Purification and *in vitro* Activity of the GAF Domain of *Herbaspirillum seropedicae* NifA Protein

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Transcriptional regulation of nitrogen fixation genes in *Herbaspirillum seropedicae* is mediated by NifA, which activates transcription from *nif* promoters and is regulated by fixed nitrogen and oxygen levels. *H. seropedicae* NifA consists of three structural domains; an amino terminal GAF domain, a central AAA+ domain and a carboxy-terminal DNA-binding domain. The N-terminal GAF domain exerts a regulatory role on the other domains, since a truncated NifA protein, lacking this domain, is active and insensitive to the level of fixed nitrogen. Previous results suggested that regulation of NifA activity in response to fixed nitrogen is probably dependent on an interaction between the GAF domain and members of the PII family of nitrogen regulatory proteins. *In vitro* studies on the *H. seropedicae* NifA GAF domain described so far have used protein purified under denaturing conditions. Here, we describe a non-denaturing purification protocol in which expression of a his-tagged form of the GAF domain is induced at low temperature to improve solubility and the isolated domain is purified by nickel affinity chromatography. Interaction between the purified GAF domain and GlnK was analyzed by protein footprinting assays. We show that both the uridylylated and non-uridylylated form of GlnK interact with the GAF domain of NifA, but the nature of the complexes formed with the two forms of GlnK are different. Our results will contribute to our understanding of the signalling components that transduce the fixed nitrogen signal to NifA *in vivo*.

Palavras Chave: *Herbaspirillum seropedicae*, NifA and Nitrogen fixation
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