03–Crystallography of Biological Macromolecules

03.05 – Enzymes

MS–03.05.01 TOWARDS THE ATOMIC STRUCTURE OF 2-OXO ACID DEHYDROGENASE MULTI-ENZYME COMPLEXES
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Multi-enzyme complexes of the 2-oxo acid dehydrogenase family include pyruvate dehydrogenase, 2-oxo keto glutarate dehydrogenase and branched chain 2-oxo acid dehydrogenase. There are involved in several crucial metabolic pathways and defects in these enzymes are related to numerous genetic disorders in man. The complexes are composed of multiple copies at least three different enzymes: E1 or pyruvate decarboxylase, a TDP containing enzyme; E2 or dihydrolipoyl acyl transferase, containing one to three covalently bound lipoyl moieties; and E3 or dihydrolipoamide dehydrogenase, a flavoenzyme. The total complex has a molecular weight in the range of 3-6 million daltons, depending on the species. The crystal structure of three E3's have been solved which show surprisingly large differences compared with glutathione reductase which is 22% identical in amino acid sequence.

The core of the multienzyme complex is formed by the catalytic domain of the acyl transferase. In Azotobacter vinelandii this core has 432 symmetry and the crystal structure revealed a marvelous, hollow, truncated cube consisting essentially of 8 trimers at the corners of the cube. Monomer and trimers have great structural similarity with chloramphenicol transferase, or CAT, in spite of a relatively low sequence homology. The structures of the catalytic domain has been solved in complex with seven different ligands and substrates. This gave detailed insight into the catalytic mechanism. It also showed two surprisingly different binding modes of CoA with the AMP moiety virtually at the same position but with the essential SH groups approximately 10 Å apart.

In the productive binding mode of CoA, it is intriguing that a hydrogen bond donor of the pantetheine arm helps positioning a serine residue of the enzymes. This serine is involved in transition state stabilization. One might, therefore, say that the substrate is assisting the enzyme in catalyzing the transfer of the acyl group from lipoamide to CoA.

Structural studies of mutants of the catalytic domain are in progress. Preliminary results indicate that mutations in the active site channel induce binding of CoA in the unproductive mode even when the "new" amino acid is smaller than that of wild type enzyme. We are studying this intriguing observation in detail. Investigations on the structure of Bacillus stearothermophilus E3 are in progress as are numerous crystallization experiments of components of various complexes.

We would like to thank our numerous collaborators in this project: Aart de Kok and Adri Westphal in Wageningen, John Sokatch in Oklahoma, Richard Perham in Cambridge, Mulchand Patel in Ohio, and David T. Chuang in Dallas.

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MS–03.05.02 TWO COMPLEXES BETWEEN ELECTRON TRANSFER PARTNERS, CYTOCHROME C PEROXIDASE AND CYTOCHROME C. H. Pelletier, Department of Chemistry, University of California/San Diego, La Jolla, California.

The crystal structure of a 1:1 complex between yeast cytochrome *c* peroxidase and yeast iso-1-cytochrome *c* has been determined at 2.3 Å resolution and refined to a crystallographic R-factor of 17.2%. This structure reveals a possible electron transfer pathway unlike any previously proposed for this extensively studied redox pair. In one of the closest interprotein contacts the exposed heme methyl group (CBC) of cytochrome *c* is in van der Waals contact with the peptide bond between the surface residues Ala-193 and Ala-194 of the peroxidase and is wedged between the methyl sidechains of these residues. If the shortest straight line were drawn from this contact point to the peroxidase heme, it would closely follow the backbone chain of residues Ala-193, Gly-192, and finally Trp-191, the indole ring of which is perpendicular to and in van der Waals contact with the heme. Previous mutatgenesis experiments show Trp-191 of cytochrome *c* peroxidase plays a key role in electron tranfer reactions with cytochrome *c*. All this strongly suggests electron transfer occurs through this short backbone segment of the peroxidase which is in direct contact with both hemes via van der Waals junctions at each end of the chain.

The crystal structure of a complex between yeast cytochrome *c* peroxidase and horse heart cytochrome *c* has also been determined at 2.8 Å resolution and refined to a crystallographic R-factor of 17.9%. Although crystals of the two complexes, CCP:cc(yeast) and CCP:cc(horse), grew under very different conditions and belong to different space groups, the two complex structures are closely similar, suggesting that cytochrome *c* interacts with its redox partners in a highly specific manner.

MS–03.05.03 THE ACTIVE SITE OF YEAST ASPARTYL-tRNA SYNTHETASE. by J. Cavarelli*, B. Rees, M. Boeglin, M. Ruff, J. C. Thierry, D. Moras, UPR de Biologie Structurale, IBMC, 15 rne rene Descartes, 67084 Strabourg Cedex, France

The crystal structure of the yeast binary complex formed by the Aspartyl-tRNA synthetase and the $tRNA^{Asp}$ has been solved and refined to 2.9Å. Cytoplasmic AspRS from yeast is an $\alpha 2$ homodimeric enzyme like seven other members of class II aminoacyl-tRNA synthetases. Each monomer contains 557 amino acids and can be structurally divided into 3 domains. The N-terminal domain (residues 1 to 207) is built around a five stranded β-barrel and an α helix inserted between the third and the forth strands. This module recognizes the anticodon of the $tRNA^{Asp}$.
The second domain is a small hinge module (residues 207 to 241) composed of small α helices. This region anchors the $tRNA^{Asp}$ at the level of the D stem (G10-U25). The C-terminal domain is the core of the protein and contains the active site of the enzyme. It is formed around a six stranded antiparallel β sheet and partly closed by loops and α helices. This crystal structure confirmed the structural homology among class II enzymes and brought the first functional correlation between

