Effect of Glutamine on the Uridylylation and Deuridylylation of *Azospirillum brasilense* GlnB and GlnZ Proteins

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Azospirillum brasilense is a nitrogen-fixing bacterium that associates with important agricultural crops such as maize, wheat and rice. In this diazotroph, nitrogen fixation is strictly controlled by nitrogen and oxygen levels. The intracellular nitrogen status is signaled by the uridylylation status of the GlnB and GlnZ proteins, which in turn is controlled by GlnD acting as uridylyl transferase or uridylyl removing enzyme depending on nitrogen level. *In vitro* uridylylation of GlnB and GlnZ proteins by GlnD also requires ATP and α-ketoglutarate. Here, we analyze the effect of glutamine on the uridylylation and deuridylylation of *A. brasilense* GlnB and GlnZ proteins. The native GlnB and GlnZ proteins and the His-tagged GlnD were purified from *E. coli,* reaching over to 95% purity. The results showed that the maximum rate of both GlnB and GlnZ uridylylation occurs in the absence of glutamine. Addition of glutamine inhibits uridylylation: at 10 mM glutamine less than 40% of PII uridylylation was observed. This inhibition effect seems to be higher for GlnZ than GlnB. To analyze the effect of glutamine on the deuridylylation activity of GlnD, PII proteins were fully uridylylated, purified and then incubated with GlnD in the presence of increasing concentration of glutamine. Our results showed glutamine is required for the deuridylylation reaction. Also, deurylylation of GlnZ-UMP_3 was more effective than that of GlnB-UMP_3 at lower glutamine, suggesting the differences in the uridylylation-deuridylylation patterns of GlnB and GlnZ might be important for fine tuning of the signaling pathway of the cellular nitrogen status in *A. brasilense*.

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