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

Jie Wei Zhang, Deborah B. Zamble

Department of Chemistry, University of Toronto

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 hydrogeanse 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 Δ*slyD* 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.