Azospirillum brasilense PII proteins GlnB and GlnZ do not form heterotrimers

Inaba,J.[a], Huergo, L.F.[a], Bonatto, A. C.[a], Chubatsu, L.S.[a], Monteiro,R.A.[a], Steffens, M.B.[a], Klassen, G.[b], Rigo, L.U.[a] ,Pedrosa, F. O.[a] , Yates, M.G., Souza, E.M.[a]

[a]- Departamento de Bioquímica e Biologia Molecular- UFPR ; [b]- Departamento de Patologia Básica- UFPR, Centro Politécnico, C. Postal 19046

81531-990, Curitiba, PR.

E-mail: juinaba@hotmail.com

Proteins of the PII are intracellular transducers of the prevailing nitrogen levels. These trimeric proteins are highly conserved across all kingdoms of life, and in Proteobacteria are covalently modified by uridylylation in response of the nitrogen status. *Escherichia coli* contains two paralogues PII proteins, GlnB and GlnK, which are capable of forming mono, di and tri-uridylylated trimers. These proteins can also form heterotrimers both *in vivo* and *in vitro,* which have intermediate capacity of signalling to the PII target proteins such as GlnE and the NtrB proteins. *Azospirillum brasilense,* a free-living nitrogen-fixing bacterium, contains two PII-like proteins, GlnB and GlnZ, both involved in the regulation of nitrogen fixation. In this work, purified GlnB, GlnB-UMP, GlnZ and GlnZ-UMP proteins were mixed in pairs and subjected to a thermic denaturation/renaturation procedure to allow the disruption and reassembly of the trimeric protein. Surprisingly, the results showed that when GlnZ and GlnB monomers were mixed and denatured, only the GlnB and GlnZ homotrimers were re-assembled. No heterotrimer GlnZ/GlnB was formed under any condition tested. Furthermore, when the unmodified and uridylylated forms of GlnB were mixed and subjected to denaturation/renaturation only the fully unmodified or fully uridylylated forms were recovered, in contrast with GlnZ and PII from *E. coli* which can readily form form partially uridylylated trimers.

Keywords: 1) *Azospirillum brasilense*; 2) heterotrimer; 3) GlnB; 4) GlnZ.

Supported by: CNPq/ MCT, Fundação Araucária and Paraná Tecnologia