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, photooactive 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).

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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 sp² hybridized carbonyl carbon to an sp³ 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.

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