Effects of combinations of ATP, ADP, 2-OG on GlnZ-DraG, GlnZ-AmtB and GlnB-AmtB complex formation in vitro in *Azospirillum brasilense*


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*Azospirillum brasilense* is a nitrogen-fixing bacterium found in association with several crops. Nitrogen fixation is highly regulated in this organism at many levels; the activity of the nitrogenase enzyme is post-translationally regulated by adding/removing an ADP-ribose, mediated by the DraT and DraG enzymes, which are themselves regulated through interaction with PII proteins, GlnB and GlnZ, respectively. DraG activity is regulated by ternary complex formation DraG-GlnZ-AmtB. Complex formation is regulated by ADP, ATP and 2-OG levels. In order to verify how these effectors interfere in PII interactions, different ATP/ADP ratios and 2-OG concentrations were tested *in vitro* by pull-down. ATP/ADP ratio affects DraG-GlnZ, AmtB-GlnZ and AmtB-GlnB complex formation, mainly in 2-OG presence. In 2-OG high concentrations, interactions only occur when using ADP only. In absence or low 2-OG concentrations, complex formation is observed even at 0.9 ATP/ADP ratio. So, 2-OG concentration is a determinant factor to occupation of the GlnB and GlnZ nucleotide binding sites by ATP or ADP, increasing 2-OG favors ATP binding and reduced 2-OG facilitate the ADP binding. GlnB and GlnZ apparently can sense the D/T ratio, in the presence of 0.1mM 2-OG, fluctuations of the D/T ratio within the reported physiological range alter the DraG-GlnZ, AmtB-GlnZ and AmtB-GlnB interactions significantly.

Keywords: Nitrogen fixation/effectors/PII

Supported by the Brazilian Program of National Institutes of Science and Technology-INCT/Brazilian Research Council-CNPq/MCT/CAPES/Fundação Araucária