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Escherichia coli SlyD, more than a Ni(II) reservoir

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Abstract

SlyD interacts with HypB and contributes to nickel insertion during [NiFe]-hydrogenase biogenesis. Herein, we provide evidence for SlyD as a nickel storage determinant in *E. coli* and show that this Ni(II) can be mobilized to HypB even under competitive conditions. Furthermore, SlyD enhances the GTPase activity of HypB and acceleration of Ni(II) release from HypB is more pronounced when HypB is GDP-bound. The data support a model in which a HypB-SlyD complex establishes communication between GTP hydrolysis and nickel delivery and provide insight into the role of the HypB-SlyD complex during [NiFe]-hydrogenase biosynthesis.

Central to microbial energy metabolism as well as fuel cell applications, [NiFe]-hydrogenases catalyze the oxidation of hydrogen gas and/or the reverse reaction.[1] This challenging chemistry occurs at an intricate organometallic centre that is assembled by several accessory proteins.[1,2] In *E. coli* the HypCDEF proteins are responsible for the synthesis of diatomic ligands and delivery of the decorated iron centre to the hydrogenase precursor protein.[1] The subsequent nickel insertion is carried out by HypA, HypB and SlyD.[1,2]

While HypA is anticipated to serve as a scaffold for the nickel insertion complex during enzyme maturation,[2,3] HypB catalyzes the GTP hydrolysis that is essential for hydrogenase maturation.[4–6] *E. coli* HypB also has two metal-binding sites (Figure S1), one that includes the CXXCXC sequence at the N-terminus of the protein and binds nickel with picomolar affinity (thus termed the high-affinity site, HAS) and a second in the GTPase domain (G-domain) that binds nickel or zinc with low-micromolar affinity (low-affinity site, LAS).[7] SlyD is a multi-domain protein harbouring both protein folding and metal-binding activities.[8–10] The unique C-terminal domain of SlyD (Figure S1) features a high concentration of potential metal-binding residues and chelates multiple nickel ions in vitro.[9] Mutation of the residues contributing to either of the metal sites of HypB or deletion of the metal-binding domain (MBD) of SlyD compromises hydrogenase maturation.[11,12]

E. coli HypB lacks the poly-histidine stretch that allows some HypB homologues to bind nickel with high capacity but low affinity.[2,13] Consequently, nickel storage in this organism is a proposed role of SlyD.[8,14] To test this hypothesis, we compared the Ni(II) content and

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Supporting Information. Detailed experimental procedures. Tables with nickel content and hydrogenase activity, Representative reconstructed mass spectra, Metal release profiles for SlyD variants, Proposed model for the Ni(II) insertion to the hydrogenase precursor. This material is available free of charge via the Internet at http://pubs.acs.org.

hydrogenase activity of a wildtype strain with those of a *ΔslyD* strain. Cell cultures were first exposed to Ni(II) under anaerobic conditions to allow the *E. coli* to accumulate the metal and potentially store Ni(II) in SlyD. Bacteria were then washed and transferred to untreated TYEP media or TYEP media treated with dimethylglyoxime (DMG), and activity was assessed after an additional growth step. DMG treatment is expected to mimic nickel-limiting conditions because this chelator selectively binds nickel with high affinity.[15,16] Under these conditions, the cytoplasmic nickel content and the hydrogenase activity of the *ΔslyD* strain decreased to 8% and 11% that of the wildtype strain, respectively, an effect that is much more dramatic than when the bacteria were cultured without DMG (Tables S1 and S2). These results afford physiological evidence that SlyD is a Ni(II) reservoir in *E. coli* and that bacteria without SlyD are highly-dependent on exogenous nickel for [NiFe]-hydrogenase assembly.

