Main Lectures

ML-01.03  TIME-RESOLVED PROTEIN CRYSTALLOGRAPHY
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A complete understanding of mechanisms at the molecular level demands knowledge, not just of long-lived, readily observable structures, but also of short-lived intermediates in processes such as catalysis, ligand binding and release, protein unfolding, and photoinduced reactions. Can X-ray crystallography, long regarded as a static technique, in fact encompass dynamic processes? A time-resolved crystallographic experiment (Moffat et al., 1989 Am. Rev. Biophys. Biophys. Chem. 18, 309-332; Time-Resolved Macromolecular Crystallography (Ed. D.W.J. Crickthael, J.R. Hellwell and L.H. Johnson), Oxford University Press (1992)) has five main steps: the X-ray source; reaction initiation; reaction monitoring by optical or other techniques; X-ray data acquisition is real time; and data reduction and analysis. These are illustrated by our recent work on photoreactive yellow protein, on protein unfolding, and on the acquisition and analysis of crystalllographic data using 120 ps exposures (Szubonski, D.M.E., Bildeback, D.H., LeGrand, K., Moffat, K., Sildkamp, W., Shoich Temple, B. and Tong, T.Y. (1992) J. Mol. Biol. 225, 414-423).

ML-03.01  CONTRIBUTION OF CRYSTALLOGRAPHY TO THE ELUCIDATION OF HYDROLYTIC ENZYME MECHANISMS.
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Biopolymers such as proteins, nucleic acids and polysaccharides are stable chemical entities that are resistant to hydrolytic cleavage into their constituent building blocks. However, the dictates of various cellular metabolic processes requires the breakdown of the biopolymers once they have served their use. Enzymes such as proteinases, nucleases and glycosidases have evolved to catalyze the degradation of these macromolecules. The hydrolytic cleavage of proteins and peptides is carried out by proteolytic enzymes. They have been categorized into four separate classes, namely the serine, aspartic, metallo- and cysteine proteinases. There is a wealth of structural information for each of these diverse enzymes. Hydrolytic mechanisms have been deduced from the vast biochemical and kinetic literature. Coupled closely with the structural data provided by high-resolution X-ray crystallographic studies of the uncomplexed native enzymes as well as those of enzyme-inhibitor or enzyme-transition state analogue complexes, both the serine and cysteine proteinases have covalently attached acyl-enzyme intermediates on their reaction pathways whereas the aspartic and metalloproteinases catalyze the hydrolysis without invoking covalently attached intermediates. Hydrolysis of peptide bonds involves an attack by an appropriate nucleophile on the carbonyl-carbon atom of the substrate with the resultant formation of a transient tetrahedral intermediate. Generation of the attacking nucleophile from water or an amionic side chain is assisted by a general base which subsequently transfers the abstracted proton to the nitrogen of the leaving group. Electrophilic assistance for the nucleophilic attack is also accompanied by the stabilization of the developing negative charge on the carbonyl-oxygen atom of the tetrahedral intermediate. The detailed stereochemistry of the formation and breakdown of the tetrahedral transition state of the hydrolytic reaction is established. Comparison of the structures of the various enzymes in the four proteinase classes shows that the serine, aspartic and metalloproteinases have converged on analogous hydrolytic mechanisms in which nucleophilic attack of the peptide carbonyl-carbon atom is on the Cα face. In contrast, the cysteine proteinases of known structure likely have the thiolate attack on the Si face of the peptide bond. For the proteinases, only serine residues, cysteine residues and water molecules are used to generate the attacking nucleophiles. Imidazole rings and carbonyl groups are used in the role of general base. Electrophilic assistance to hydrolysis is provided by a wide variety of functional groups; even metal co-factors (e.g., Zn²⁺) have been recruited to this role. (Research supported by the MRC Canada.)