

[s8a.m7.p3] Structure of glutamate synthase, a complex iron-sulphur flavoprotein. C. Binda¹, R. Bossi¹, S. Wakatsuki², S. Artz², B. Curti³, M.A. Vanoni³ and A. Mattevi¹, ¹Dept. Genetics & Microbiology, University of Pavia, via AbbiateGrasso 207, Pavia (Italy), ²ESRF, BP220 Grenoble F-38043, France, ³Dept. General Biochemistry and Physiology, University of Milano, via Celoria 26, 20133 Milano (Italy).

Keywords: metalloproteins.

Flavoenzymes are widely distributed in Nature. The reasons of the success of flavin cofactors in biological processes reside in their versatility. Flavine molecules can function in one- and two-electron transfer reactions involving highly diverse substrates. Furthermore, they are able to react with oxygen in a tuneable manner so that flavoenzymes can act as oxidases, dehydrogenases and electron-transferases.

In our laboratory, we have an ongoing project focused on the structural biology of FAD-dependent enzymes. Our main aim is the investigation of the mechanisms of catalysis and the structural features which allow flavoenzymes to act on a variety of substrates¹. In the framework of this project, we are pursuing the structure determination of glutamate synthase, a complex "multicenter" flavoprotein.

Glutamate synthase (GltS) is a crucial enzyme for nitrogen metabolism and assimilation in plants and bacteria. In bacteria GltS is a tetramer of ___ protomers (molecular mass 4x200 KDa) and contains three iron-sulphur clusters, one molecule of FMN and one of FAD. The catalytic cycle involves two electron reduction of the FAD by NADPH, followed by electron transfer to the FMN through the iron-sulphur clusters. Next, this cofactor reduces the iminoacid formed by addition onto 2-oxoglutarate of the ammonia resulting from glutamine hydrolysis. Thus, GltS is able to employ the electron transfer from an "entry site" (the FAD reduced by NADPH) to an "exit site" (the FMN) as driving force for the hydrolysis of Gln by a glutamine amidotransferase active centre. In this way, the directionality in the electron flow makes the enzyme catalysed reaction essentially irreversible.

Crystals of *Azospirillum brasilense* GltS have been obtained. They belong to space group P3₁21 with unit cell parameters $a=b=234$ Å and $c=307$ Å. We have collected a 3.0 Å native data set at the beam line ID14-EH3 at ESRF. Furthermore, we have very recently carried out a MAD experiment at 4.3 Å resolution on Fe edge at the beam line BM14. The MAD phases were extended by solvent flattening and density averaging to 3.0 Å resolution. The resulting electron density map allows the unambiguous identification of secondary structure elements and a three-dimensional model for the protein is currently being built.

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[s8a.m7.p4] The fate of molybdate inside the cell: *Azotobacter vinelandii* ModG binding specificity. L. Delarbre, C.E.M. Stevenson, D.J. White, L.A. Mitchenall, R.N. Pau & D.M. Lawson, John Innes Centre, Norwich NR4 7UH, UK.

Keywords: molybdenum, MAD, binding specificity.

Azotobacter vinelandii ModG belongs to a novel family of intracellular molybdate-binding proteins that also bind tungstate but not sulphate, and are described as molbindins. They appear to be involved in intracellular molybdenum homeostasis, and in diazotrophs such as *A. vinelandii*, may have a role in nitrogenase cofactor biosynthesis.

The ModG subunit consists of a tandem repeat which is also found in ModE, the regulatory protein of the high-affinity ABC transport system for Mo. The structure of *E. coli* apo ModE has been solved as a dimer [1]. We have solved the structure of the ligand-bound form of ModG at 2.2 Å resolution by MAD phasing on tungsten and subsequently refined it against 1.8 Å resolution data [2, 3]. The ModG monomer consists of two discontinuous Oligosaccharide/oligonucleotide Binding folds (OB fold) and displays a strong non-crystallographic two-fold symmetry. ModG forms a trimer arranged around a crystallographic three-fold axis. The first type of binding site is found at the interface between two neighbouring subunits and consequently occurs three times. The second type of binding site is located on the crystallographic three-fold axis. Pseudo-equivalent binding sites are also found in the second half of the assembly so that ModG trimer binds eight molybdate molecules. The structure of the apo form of ModG was subsequently solved to 2.4 Å resolution by molecular replacement. Although all binding sites are located at subunit interfaces, apo ModG was found to remain a trimer.

Comparison between apo and ligand-bound structures of ModG as well as with that of ModA [4], the monomeric periplasmic component of Mo high-affinity transport system, will allow assumptions to be made regarding the criteria driving ModG binding specificity.

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