

**PS04.01.106 CRYSTALLIZATION AND PRELIMINARY X-RAY STRUCTURE ANALYSIS OF *D*-DOPACHROME TAUTOMERASE.** Hiroshi Sugimoto, Atsushi Nakagawa, Isao Tanaka, Jun Nishihira\*, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan, \*Central Research Institutes, School of Medicine, Hokkaido University, Sapporo 060, Japan.

*D*-dopachrome tautomerase converts *D*-dopachrome into 5,6-dihydroxyindole (DHI). It was first observed in experiments when *D*-isomer of dopachrome was used as a control substrate for the study of isomerization of naturally occurring *L*-isomer in cultured melanoma cells. It is composed of 114 amino acids ( $M_r=12\text{kDa}$ ). *D*-dopachrome tautomerase has no homology in amino acid sequence to *L*-dopachrome tautomerase. However it exhibits 27% sequence homology to macrophage migration inhibitory factor (MIF), a cytokine involved in inflammation and immune system. The three dimensional structure of MIF has been solved by ourselves. It has a similar trimeric b-prism structure to 5-carboxymethyl-2-hydroxyruconate isomerase (CHMI) from *Escherichia coli*. These findings prompted us to start the three dimensional structure analysis of *D*-dopachrome tautomerase.

*D*-dopachrome tautomerase has been crystallized at 18°C by hanging drop vapor diffusion method. The reservoir solution contained 10% (w/v) PEGMME (polyethyleneglycol monomethylether) 2000, 0.1M Tris-HCl buffer (pH8.8) and 0.1M nickel chloride. Equal volumes of the reservoir solution and 20mg/ml protein solution in deionized water were mixed to give 8ml drops. Rod shaped crystals grew up to 0.3 x 0.3 x 0.8mm within a week. They diffract X-ray up to 3.0Å resolution. A preparation of selenomethionyl crystals for multiwavelength anomalous diffraction (MAD) method is under way.

**PS04.01.107 CRYSTAL STRUCTURE OF THE CATALYTIC DOMAIN OF *PSEUDOMONAS AERUGINOSA* EXOTOXIN A COMPLEXED WITH AN NAD ANALOGUE: IMPLICATIONS FOR THE ACTIVATION PROCESS AND FOR ADP-RIBOSYLATION.** Mi Li\*, Fred Dyda\*, Itai Benhar-, Ira Pastan-, David R. Davies\*, Laboratory of Molecular Biology, NIDDK and -Laboratory of Molecular Biology, DCBDC, NCI, National Institutes of Health, Bethesda MD 20892

The isolated third domain of *pseudomonas aeruginosa* exotoxin A (PEIII) catalyses the transfer of ADP-ribose from NAD to elongation factor-2 in eukaryotic cells. A previous structure of PEIII crystallized in the presence of NAD showed only hydrolysis products of NAD bound to the enzyme. In order to define the site of NAD binding, we have now crystallized PEIII in the presence of a less hydrolyzable NAD analogue,  $\beta$ -thiazole-4-carboxamide adenine dinucleotide ( $\beta$ -TAD), and refined the complex structure at 2.3 Å resolution. There are two independent molecules of PEIII in the crystal, and the conformations of  $\beta$ -TAD show some differences in the two binding sites. The TAD attached to monomer 2 appears to have been hydrolyzed between the pyrophosphate and the nicotinamide ribose. The other molecule, monomer 1, shows an intact TAD and has no crystal packing contacts in the vicinity of  $\beta$ -TAD, so that the observed conformation probably most resembles that in the free PEIII in solution.

Li, Dyda, Benhar, Pastan & Davies: The Crystal Structure of *pseudomonas aeruginosa* exotoxin Domain III with nicotinamide and AMP: Conformational differences with the intact exotoxin. PNAS, 92: 9308-9312, 1995.

**PS04.01.108 STRUCTURE AND FUNCTION OF RIBONUCLEOTIDE REDUCTASE R1 PROTEIN.** Eriksson, M., Ramaswamy, S., Uhlin, U. and Eklund H., Department of Molecular Biology, Biomedical Center, Swedish Univ. Of Agricultural Sciences, Uppsala, 75124. SWEDEN.

The effector binding site, the reduced and the oxidised forms of the large subunit of the R1 protein of Ribonucleotide Reductase (RR1) and the refinement of the native protein is the subject of this abstract. RR1 is the enzyme that catalyses the reaction of all the four ribonucleotides into its corresponding deoxy form. The enzyme is therefore very crucial in the synthesis of the precursor molecules of DNA. The enzyme is an  $\alpha_2\text{-}\beta_2$  enzyme. One subunit called the R2 or the smaller subunit has the di-iron center and generates the free-radical which is required for its activity, the other subunit R1, is the larger subunit and houses the active site and the allosteric sites. The structures of both these have been determined and published earlier.

We have now refined the structure of the R1 protein at 2.5Å resolution with strict ncs symmetry. The final R-factor is 21%. We also have determined the structure of various mutants of this enzyme and refined them. One of the mutants Y730F, has the catalytic disulphide in the reduced form. A structure has also been determined which has the effector bound to it. These have enabled us to further our understanding of the mode of action of this subunit. We have also docked the two subunits and have now a hypothetical model for the possible mode of transfer of the free-radical from one subunit to the other. The results of these studies will be presented.

**PS04.01.109 A DETAILED STUDY OF THE ACTIVE SITE OF RUBISCO BY X-RAY CRYSTALLOGRAPHY** Anthony P. Duff\*, T. John Andrews\*\* & Paul M. G. Curmi\*, \*IBIS, School of Physics, The University of New South Wales, NSW 2052, Australia \*\*Research School of Biological Sciences, The Australian National University, Canberra, ACT 2601, Australia.

We are creating new complexes with rubisco by co-crystallisation to further study its complex reaction. Rubisco is very well known amongst plant enzymes, for its abundance (50% soluble leaf protein), for its inefficiency (reaction rate 2 per second), and for the many years that many groups worldwide have put into its study. Despite previous studies, the active site, and protein as a whole is not satisfactorily understood. That is why we are intent on completing probably the most difficult task yet to be done - the co-crystallisation of rubisco complexed with new intermediate analogues, so as to complete a set of snapshots of the five-step reaction. Crystallisation of new complexes has been achieved, and optimisation and x-ray diffraction is continuing. Electron density has been calculated in one case, giving us more definite knowledge of the movements of an important loop. Differences and similarities in crystallisation of different reaction states suggest some general properties of the enzyme. We are also eager to discuss difficulties, techniques and inventions in the methods of purification, crystallisation, and wet and cryo x-ray diffraction.