Dual Roles of *Bacillus subtilis* Glutamine Synthetase

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Glutamine synthetase (GS) catalyzes the ATP-dependent synthesis of glutamine from glutamate and ammonium. In the low-GC Gram-positive bacterium *Bacillus subtilis*, GS is not only required for the assimilation of ammonium, but it also plays a direct role in regulating gene expression in response to nitrogen availability.

The TnrA transcription factor controls the expression of a large number of *B. subtilis* genes involved in nitrogen metabolism. During nitrogen-limited growth, TnrA is active and can function as either a transcriptional activator or repressor. TnrA is inactive under conditions of nitrogen excess due to a protein-protein interaction with feedback-inhibited GS that prevents TnrA from binding to DNA.

One striking difference between GS from *B. subtilis* and enteric bacteria is that the *B. subtilis* enzyme is subject to feedback inhibition by glutamine. Kinetic experiments have demonstrated that glutamine inhibition is competitive with respect to glutamate. Additional studies have revealed that the binding of glutamine to *B. subtilis* GS only occurs in the presence of phosphate. These results, combined with structural studies of *Salmonella typhimurium* GS, argue that glutamine inhibits GS activity by binding to the active site.

A novel *B. subtilis* mutant with constitutive TnrA activity was isolated. This mutant is unique in that is crossfeeds Gln^- cells. Genetic mapping and DNA sequence analysis revealed that this mutant contains an alteration in *glnA*, the structural gene for GS, and replaces residue Ser-186 with phenylalanine. This amino acid residue is located within a β-strand that lines the active site. Enzymatic analysis revealed that the S186F GS is resistant to feedback inhibition. The mutant enzyme is defective in its ability to bind glutamine or inhibit TnrA DNA binding *in vitro*. The isolation and properties of this *glnA* mutant supports the model in which the feedback-inhibited form of GS plays a direct role in regulating TnrA activity *in vivo*.