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TIME-RESOLVED MACROMOLECULAR CRYSTALLOGRAPHY : MOLECULAR MOVIES ?

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Time-resolved crystallographic experiments on light-sensitive crystals of biological macromolecules have been conducted at both the European Synchrotron Radiation Facility and the Advanced Photon Source in pump - probe mode, spanning the time range from hundreds of picoseconds to milliseconds and longer. Results on the photodissociation and rebinding of carbon monoxide from myoglobin and the photocycle of the bacterial blue-light photoreceptor, photoactive yellow protein, may be presented as time-dependent 'movies' of (difference) electron density. The time dependence arises from the variation with time of the populations of the intermediates in the reactions, each of which possesses a time-independent structure. The time-dependent data may be further analyzed by singular value decomposition to reduce the noise and to aid in determining the chemical kinetic mechanisms and the structures of the short-lived intermediates. This process may be denoted 'analytical trapping' of the intermediates, to distinguish it from the related techniques of 'chemical trapping' and 'physical trapping'.
See for example Moffat, Chem. Revs. 101, 1569-1581 (2001).

Keywords: TIME RESOLVED CRYSTALLOGRAPHY LIGHT SENSITIVE PROTEINS LAUE DIFFRACTION

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COMPLEXITY OF THE STRUCTURAL DYNAMICS OF MYOGLOBIN: A STUDY BY TIME RESOLVED LAUE CRYSTALLOGRAPHY

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Myoglobin maintains a central position in protein science being ideal to study internal dynamics and its role in controlling function. Thanks to the photosensitivity of the iron-ligand bond, the structure of “frozen” intermediates states of Mbs showed photolysised CO either in the distal heme pocket or in one of two alternative cavities, accessible to Xe atoms. These results convey the picture that packing defects are involved in controlling the dynamics and reactivity of Mb with gaseous ligands. Time-resolved Laue diffraction allowed us to follow globin relaxations after CO photodissociation of a triple mutant of Mb. As in wild-type Mb, photolysis induces an immediate motion of the iron out of the heme plane, bending of the pyrrole ring C towards the distal pocket; in the mutant motion of Y(B10)29 towards the iron is followed by rotation of Q(E7)64, dragging the whole helix E towards its deoxy state position. On the nsec timescale, a transient weak occupation of the 'Xe 1' cavity on the proximal site is attributed to CO trapped in the matrix. The extended dynamics of the internal motions and the conformational change of Helix E seem in agreement with the idea that the protein populates conformational substates, and that internal motions occur at different rates. It may be assumed that the heterogeneous nature of the protein core is the basis for the extended time-structure of the internal conformational changes. These results may correlate to data obtained by time-resolved spectroscopy, and represent direct evidence for the 'rugged' protein energy landscape.

Keywords: MYOGLOBIN, DYNAMICS, FUNCTION

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SNAPSHOTS OF SERINE PROTEASE CATALYSIS: UNRAVELLING THE MECHANISM OF DEACYLATION

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Studies on the catalytic mechanism and inhibition of serine proteases are widely used as paradigms for teaching purposes. Ground-breaking work on the structure of chymotrypsin and subtilisin led to the idea of a conserved catalytic triad formed by active site serine, histidine and aspartic acid residues. An oxyanion hole, consisting of the peptide amide of the active site serine and a neighboring glycine, was identified and it was suggested that hydrogen bonding in the oxyanion hole stabilizes the two proposed tetrahedral intermediates on the catalytic pathway. We have developed a method of initiating the reaction in a co-crystal complex of the acyl-enzyme intermediate between a serine protease, porcine pancreatic elastase, and an unmodified peptide, β-casomorphin-7, using a pH jump protocol. Electron density changes were consistent with the formation of a tetrahedral intermediate during the hydrolysis of the acyl-enzyme complex. A comparison of the acyl-enzyme and tetrahedral intermediate structures indicates an early transition state for deacylation with a predominantly planar geometry. The results also suggest a mechanism for the synchronization of hydrolysis and peptide release triggered by the conversion of the sp^2 hybridized carbonyl carbon to an sp^3 carbon in the tetrahedral intermediate. This affects the location of the peptide in the active site cleft, triggering the collapse of a hydrogen-bonding network between the peptide and the β-sheet of the active site.

Keywords: SERINE PROTEASES, TIME RESOLVED, PROTEIN CRYSTALLOGRAPHY

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CRYOPHOTOLYSIS OF CAGED COMPOUNDS AND UV-VISIBLE FLUORESCENCE SPECTROPHOTOMETRY AS A TOOL TO STUDY THE DYNAMICS OF CRYSTALLINE PROTEINS

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Kinetic crystallography consists in allowing an enzyme to turnover in the crystalline state so that intermediate structures along the reaction pathway may be trapped and analyzed at atomic resolution. When reaction initiation cannot be made much faster than the enzyme turnover at close to ambient temperature (which would result in asynchrony between the molecules in the crystal), an alternative approach consists in triggering the reaction at a temperature where the enzymatic activity is blocked, followed by a transient temperature increase to allow a transition barrier to be passed and an intermediate to accumulate in the crystal. Such strategies have been used in the case of proteins displaying built-in photosensitivity. We demonstrate here that they can be extended to the case of many non-naturally photosensitive and rapid enzymes. Firstly, we have shown that reaction triggering in the frozen state may be achieved by UV-photolysis of caged substrates at 100-150 K. Secondly, we used fluorescence microspectrophotometry to determine the appropriate temperature window in which intermediate states may accumulate. This window should generally stand in between the temperature of the protein dynamical transition and the temperature of solvent crystallization. Indeed, in these conditions the macromolecule is expected to recover sufficient flexibility to carry forward its reaction while non-reversible crystal damage due to ice formation is avoided. By diffusing fluorescein into the crystals, we observed that the fluorescence peak emission is a sensitive reporter of temperature dependant transitions, thereby allowing identification of the suitable window. These results are of general interest to the study of protein dynamics.

Keywords: CAGED COMPOUNDS FLUORESCENCE MICROSPECTROPHOTOMETRY PROTEIN DYNAMICS