IN VITRO URIDYLYLATION AND DEURIDYLYLATION OF PII PROTEINS FROM *HERBASPIRILLUM SEROPEDICAE* BY THE GLND PROTEIN

Bonatto, A. C., Souza, E. M., Pedrosa, F. O., Couto, G. H., Araújo, L. M., Benelli, E. M.
Departamento de Bioquímica e Biologia Molecular – UFPR, Centro Politécnico, C. Postal 19046, Curitiba – PR. Email: benelli@ufpr.br

GlnD is a bifunctional uridylyltransferase/uridylyl-removing enzyme that has a primary role in the general nitrogen regulation (NTR) system. In enterobacteria, GlnD responses to nitrogen levels by modifying the PII proteins GlnB and GlnK. Under low levels of nitrogen, GlnD transfers UMP groups to GlnB and GlnK (uridylylation). When the nitrogen levels rises, GlnD removes UMP from these proteins (deuridylylation). GlnB and GlnK are signal transduction proteins that integrate the signals of nitrogen, carbon and energy, and transduce this information to other proteins involved in nitrogen metabolism. In *Herbaspirillum seropedicae*, an endophytic diazotroph isolated from grasses, several genes coding for proteins involved in nitrogen metabolism have been identified. In this work, *H. seropedicae* GlnB, GlnK and GlnD proteins were expressed and purified in their native forms and used to reconstitute the uridylylation system *in vitro*. The uridylylation states of GlnB and GlnK were determined by native gel electrophoresis. The results show that *H. seropedicae* GlnD uridylylates GlnB and GlnK in the presence of 2-oxoglutarate and ATP. These reactions were inhibited by glutamine, and this inhibition increased with the glutamine concentration. The deuridylylation reaction was also reproduced *in vitro*; it was considerably slower than uridylylation.

Supported by CAPES and CNPq/Instituto Milênio