temperatures. The use of femtosecond pulses at an X-ray free electron laser (XFEL) allows one to probe reactions to yield atomic and electronic structures, without X-ray radiation-induced changes to sensitive sites such as an active site metal centre. To this end, our collaboration has developed a drop-on-demand sample delivery system[1] to enable us to simultaneously collect and correlate X-ray diffraction data and X-ray emission spectroscopy. Crystallographic data allow us to monitor conformational changes and movements throughout the crystal during turn-over, whereas the changes to the electronic structure of an active site metal is probed by emission spectroscopy.

Here we present structural and spectroscopic results from an important metalloenzyme, Isopenicillin N synthase (IPNS), which catalyses the iron-dependant four electron oxidation of the linear tripeptide δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (ACV) into isopenicillin *N* - the precursor of all natural β-lactam antibiotics.[2,3] The work includes microcrystal slurry optimization, the synthesis of isotopically labelled ACV (to exploit high kinetic isotope effects that build-up key intermediates) and new methods in sample delivery. An unique feature of the proposed reaction mechanism is the role of two high-valent iron species - an Fe(III)-superoxo and a high-spin Fe(IV)=O species that promote the first and second ring closures of the β-lactam, respectively. We present results for these intermediates obtained during O_2-catalyzed turnover of the IPNS•ACV complex revealing unexpected dynamics throughout the whole structure making these fascinating constrained ring-forming steps possible.[3] These correlated motions initiated upon O_2 binding are supported by extensive solution ^{19}F NMR and EPR studies. Recent crystallographic data with the isotopically labelled substrate AC[^{2}H]V in H_2O and D_2O suggest a revision of the mechanism for the late-stage intermediates in the IPNS mechanism.

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