

Regulation of Deuridylylation Activity of *Herbaspirillum seropedicae* GlnD Protein

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INTRODUCTION: The nitrogen metabolism of Proteobacteria is controlled by the general Ntr system in response to nitrogen quality and availability. The PII proteins play an essential role on this system by modulating the cellular metabolism through regulation of target proteins by protein-protein interaction. *Herbaspirillum seropedicae*, a nitrogen-fixing bacterium, has two PII proteins, GlnB and GlnK. *H. seropedicae* PII proteins activity is regulated by allosteric ligands and by post-translational uridylylation. Both uridylylation and deuridylylation reactions are catalysed by the same enzyme, GlnD. The mechanism of regulation of GlnD activity is still not fully understood. **OBJECTIVES:** Here, we characterized the deuridylylation activity of *H. seropedicae* activity *in vitro*. **MATERIAL AND METHODS:** The fully modified PII proteins were submitted to kinetics analysis of its deuridylylation catalysed by purified GlnD. **RESULTS AND DISCUSSION:** GlnD removed the uridylyl group of PII in presence of either ATP or ADP. Also, the deuridylylation activity was strongly stimulated by glutamine, allowing GlnD modify the uridylylation status of PII in response to nitrogen availability. In addition, the deuridylylation activity was repressed by 2-oxoglutarate (2-OG), and, this repression was strong enough to overcome the glutamine stimulus of enzymatic activity. We also constructed and analysed a truncated version of GlnD, lacking the C-terminal regulatory domain ACT. The GlnD Δ ACT protein was unable to sense any molecular effector, catalysing the futile cycle of uridylylation and deuridylylation of PII regardless of glutamine and 2-OG levels. **CONCLUSIONS:** The results presented here suggest that GlnD can sense the glutamine/2-OG ratio to fine tune the PII activity. Also, we suggest that the ACT domain of GlnD is the protein sensor of environment clues of nitrogen availability.

Key words: *Herbaspirillum seropedicae*, PII proteins, GlnD

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