

THE CRYSTAL STRUCTURE OF PHOTOSYNTHETIC A_2B_2 -GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE DISCLOSES THE MECHANISM OF THIOREDOXIN REGULATION

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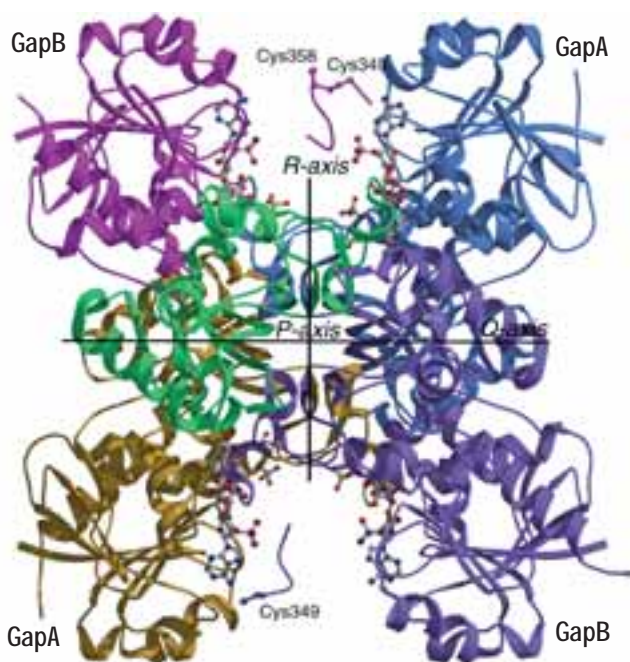
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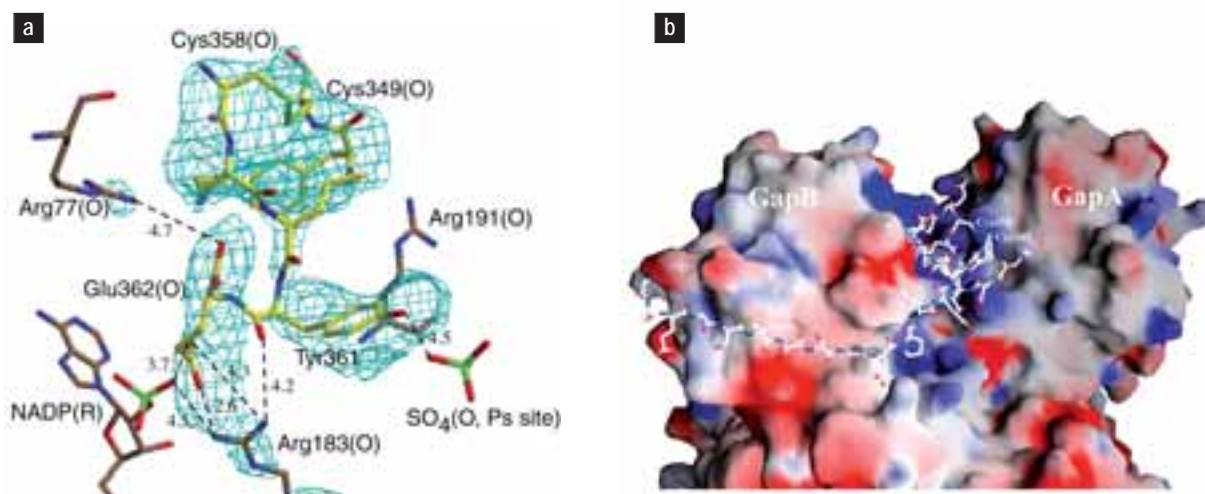
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Figure 1. Ribbon model of a single tetramer of oxidized A_2B_2 -GAPDH. The coenzyme molecules, and the sulphate ions of each subunit and the cysteines of the CTE are represented as ball and sticks. For one GapB subunit the coenzyme-binding domain (magenta) and the catalytic domain (green) are represented.

