Structural and spectroscopic observation of an enzyme at work

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Observing enzymes at work is a difficult task. Yet, scientists at the University of Pavia in Italy, in collaboration with colleagues from the ESRF, the IBS and the University of Groningen in The Netherlands succeeded in generating and characterizing several biologically relevant intermediate states of a Baeyer-Villiger monooxygenase, a promising target for biocatalytic applications in synthetic and pharmaceutical chemistry. Their studies, combining X-ray crystallography, single-crystal microspectrophotometry, and X-ray induced photoreduction, have been published in the Journal of Biological Chemistry.

At the end of the 19th century, Baeyer and Villiger discovered that cyclic ketones react with oxidants such as peroxymonosulphuric acid to yield lactones. Baeyer-Villiger reactions are of enormous value in synthetic organic chemistry and the number of their applications is countless. Several microorganisms produce enzymes able to catalyse Baeyer-Villiger reactions. Baeyer-Villiger monooxygenases are FAD-dependent proteins that use NADPH and molecular oxygen to insert an oxygen atom into their substrate. We have identified a microbial Baeyer-Villiger enzyme, phenylacetone monooxygenase, which offers several unique and attractive features: it is thermostable and tolerant towards organic solvents, and catalyzes enantioselective Baeyer-Villiger oxidations and sulfoxidations. Researchers at the University of Pavia in Italy, at the ESRF and the Institut de Biologie Structurale in Grenoble, and at the University of Groningen in The Netherlands were able to generate several intermediate states of the colored enzyme phenylacetone monooxygenase and to characterize them by X-ray crystallography and single-crystal microspectrophotometry both at the synchrotron beamline (online) and at the dedicated Cryobench laboratory (offline). The experimental strategy recruited otherwise detrimental X-ray induced changes of biomolecules to trigger reduction of the X-ray sensitive flavin moiety of phenylacetone monooxygenase. The resulting intermediate state was captured and structurally characterized by collecting X-ray crystallographic data at a very low temperature of -170°C. These studies highlight the fascinating ability of Baeyer-Villiger monooxygenases to catalyze a complex multi-step catalytic reaction originates from concerted action of two cofactors (NADPH and FAD) and the protein active site to subsequently promote flavin reduction, oxygen activation, tetrahedral intermediate formation, and product synthesis and release. The emerging picture is that these enzymes are mainly oxygen-activating and "Criegee-stabilizing" catalysts that act on any chemically suitable substrate that can diffuse into the active site, emphasizing their potential value as toolboxes for biocatalytic applications.

A movie related to this research is available at www.unipv.it/biocry. Supported by the EU-FP7 project "Oxygreen".

Principal publication and authors

Orru, R. (a), Dudek, H.M. (b), Martinoli, C. (a), Torres Pazmino, D.E. (b), Royant, A. (c, d), Weik, M. (c, d), Fraaije, M.W. (b) and Mattevi, A. (a) Snapshots of Enzymatic Baeyer-Villiger Catalysis: Oxygen activation and intermediate stabilization *J Biol Chem*, 286, 29284 (2011).

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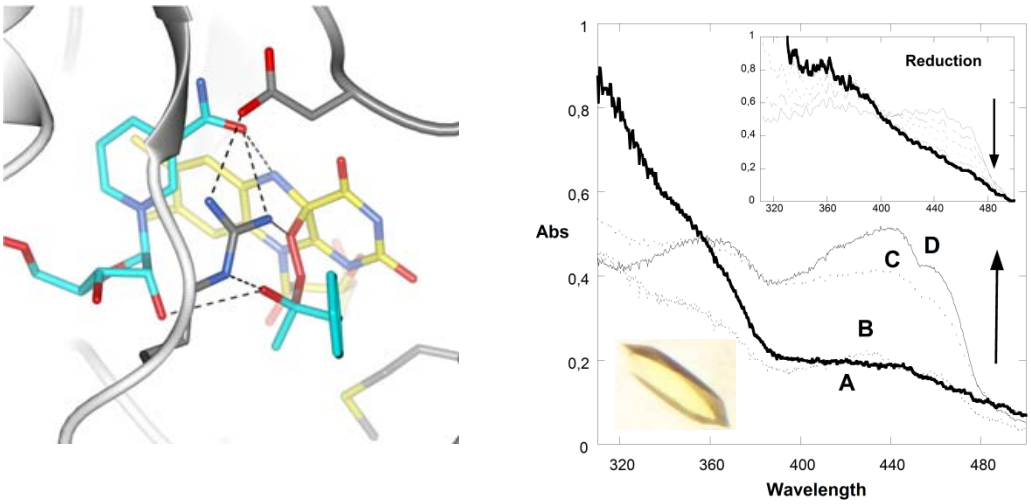

(d) European Synchrotron Radiation Facility, Grenoble (France)

Figure (*Left*) Model of the crucial oxygenating "Criegee" intermediate on phenylacetone monooxygenase (PAMO) and (*Right*) Microspectrophotometry of PAMO crystals (inset) measured at 100 K. X-ray exposure of the crystals leads to their reduction (spectrum A, bold line and inset). Reduced crystals can then be re-oxidized by soaking at room-temperature in aerated solutions as shown by spectra B, C, D which were collected on crystals that were cryo-cooled 2, 4, and 6 minutes after beginning of re-oxidation.

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