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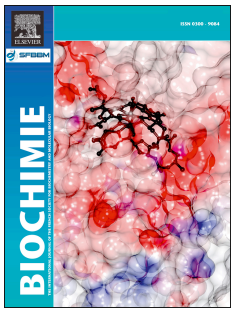

Elucidating paramylon and other carbohydrate metabolism in *Euglena gracilis*: Kinetic characterization, structure and cellular localization of UDP-glucose pyrophosphorylase

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Many oligo and polysaccharides (including paramylon) are critical in the *Euglena gracilis* life-cycle and they are synthesized by glycosyl transferases using UDP-glucose as a substrate. Herein, we report the molecular cloning of a gene putatively coding for a UDP-glucose pyrophosphorylase (*Egr*UDP-GlcPPase) in *E. gracilis*. After heterologous expression of the gene in *Escherichia coli*, the recombinant enzyme was characterized structural and functionally. Highly purified *Egr*UDP-GlcPPase exhibited a monomeric structure, able to catalyze synthesis of UDP-glucose with a V_{max} of 3,350 $U.mg^{-1}$. Glucose-1P and UTP were the preferred substrates, although the enzyme also used (with lower catalytic efficiency) TTP, galactose-1P and mannose-1P. Oxidation by hydrogen peroxide inactivated the enzyme, an effect reversed by reduction with dithiothreitol or thioredoxin. The redox process would involve sulfenic acid formation, since no pair of the 7 cysteine residues is close enough in the 3D structure of the protein to form a disulfide bridge. Electrophoresis studies suggest that, after oxidation, the enzyme arranges in many enzymatically inactive structural conformations; which were also detected *in vivo*. Finally, confocal fluorescence microscopy provided evidence for a cytosolic (mainly in the flagellum) localization of the enzyme.