the signature motifs and the binding of substrates (ATP, tRNA) or the oligomeric state of the protein. It also showed that this family of enzymes is modular and can be built around a central active site for ATP, amino acid and CCA recognition, with the addition of extra domains for functions specific of each synthetase.
The crystal structure of the complex formed by yeast aspartyl-tRNA synthetase, ATP, aspartic acid and $tRNA^{Asp}$ will be presented, with a detailed description of the active site of the enzyme. The class II synthetases exhibit a novel ATP binding fold, where the ATP molecule is anchored by highly conserved motif II and motif III residues. The binding pocket of the amino acid which has been inferred by model building and sequence analysis will be described. This model has been tested and confirmed by a number of site-directed mutations. The mode of binding of the receptor stem of the tRNA which is also class specific, explains the correlation of the two classes of synthetases with the primary site of attachment of the amino acid to the terminal adenosine.

MS-03.05.04 CATALYTIC MECHANISM OF HALOALKANE DEHALOGENASE: CRYSTAL STRUCTURES OF ENZYME-SUBSTRATE, ALKYL-ENZYME AND ENZYME-PRODUCT COMPLEXES

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Haloalkane dehalogenase, an enzyme of molecular weight 36,000, catalyzes the conversion of 1,2-dichloroethane into chloroethanol and chloride:
$Cl\text{-}CH_2\text{-}CH_2\text{-}Cl + H_2O \rightarrow Cl\text{-}CH_2\text{-}CH_2\text{-}OH + H^+ + Cl^-$
Dehalogenase is a member of the α/β hydrolase-fold family[1], to which family also belong acetylcholinesterase, lipases, dienelactone hydrolase and serine carboxypeptidases. These other enzymes are all esterases or peptidases, whereas the dehalogenase hydrolyses carbon-halogen bonds. Moreover, the proposed nucleophilic residue in dehalogenase (Asp124)[2] is quite different from the nucleophile in the other enzymes (Ser or Cys). Therefore, we analysed the reaction mechanism in detail by soaking dehalogenase crystals in the substrate 1,2-dichloroethane at different temperatures and pH values.
1. Substrate could be bound in the active site of the dehalogenase at 0°C and pH 5.0 without appreciable conversion. One chlorine atom is bound between the sidechain nitrogen atoms of two tryptophan residues. For the other chlorine no clear density is available suggesting that part of the molecule is flexible and not bound very tightly.
2. Soaking a crystal at room temperature and pH5.0 produces a covalent intermediate, bound to the Asp124 side-chain. This shows that Asp124 is the nucleophile attacking the C1 atom of the substrate. A chloride ion is produced, the density of which is clearly visible between the sidechains of the two tryptophans in the active site. A hydrolytic water molecule is in attacking position from the Cγ atom of Asp124.
3. Soaking crystals at room temperature and pH6.0, produces a chloride ion. No density is visible anymore for the alkyl-enzyme, nor for the product chloroethanol. Also no density for the hydrolytic water molecule is present anymore, suggesting that one reaction cycle has nearly completed.

From the above we conclude: Asp124 is the nucleophile in the active site of dehalogenase. It attacks the C1 carbon atom of the substrate. The negative charge which develops on the chlorine atom is stabilized by the side-chains of two tryptophan residues. An intermediate, covalently bound to the Asp124 side-chain in an ester bond, is formed. This ester is subsequently hydrolysed by a water molecule, assisted by His289 as a general base.

1 Ollis, D. L. et al. Protein Engin. 5, 197-211 (1992)
2 Franken, S. M. et al. EMBO J. 10, 1297, 1302 (1991)

MS-03.05.05 ELUCIDATING THE MECHANISM OF β-LACTAMASE BY CRYSTALLOGRAPHIC STUDIES. Osnat Herzberg*, Celia C.H. Chen & John Moult. Center for Advanced Research in Biotechnology, Maryland Biotechnology Institute, University of Maryland, 9600 Gudelsky Dr., Rockville MD 20850, USA.

The hydrolysis of β-lactams by β-lactamases constitutes the major bacterial defence against this class of antibiotics, diminishing the effectiveness of some of the most powerful therapeutic compounds. Following the determination of the crystal structure of a class A β-lactamase from *Staphylococcus aureus* we have proposed a catalytic mechanism that shares common features with that of the serine proteases. However, unlike the latter group of enzymes, the assisting catalytic machinery for acylation of β-lactamase differs from that of deacylation (Herzberg and Moult, *Science*, (1987), 236, 694). Support for this proposal has been provided by solution and crystallographic studies, investigating substrate and inhibitor binding, as well as mutant structures. The structure determination of the mutant protein P54 revealed that its activity is impaired due to a disordered loop at the active site. This disorder includes a glutamic acid residue, Glu166, whose main role is in the deacylation step, but not in acylation. Stopped flow measurements substantiated the crystallographic results, showing that the deacylation step becomes the rate-limiting step. The crystal structure of a phosphonate-inhibited β-lactamase, an analog of the tetrahedral transition state/intermediate of β-lactam hydrolysis enabled the modeling of the tetrahedral transition state associated with the acylation step of the true substrate. Because of the conformational rigidity of the fused rings of the penicillin molecule, the orientation of the substrate is fixed. The model supports our previously proposed mechanism of hydrolysis and provides new information about the mode of binding. We have also succeeded in trapping the acyl-enzyme products of clavulanate, a naturally occurring inhibitor, using cryo-crystallographic techniques. The crystal structure has been interpreted as containing a mixture of two acyl-enzymes representing two different stages of inhibitor degradation. This helped in differentiating between various proposed mechanisms of inactivation.