Time-resolved serial femtosecond crystallography of the early intermediates in the isopenicillin N synthase reaction with ACV and O_2

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The femtosecond pulses at an X-ray free electron laser (XFEL) allow experimental access to enzyme reaction cycles and reveal time-resolved 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 that enables simultaneous collection and correlation of time-resolved femtosecond crystallography (tr-SFX) data with X-ray emission spectroscopy (tr-XES) data.[1] High resolution tr-SFX data yields atomic models throughout the crystals, whereas tr-XES monitors the changes to the electronic structure of the active site metal ion.

Isopenicillin N synthase (IPNS) catalyses the nonheme iron-dependant, four electron oxidation of the linear tripeptide δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (ACV) into isopenicillin N.[2] A unique feature of the proposed reaction mechanism is the role of two reactive 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. High valent iron intermediates are of exceptional importance throughout biology where they function as key intermediates, including those that form antimicrobial compounds and in human enzymes, including in hypoxia sensing/response and DNA damage repair.[3]

We present results for the early reaction intermediates obtained during O[sub]2[/sub]-catalysed turnover of the IPNS•Fe(II)•ACV complex. Our collaboration has collected dozens of diffraction datasets at several XFELs (LCLS, SACLA, PAL-XFEL) and at Diamond Light Source with room temperature serial crystallography, as well as with freeze-quench cryogenic methods. The room temperature results shown that O[sub]2[/sub] binding to the IPNS•ACV complex results in an ACV-Fe(III)-O-O[sup]-[/sup] complex. As the reaction cycle progresses, conformational changes originating at the iron-ACV complex increase the dynamics of ACV as a result of catalysis. The conformational changes propagate outward to the exterior of the enzyme, where they eventually promote rearrangement of an exterior α-helix with increased thermal parameters. Analogous perturbations to the α-helix are also observed in the anaerobic IPNS•ACV•NO complex under cryogenic conditions, wherein the NO serves as a surrogate for O[sub]2[/sub]. Using solution state [sup]19[/sup]F NMR and specifically labelled protein, we observe increased dynamics of the exterior α-helix in the IPNS•ACV•NO complex. Together, these results indicate several roles for O[sub]2[/sub] biding that influence enzyme and substrate dynamics that ultimately impact the reaction coordinate catalysed at the iron centre by the enzyme.

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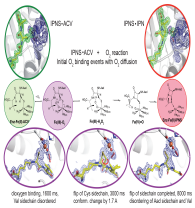

Figure 1