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REDOX-SENSITIVE STRUCTURAL FEATURES OF TRANSKETOLASE FROM *CHLAMYDOMONAS REINHARDTII*

Tedesco, D.;[a] Naldi, M.;[a,b] Pasquini, M.;[c] Zaffagnini, M.;[c] Francia, F.[c] and Bertucci, C.[a]

[a]Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126, Bologna, Italy; [b]Center for Applied Biomedical Research (C.R.B.A.), S. Orsola-Malpighi Hospital, Via Massarenti 9, 40138, Bologna, Italy; [c]Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio 42, 40126, Bologna, Italy.

daniele.tedesco@unibo.it

Several studies identified the Calvin–Benson cycle (CBC), the photosynthetic pathway responsible for carbon fixation (*Figure 1*), as a redox-regulated process. New biochemical and proteomic approaches suggest that all the CBC enzymes may withstand redox regulation through multiple redox post-translational modifications (PTMs), like disulfide bond formation, glutathionylation and nitrosylation[1]. This redox control is likely involved in the adaptative response to environmental conditions, including light/dark transitions and abiotic/biotic stress.

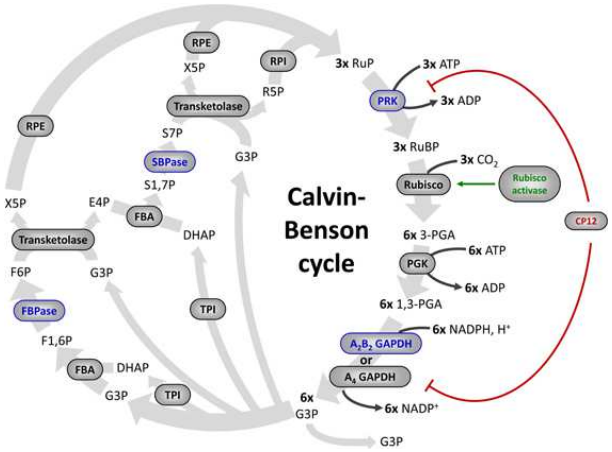

Figure 1. Scheme of the Calvin–Benson cycle[1].

This ongoing work aims at investigating the redox regulation of the chloroplastic CBC enzyme transketolase from the green microalga *Chlamydomonas reinhardtii* (CrTK). Recombinant CrTK was heterologously expressed in *E. coli* and purified to homogeneity through metal affinity chromatography. *In vitro* activity assays showed that the purified enzyme displays a redox-sensitivity being partially inhibited by oxidizing treatments. Moreover, the 3D-structure of the reduced protein (solved at 1.8 Å resolution) revealed the presence of several cysteine couples located at suitable distance for disulfide bond formation and in close proximity to catalytic residues. The influence of the redox state on the structural features of *Cr*TK was further investigated by LC-ESI-MS/MS analysis, allowing the identification of the regulatory cysteines. Concomitantly, circular dichroism (CD) analysis in the far-UV spectral region allowed evaluating redox-dependent changes in the secondary structure of the enzyme. Furthermore, CD analysis in the near-UV region showed the onset of an induced circular dichroism (ICD) signal[3] arising from the interaction between CrTK and the cofactor thiamine pyrophosphate (TPP), which was exploited to monitor CrTK-TPP binding under different redox states of the enzyme.

References

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