s8a.m1.p45 **Crystal structures of Wild-Type and D30A thioredoxin *h* from *Chlamydomonas reinhardtii* enlighten the role of water molecule in catalysis.** V. Menchise[ab], C. Corbier[a], C. Didierjean[a], M. Saviano[b], E. Benedetti[b], J-P. Jacquot[c] and A. Aubry[a]* [a] *Laboratoire de Cristallographie et Modélisation des Matériaux Minéraux et Biologiques, Groupe Biocristallographie, ESA 7036, Université Henri Poincaré-Nancy I, BP 239, 54506 Vandoeuvre-lès-Nancy Cedex, France.* [b]*Centro di Studio di Biocristallografia del CNR, Dipartimento di Chimica, Via Mezzocannone 4, 80134 Napoli, Italy.* [c]*Laboratoire de Biologie Forestière, Université Henri Poincaré-Nancy I, BP 239, 54506 Vandoeuvre-lès-Nancy Cedex, France.*
Keywords: thioredoxin,, molecular dynamics, acid-base catalysis.

Thioredoxins are ubiquitous proteins which catalyse the reduction of disulphide bridge on target proteins. The catalytic mechanism proceeds via a mixed disulphide intermediate whose breakdown should be enhanced by the involvement of residue Asp30 as a base catalyst towards residue Cys39[1]. We report here the crystal structure of wild-type and D30A mutant thioredoxin *h* from *Chlamydomonas reinhardtii*, which constitutes the first crystal structure of a thioredoxin isolated from an eukaryotic plant organism. The role of residue Asp30 in catalysis has been revisited since the distance between Asp30-OD1 and Cys39-SG is too long to support the hypothesis of a direct proton transfer: a careful analysis of all available crystal structures reveals that the relative positioning of residue Asp30 and Cys39 as well as hydrophobic contacts in the vicinity of residue Asp30 does not allow a conformational change that would bring the two residues close enough for a direct proton transfer. This suggests that deprotonation of Cys39 should be mediated by a water molecule. Molecular dynamics simulations, carried out either *in vacuo* or in water, support this hypothesis. The results are discussed with respect to biochemical and structural data.

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s8a.m1.p46 **Crystal structure of the NAD-dependent erythrose-4-phosphate dehydrogenase from *Escherichia coli*.** B. Kauffmann[1], D. Cobessi[1], S. Boshi-Muller[2], S. Azza[2], G. Branlant[2] and A. Aubry[1]. [1]*LCM3B and* [2]*MAEM, Faculté des Sciences, BP 239, 54506 Vandoeuvre-lès-Nancy, France.*
Keywords: E4PDH, NAD, evolution.

Erythrose-4-phosphate dehydrogenase (E4PDH) is an NAD-dependent tetrameric enzyme of 338 residues per monomer which catalyses the irreversible oxidation of D-erythrose-4-phosphate (E4P) into 4-phospho-erythronate (4PE). E4PDH could be a key enzyme of the pyridoxine (vitamin B6) biosynthesis, a direct precursor of the ubiquitous, essential coenzyme pyridoxal 5' phosphate (PLP), used by several enzymes involved in the amino acids metabolism [1]. The reaction rate of E4PDH differs according to that the reaction is studied with glyceraldehyde-3-phosphate (G3P) or E4P. The same observation is reported when E4P instead of G3P is used as GAPDH substrate [2]. E4P and G3P differs only by one CHOH group. So, the substrate structure modulates the enzyme activity. Concerning the cofactor, E4PDH has a lower affinity for NAD than the one of GAPDH [2].

E4PDH apoenzyme was crystallised in several crystallisation conditions, one of them leading to crystal sizes suitable for X-ray studies. Data were collected at 2.7 Å resolution on beamline BM30 at ESRF. Crystals belong to the space group $P2_12_12_1$. The unit cell parameters is a= 90.5, b= 111.8, c= 137.6 Å. The structure was solved by molecular replacement using AMoRe with the model of GAPDH from *B. stearothermophilus.* Correlation factor and R-factor were 54.3 % and 39.6 % respectively. The structure was refined using CNS_SOLVE. The free-R and R-factor are 26.3% and 21.1 % respectively. Structure analysis reveals distance between the catalytic residues differing from the ones observed in GAPDH (7 Å *versus* 4 Å in GAPDH). Structure comparison shows also the residues probably involved in the NAD binding and should provide response on the difference observed for the cofactor affinity. Catalytic site comparison should allow to propose mutations in order to efficiently transform a non-phosphorylating dehydrogenase into phosphorylating dehydrogenase and *vice versa*.

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