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.

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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

