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Bacterial Nickel Metabolism and Storage

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Nickel is required for the growth of many microorganisms, as the metal functions as a key component of a metal center for several enzymes. However, after its sequestration, nickel must undergo poorly-defined processing reactions that are dependent on "accessory" proteins. The proposed research objective was to understand the number, nature, and function of the components involved in nickel metabolism for Ni-hydrogenase biosynthesis. The work has focused on the nickel-metabolizing attributes of the bacterium *Bradyrhizobium japonicum* that are different than the other H₂-oxidizing systems. This has included the study of the nickel-dependence of H₂ uptake hydrogenase (*hup*) gene transcription, properties of the Ni and H₂ sensory complex HupUV, the roles of the *his*-rich Ni-storage protein HypB in nickel mobilization and nickel dependent gene transcription. Identification of *hup* gene transcription activating factors that function symbiotically as compared to those needed for free-living *hup* expression has been achieved, and further roles of the nickel-storage protein HypB in nickel mobilization into hydrogenase have been clarified.

The major goals of the research closely followed the original proposal and involved primarily the following three areas of research:

1) Association of the dual functions of HypB with specific domains of the protein.

The HypB protein from *B. japonicum* can sequester 18 Ni ions per dimer of the protein, and this Ni-storage role is associated with 24 clustered histidine residues (per monomer). Specific removal of the *his*-rich area (but retaining the rest of the protein) results in a phenotype such that the bacterium cannot store nickel for hydrogenase expression later during the derepression-incubation. The nickel-mobilizing role, associated with a different area of the protein, was shown to be dependent on the intrinsic GTPase activity. Ni-mobilization is not dependent on the histidine-containing area, and Ni-storage is not dependent on the GTPase activity. A single Ni ion remains bound to the protein lacking the clustered *his* residues, and that version retains GTPase activity and hydrogenase activity. It is possible that the single nickel atom remaining is the one donated to the hydrogenase apoprotein. The GTPase is the portion common to all HypB proteins, whereas the histidine-rich cluster responsible for nickel storage is present in just some H₂-metabolizing bacteria. In the context of bacterial nickel metabolism, for all known Ni-enzymes, the results are in agreement with the concept of an energy (GTP) utilizing step, separate from a Ni-sequestering domain. The model for HypB function is that the

GTPase Ni-donation role of HypB adds a single nickel atom to hydrogenase as well as to the hydrogenase-like nickel and H₂-sensor regulatory protein HupUV.

2) Regulation of Hydrogenase expression

We proposed that HupUV is a hydrogenase-like sensory protein involved in sensing the environmental signals hydrogen, oxygen, and nickel. This was based on mutant analysis and predicted sequence, and many attempts were made to purify HupUV. It was overexpressed in *E. coli* (in pET) and later in *Azotobacter vinelandii* (by use of the *nifH* promoter) but the expressed proteins were always in inclusion bodies (despite varying many of the culture conditions). The HupU and HupV were even singly expressed but were not stable. Attempts to purify small amounts of soluble HupUV that was on occasion expressed in an heterologous system, or from microaerobically incubated *B. japonicum*, were also unsuccessful. Nevertheless, useful antisera against HupU and HupV was generated by using inclusion body preparations and proteins separated and then excised from SDS gels as the antigen. At the same time, progress proceeded on studying the pure HupUV of *R. capsulatus* or the analogous protein HoxBC from *A. eutrophus* so we did not pursue the HupUV project in the final year. Nevertheless, our initial proposal that HupUV played a unique role as an environmental sensor to detect the conditions favorable for hydrogenase synthesis has so far been substantiated by the work of others in the other bacterial systems. The three regulatory signals (H₂, O₂, nickel) affecting hydrogenase expression in *B. japonicum* exert their effect at a 50 bp region of DNA upstream of the start site of the hydrogenase structural gene. The *hoxA* gene encodes a transcriptional activator protein that is like the HoxA of *A. eutrophus*, HupR of *Rhodobacter capsulatus* and HydG of *E. coli*. These are all members of the NtrC-family of response regulators. HoxA is involved in binding to a region in the 50 bp regulatory area in free-living *B. japonicum*. However, bacteroids from nodules formed by *B. japonicum* HoxA mutants exhibited wild type levels of hydrogenase activity, and extracts from wild type (Hup⁺) bacteroids did not cause a shift of the 50 bp regulatory region. Therefore, we searched for a symbiosis-specific regulator (activator) of hydrogenase activity. It was determined that FixK₂, and not FixK₁, or NifA, is a transcriptional activator for symbiotic hydrogenase expression. By use of transcription assays we found that FixK₁ can induce *hup* transcription at rates similar to that of HoxA, the "free-living" transcriptional activator. Also, the FixK₂ binding site upstream of *hupSL* was identified by truncated promoter-fusion analysis.

2) Characterization of metal-binding by HypB

HypB from *B. japonicum* can bind many divalent cations in addition to nickel. We (over) expressed HypB in *E. coli* and from these (*E. coli*) extracts can purify large amounts of HypB. The affinity of HypB for ions such as Cu⁺⁺, Mn⁺⁺, and Co⁺⁺, is slightly

less than that for Ni^{++} , whereas Zn^{++} binding affinity is similar to that for nickel. Although Ni^{++} -binding is thought to be the most physiologically relevant ion, the binding of the other metals is important to metallobiochemists as well as to environmentalists (it should be noted that a research group in Irvine, CA is trying to express *B. japonicum* HypB in plants for bioremediation of metal-contaminated sites). Along the same lines of interest, HypB also binds cadmium with high affinity. The metal-binding sites of *B. japonicum* HypB have been determined to be imidazole groups, as determined by EXAFS.

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Olson, J.W. and R. J. Maier. 1997. The sequences of hypF, hypC and hypD complete the hyp gene cluster required for hydrogenase activity in *B. japonicum* Gene 199: 93-99.

Olson, J.W., C. Fu, and R.J. Maier. 1997. The HypB protein from *B. japonicum* can store nickel and is required for the nickel-dependent transcriptional regulation of hydrogenase. Molec. Microbiol. 24:119-128

Durmowicz, M.C., and R.J. Maier. 1997. Roles of HoxX and HoxA in biosynthesis of hydrogenase in *Bradyrhizobium japonicum*. J. Bacteriol. 179:3676-3682.

Maier, R.J., Olson, J.W. and Fox, J.D. 1998. Nickel-dependent expression and maturation of hydrogenases, in Biosynthesis and Function of Metal Clusters for Enzymes, 25th Steenbock Symp. Proceed. Madison, WI, pp 133-142

Durmowicz, M.C., and R.J. Maier. 1998. The FixK₂ protein is involved in regulation of symbiotic hydrogenase expression in *Bradyrhizobium japonicum*. J. Bacteriol. 180:3253-3256.

Olson, J.W, and R.J. Maier. 2000. Dual roles of *Bradyrhizobium japonicum* nickelin protein in nickel storage and GTP-dependent Ni-mobilization. J. Bacteriol. 182:1702-1705.