Metal Binding and PPIase Activities of SlyD Are Essential for the Biosynthesis of Hydrogenase in *Escherichia coli*

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Three [Ni-Fe] hydrogenase enzymes are expressed in *Escherichia coli* as components of various types of anaerobic metabolism. The biosynthesis of the hydrogenase is a multi-step process in which accessory proteins assemble the intricate active site of the enzyme. The main auxiliary proteins are encoded by the *hypA-F* genes. HypA and the GTPase HypB facilitate insertion of the nickel ion to the hydrogenase 3 precursor. By using a sequential peptide affinity tagging technique, the peptidyl-prolyl cis/trans isomerase SlyD was isolated as a HypB-binding protein. Deletion of the *slyD* gene resulted in a marked reduction of the total hydrogenase activity in extracts prepared from anaerobic cultures of *Escherichia coli* and an in-gel assay revealed diminished activities of both hydrogenase 1 and 2. This deficiency could be rescued by high nickel concentrations in the growth media. Experiments with radioactive nickel demonstrated that less nickel accumulated in ΔslyD cells compared to wild type and overexpression of SlyD from an inducible promoter doubled the level of cellular nickel. Thus one potential role for SlyD is that of a nickel source. SlyD mutants lacking metal-binding or PPIase activity were generated respectively, and neither restored the hydrogenase activity in vivo. These results indicate that both the metal-binding and PPIase activities of SlyD are essential for the biosynthesis of hydrogenase.