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CHARACTERIZATION OF POPLAR PLASTIDIC P2-G6PDH

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Glucose-6-phosphate dehydrogenase (G6PDH - EC 1.1.1.49) is a regulatory enzyme of the OPPP (oxidative pentose phosphate pathway) because of its tight regulation and/or redox dependence. The most important role of OPPP is to generate NADPH to provide reductants for biosyntheses. Reductants produced in the OPPP may also be important to fight oxidative stress (Wang *et al.,* 2003), induced by nutrient starvation, drought, salinity, pathogens. The Cy- and P2-G6PDHs are essential for stress tolerance (Esposito *et al.,* 2003; Nemoto and Sasakuma, 2000; Valderrama *et al.,* 2006; Wang *et al.,* 2008; Scharte *et al.,* 2009; Cardi *et al.,* 2011).

A fundamental role of redox regulation for the plastidial P2-G6PDH has been described, this isoform appearing strictly linked to the reductant balance within the plastids and to stress response (Esposito *et al.,* 2003).

Therefore, the gene encoding for the plastidic G6PDH isoform (P2-G6PDH) from poplar (*Populus trichocarpa*) has been cloned in the expression vector pET15b (His-Tag), and the recombinant protein overexpressed in *E. coli.* Moreover we generated single mutants of all cysteines and a double mutant for both cysteines presumably involved in the redox regulation.

The main kinetic parameters and the redox sensitivity to DTT and glutathione have been determined for all purified enzymes (wild type and all the mutants). The values measured for the wild type are in the same range as those obtained for most other P2-type G6PDHs (e.g. high Ki_{NADPH}). Additionally, the recombinant poplar WT enzyme is moderately sensitive to reductants (DTT_{red}), though it exhibits a redox potential (-280 mV) favourable for control by either thioredoxins *m* or *f*.

The study of the C175S and C183S variants confirmed that these cysteines are involved in the enzyme redox regulation. As these substitutions did not affect Km_{G6P}; this suggests that these mutations do not affect the active site, while the effects of NAPDH clearly indicate an non-competitive inhibition. On the other hand, the C145S and C242S variants did not display any activity suggesting an involvement in the tetrameric assembly of the enzyme. Regarding the C194S variant, the results suggest that this residue is not involved in the redox regulation since the substitution lefts Km_{G6P} substantially unchanged. Besides, this variant completely lost inhibition by NADPH, suggesting that C194 is part of the NADPH binding site.