IN VITRO COMPLEX FORMATION BETWEEN THE AMMONIUM CHANNEL AMTB AND THE NITROGEN SIGNAL TRANSDUCTION PROTEINS PII IN *AZOSPIRILLUM BRASILENSE*

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The transport of ammonium across biological membranes is a key physiological process throughtout all kindgoms of life. Ammonium movement is facilitated by a class of ubiquitous channel proteins, the Amt family. Besides their role in transport, Amt proteins have also been implicated in cellular responses to ammonium availability in a variety of organisms. Ammonium sensing by Amt in bacteria involves the complex formation with cytosolic proteins from the nitrogen signal transduction P_{II} family. Here we have studied the *in vitro* complex formation between the AmtB and the P_{II} proteins from the nitrogen-fixer, plant-associative bacteria *Azospirillum brasilense*. An N-terminal 6xHis (His) tagged version of the *A. brasilense* AmtB protein was expressed in *Escherichia coli* C43 strain and purified from the membrane fraction. His-AmtB was linked to Ni_2^+ magnetic beads and used to pull-down native versions of the *A. brasilense* P_{II} proteins (GlnB and GlnZ). AmtB-P_{II} complex formation only occurred in the presence of adenine nucleotides and was sensitive to 2-ketoglutarate when Mg^{2+} was present. These results suggest that AmtB-P_{II} complex formation is not only influenced by the cellular nitrogen levels but can also respond to carbon and energy availability through binding 2- ketoglutarate and adenine nucleotides.