Chloroplast glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a light-regulated, NAD(P)H-dependent enzyme involved in plant photosynthetic carbon reduction. At difference with lower photosynthetic organisms which only contain A_4 -GAPDH, the major GAPDH isoform of land plants is made up of A and B subunits, the latter containing a C-terminal extension (CTE) with fundamental regulatory functions [1]. Light-activation of AB-GAPDH depends on the redox state of a pair of CTE cysteines forming a disulfide bond under control of

thioredoxin *f*, leading to specific inhibition of the NADPH-dependent activity. The crystallographic structural model of oxidized A_2B_2 -GAPDH complexed with NADP is composed of one tetramer and a dimer, which generates a second tetramer using a crystallographic two-fold axis coincident with the molecular axis P (Figure 1). A_2B_2 -GAPDH showed an overall structural organization similar to thioredoxin-independent A_4 -GAPDH [2]. These two isoforms almost identical along P and Q axes, are differently sized along R axis, where the A_2B_2 tetramer (75.7 Å) appeared shorter than A_4 -GAPDH (78.1 Å). Each A or B subunit consists of a coenzyme-binding domain and a catalytic domain. One NADP molecule was bound to each coenzyme domain, and one or two sulphate ions were found in each catalytic domain (Figure 1). The CTE of B-subunits was only partially detectable by x-ray diffraction experiments. The inspection of the electron density maps showed an elongated electron density region not continuous with any subunit, but interpretable as a protein chain slipping into the cleft bordered by a pair of A and B-subunits (Figures 1, 2a). This cleft features a positive electrostatic surface potential due to six exposed arginines and two histidines (Arg77, Arg183, Arg191 and His190), conserved in both A and B-subunits (Figure 2b). The CTE contains negatively charged residues and could be attracted by the strongly cationic cleft.





The structure of oxidized CTE was also investigated by site-specific mutagenesis and by a computational approach predicting the positioning of the whole CTE. In the final predicted model, well compatible with the crystalline packing of oxidized A_2B_2 -GAPDH, the CTE appears in an extended conformation lining the protein surface (Figure 2b). The CTE domain approaches the coenzyme binding site forming a loop stabilized by the regulatory disulfide bond between Cys349 and Cys358. The CTE is kept in place through interaction of its negative side chains with Arg191, Arg77 and Arg183 (Figure 2a). The roles played by the strong interaction between the Glu362, last charged CTE residue, and Arg183 was exploited by using recombinant regulatory B_4 -GAPDH [2]. It is known that the point mutations R183A and E362Q cause redox-insensitive forms [2,3]. Consistently, the mutation R191A strongly diminished the redox sensitivity of B_4 -GAPDH, supporting the involvement also of this residue in CTE docking [2]. Mutation R77A was also recently shown to abolish redox sensitivity and decrease NADPH-dependent activity, consistent with a crucial role of Arg77 in kinetic efficiency of the enzyme [3]. Single mutations of the CTE charged residues (D351N, E356Q, E357Q) slightly affected the redox sensitivity, while the complete redox insensitivity was achieved only with the double mutant E356Q/E357Q [2].

In conclusion, present data support the view that A_2B_2 -GAPDH inhibition by thioredoxin depends on the docking of the oxidized CTE into the cleft delimited by A/B-subunits. In this location, the CTE appears to interfere with the recognition of bound NADP by the crucial residues Arg77 and Ser188, thus leaving the tetramer in a kinetically inhibited conformation, unable to efficiently use NADPH as the preferred coenzyme.

References

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Figure 2. (a) (Fo-Fc) electron density map of CTE (belonging to chain O), calculated omitting the CTE residues and shown at 2.5 σ cut-off. The disulphide bridge between Cys349 and Cys358 and distances below 5 Å between CTE residues and protein residues or ligands are also shown. (b) Surface electrostatic potential of oxidized A_2B_2 -GAPDH complexed with NADP. Blue colour indicates a positive potential while red colour a negative one. The CTE is represented as ball and sticks.