Laue/Time Resolved Macromolecular Crystallography

MS02.02.01 TIME-RESOLVED MACROMOLECULAR CRYSTALLOGRAPHY. K. Moffat Department of Biochemistry and Molecular Biology, and the Consortium for Advanced Radiation Sources, The University of Chicago, 920 E. 58th St., Chicago, IL 60637, USA.

Time-resolved crystallography is aimed at a better understanding of reaction mechanisms, by initiation of a structural reaction in the crystal and the sequential generation of intermediates along the reaction path from reactants to products[1]. Progress along this reaction coordinate is monitored through the time dependent change in X-ray diffraction intensities, and often in another parameter such as optical absorbance. Experiments may be classed by the means of reaction initiation (e.g. photoactivation of light-sensitive systems, or reactant diffusion in a flow cell); by whether intermediates are trapped or not[2] and if so, by the means of trapping (chemical or physical); by the time scale of intermediate lifetimes (at present from nanoseconds[3] to kiloseconds or longer) and hence by whether the pulsed or quasi-continuous nature of the synchrotron X-ray source is exploited; by the use of Laue or monochromatic techniques; and by the mode of analysis and presentation of the results (a time-dependent structural average, or progression through the sequence of time-independent structures). Although time-resolved experiments are challenging, successful general strategies have been identified and applied to several macromolecular systems. Surprisingly, in many respects macromolecular systems are easier to study than small organic or inorganic systems[4].

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MS02.02.02 VISUALIZING CATALYTIC INTERMEDIATES AND STRUCTURAL MECHANISM FOR ISOCITRATE DEHYDROGENASE USING STEADY-STATE AND SINGLE-TURNOVER LAUE EXPERIMENTS. Barry Stoddard, Basic Science & Stuctural Biology, Fred Hutchinson Cancer Research Center, Seattle WA, 98104

A combination of intermediate trapping methodologies and fast diffraction techniques are showing great promise for the direct visualization of structural intermediates formed during turnover. The greatest challenge for such studies is the matter of how the experimenter might induce a relatively high occupancy population throughout the crystal that represents a predominant catalytic species. This is of particular interest when enzymes are studied that normally follow multi-intermediate pathways with efficient free-energy profiles containing no substantial rate barriers between bound substrates and final products. In this talk, we present a series of studies of the enzyme isocitrate dehydrogenase, in which three specific catalytic states are trapped and visualized. The most important experimental lesson is that a combination of several techniques may be used to conduct such studies: kinetic analysis and spectroscopy in solution and the crystal, genetic mutational engineering, steady-state and single-turnover Laue experiments. When true fast Laue diffraction experiments are conducted using photochemical triggering, particular attention must be paid to substrate on-rates in the crystal lattice and mother liquor. In addition,independent experimental verification of structural assignments and of dynamic movements and interactions must be conducted: we present the recent use of molecular dynamic simulations and secondary mutagenic and kinetic studies to test specific features of the structural mechanism of IDH.

MS02.02.03 THE SYNTHESIS OF NITRIC OXIDE IN CYTOCHROME cd_1 NITRITE REDUCTASE. Vilmos Fülöp, Pamela Williams & János Hajdu. Laboratory of Molecular Biophysics & Oxford Cenre for Molecular Sciences, University of Oxford, U.K.

Cytochrome cd_1 nitrite reductase is a bifunctional enzyme that catalyses the one-electron reduction of nitrite to nitric oxide and the four-electron reduction of oxygen to water. We recently reported the 1.55 Å resolution structure of the dimeric enzyme isolated from *Thiosphaera pantotropha*. Each subunit contains a covalent *c* haem and a unique non-covalent *d*1 haem. The d_1 haem is the mononuclear centre where both oxygen and nitrite reduction takes place. The two types of haems are located in separate domains whose arrangement suggests a mechanism requiring domain movement during catalysis. A mechanism of NO release linked to domain movement may have wider implications for haem catalysis and signalling, for example, in the NO-dependent haem-containing guanyl cyclase. Preliminary studies show that cytochrome cd_1 is active in the crystal. Crystals in the act of catalysis diffract to high resolution. The study of the structure of the reduced form indicates that there are substantial regions of conformational change. Implications for time-resolved studies will be presented.

Reference
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MS02.02.04 NANOSECOND TIME-RESOLVED MACROMOLECULAR CRYSTALLOGRAPHY: PHOTOLYSIS OF CARBONMONOXY MYOGLOBIN. V. Srajer*, T.-Y. Teng*, T. Ursby†, C. Pradervand*, Z. Ren*, S. Adachi§, W. Schildkamp*, D. Bourgeois†‡, M. Wulff† and K. Moffat* *Department of Biochemistry and Molecular Biology and CARS, University of Chicago, Chicago, IL, USA †ESRF, Grenoble, France ‡UPR 9015/ IBS, Grenoble, France §RIKEN, Saitama, Japan

The pulse structure and high brilliance of the focused white synchrotron radiation from the BL3 wundulator[1] at ESRF, Grenoble, France were utilized to explore structural changes in carbonmonoxy myoglobin (MbCO) crystals induced by a 10ns laser pulse. Several essential experimental features were implemented: a fast shutter system[1] to isolate individual single and super- pulses; a high efficiency, low-noise area detector[1] to record relatively weak diffraction patterns; uniform photolysis of crystals by ns laser pulses synchronized with x-ray pulses[1]; parallel X-ray and optical measurements on crystals to quantify reaction initiation and progress[2]; and novel Laue data processing methods[3]. Complete Laue X-ray diffraction data were collected using either single, 60ps X-ray pulses or 940ns, "super-pulses" at laser/X-ray pulse time delays of 4ns, 1µs, 7.5µs, 50µs, 350µs and 1.9ms. Data were typically about 70% complete to 1.8Å resolution with R_{merge}≈11% and yielded successful wavelength normalization and deconvolution of harmonic energy overlaps. Departure of the CO ligand upon photolysis and subsequent µs rebinding are clearly observed as well as partial iron displacement from the heme plane and other smaller, consequent tertiary structural changes in the heme pocket and the F-helix. These experimental results are compared with the structural changes inferred from numerous spectroscopic experiments and molecular dynamics simulations. They establish the feasibility of nanosecond time-resolved macromolecular crystallography.

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