s8a.m1.p47 **The X-Ray structure of the ternary complex GAPDH/NAD/G3P enlightens the role of two anion binding sites during the catalysis.** M. Fatih[1], C. Didierjean[1], C. Corbier[1], S. Boschi-Muller[2], G. Branlant[2] and A. Aubry[1], *[1]Laboratoire de Cristallographie et Modélisation des matériaux Minéraux et Biologiques, Groupe Biocristallographie, Université Henri Poincaré Nancy I, B.P. 239, 54506 Vandoeuvre lès Nancy Cedex, France, [2]Laboratoire de Maturation des ARN et Enzymologie Moléculaire, UMR 7567, B.P. 239, 54506 Vandoeuvre lès Nancy Cedex France. e-mail: didier@lcm3b.u-nancy.fr*
Keywords: GAPDH, holoenzyme-substrate complex, anion-binding site.

The glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a tetrameric enzyme which catalyzes the oxidative phosphorylation of D-glyceraldehyde-3-phosphate (G3P) to 1,3-diphosphoglycerate (1,3-dPG) in the presence of NAD and inorganic phosphate. The pathway involves two steps: firstly an oxydoreduction which leads to the formation of a thioacylenzyme and NADH via a hemithioacetal enzyme intermediate and secondly a phosphorylation which produces the 1,3-dPG. The active site of the GAPDH contains two anion binding sites which have been attributed to the phosphate binding of the substrates, namely, G3P (Ps site) and inorganic phosphate (Pi site). Two hypotheses were put to describe the formation of the hemithioacetal intermediate between substrate G3P and the catalytic residue Cys149. Moras et al. (1975) proposed that the C3 phosphate group is located in the Ps site during the two steps of the reaction. In the model proposed by Skarzynski et al. (1987) the substrate G3P would first interact with the Pi site. Before the phosphorylation step, the C_3 phosphate would shift from the Pi site towards the Ps site, making the Pi site available for the inorganic phosphate. In order to validate one of the two hypotheses we have undertaken the X-Ray structural determination of the holoform of the mutants C149A and C149S in complex with the natural substrate D-G3P. The crystal structures of two ternary complexes (mutant C149A / NAD / G3P and mutant C149S / NAD / G3P) have been solved at high resolution using the laboratory copper X-Ray source. Both structures present small differences in comparison with the wild-type structure. The substrate G3P is non covalently bounded in the active site with its C_3 phosphate located in the Ps site. Details of the structures will be presented in relation with the enzymatic results.

Moras et al. (1975) J. Biol. Chem. 250, 9137-9167.
Skarzynski et al. (1987) J. Mol. Biol. 193, 171-187.

s8a.m1.p48 **Cellular detoxification: crystal structure of a repair enzyme from *Escherichia coli* at 1.9 Å resolution**. F. Tête-Favier[1], D. Cobessi[1], S. Boschi-Muller[2], S. Azza[2], G. Branlant[2], A. Aubry[1]. *(1): LCM3B and (2): MAEM; UHP Nancy I, BP 239, 54506 Vandoeuvre, France.*
Keywords: MAD, selenomethionines, X-ray structure.

Amino acids in proteins are often oxidized by biological oxidative species, leading to ageing problems in organisms. In peculiar, methionines are easily oxidized into methionine sulfoxides. The cell overcomes this problem using an enzyme[1-2], named Peptide Methionine Sulfoxide Reductase (PMSR, EC 1.8.4.6). It catalyzes the reduction of methionine sulfoxides to methionines. More than forty PMSR sequences are available from mammalians, plants or bacteria. A search for homologous proteins on the basis of their sequences reveals a very independent family, far from any known protein. The knowledge of the three-dimensional structure of PMSR is thus highly interesting, in particular to understand the unknown catalytic mechanism of this crucial enzyme involved in repair of cell damages.

E. coli wild-type PMSR (MsrA, 211 aminoacids) was cloned, sequenced and overexpressed. The production and purification of the selenomethionine-substituted enzyme ([Se-Met]MsrA) was also achieved in order to solve the phase problem using MAD methods. Crystallisation conditions were investigated and resulted in three different crystal forms of MsrA, one of them diffracting to 1.9 Å resolution. A complete native data set was collected at this resolution on beamline BM30 at ESRF. Crystals belong to space group $P6_522$, with unit cell parameters a=b=102.5 Å, c=292.3 Å and have three monomers per asymmetric unit. The [Se-Met]MsrA crystals were grown from the same condition than MsrA crystals. Three different wavelengths were used for data collection at 3 Å resolution on beamlines BM14 at ESRF and PX31 at DESY. Using SnB, CNS, and MLPHARE, 18 selenium sites were determined which gave a readable electron density map. The atomic model is currently under building, and the structure of MsrA refined at 1.9 Å resolution will be described.

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