Given the observation that SlyD could store Ni(II) ions and that it forms a complex with HypB,[12,14] we postulated that this protein could function as a source of Ni(II) for metallating HypB. To test this model, SlyD bound to 1 equivalent of Ni(II) was mixed with apo HypB and metal transfer from SlyD to HypB was assessed. We used ESI-MS for analysis, which enabled simultaneous detection of the metallation state of both proteins (Figure S2). Incubating holo SlyD with apo HypB led to accumulation of Ni(II)-bound HypB with a concomitant decrease in the amount of holo SlyD (Figures 1A and S2). Although HypB has two metal-binding sites,[7] only a single Ni(II) per HypB monomer is detected in these spectra. That SlyD only populates the HAS of HypB was confirmed by the lack of metal transfer from holo SlyD to the HypB mutant C2,5,7A (data not shown), which has a disrupted HAS but an intact LAS.[7]

To determine if heterodimer formation is necessary for the metal transfer, SlyD bound to 1 equivalent of metal was dialyzed against an apo HypB sample. The rate of nickel transfer to HypB was considerably reduced compared to that achieved when the two proteins were incubated together (Figure 1A). To assess whether metal transfer could occur under more competitive conditions, Ni(II) transfer from SlyD to HypB was monitored in the presence of two metal chelators, EGTA or glycine. EGTA forms a 1:1 complex with Ni(II) with an apparent K_D ~10^{-11} M at pH 7.5 whereas glycine coordinates Ni(II) with a weaker affinity in the micromolar range.[17] While transfer of nickel from SlyD to HypB remained unchanged in the presence of 1 mM glycine (data not shown), inclusion of EGTA lead to a decrease in the Ni(II) transfer rate compared to that in the absence of competitor (Figure 1 and 2S). However, HypB metallation was faster with the Ni(II)-SlyD complex than if Ni(II) was provided as a $[Ni\text{-}EGTA]^{2-}$ complex (Figure 1B). Together, these data indicate that Ni(II) can relocate from SlyD to the HAS of HypB even under competitive circumstances and heterodimer formation is a pre-requisite to achieve favourable conditions.

The intrinsic GTP hydrolysis of *E. coli* HypB is very slow ($k_{cat} \approx 0.2\ min^{-1}$).[6,18] NTPases can utilize protein-protein interactions to accelerate low hydrolysis rates,[19,20] so we tested whether SlyD could enhance the HypB GTPase activity. While HypB with Ni(II) bound at the HAS (holo HypB) hydrolyzed GTP with a k_{cat} of $0.27 \pm 0.06\ min^{-1}$, the addition of SlyD resulted in a 3-fold increase in the catalytic rate (Table 1). SlyD is the first factor observed to positively contribute to the enzyme activity of HypB. The MBD of SlyD is

necessary for this process because the same effect was not observed with $SlyD_{1–146}$ (Table 1). The k_{cat} of the HypB mutant C2,5,7A is analogous to that of wildtype HypB, indicating that the HAS does not influence the GTPase activity of the protein (Table 1). In agreement with this conclusion, SlyD produces an increase in the GTPase activity of C2,5,7A HypB comparable to that of wildtype HypB (Table 1).

To assess the influence of SlyD on GTP hydrolysis when HypB has metal loaded in both metal sites, holo HypB with either Ni(II) or Zn(II) bound to the LAS was examined. The GTPase activity was reduced when the LAS was metallated, as expected (Table 1).[18] Inclusion of SlyD in the reaction mixture reversed the metal-dependent inhibition, leading to a ~10-fold increase in hydrolysis rate when HypB containing Zn(II) at the LAS was used (Table 1). In contrast, $SlyD_{1–146}$ could not rescue the compromised GTP hydrolysis activity of HypB with metal loaded in the LAS. Given that wildtype SlyD can bind multiple Ni(II) or Zn(II) ions while $SlyD_{1–146}$ is unable to do so (reference 9 and unpublished data), we postulated that metal-dependent inhibition of HypB enzymatic activity is reversed due to competitive metal chelation of the LAS metal of HypB by the MBD of SlyD. In agreement with this model, ESI-MS revealed that metal transfer from the LAS of HypB to SlyD was ~95% complete within 15 minutes (Figure S3) and remained unaffected when HypB was bound to GDP or GMP-PNP, or if SlyD was pre-loaded with 1 equivalent of Ni(II) (data not shown). $SlyD_{1–146}$ does not lead to any detectable metal loss from HypB (Figure S4).

Previous work demonstrated that heterodimer formation with SlyD increases the rate of metal-release from the N-terminal HAS of HypB to a colorimetric chelator.[12] To establish features of SlyD that are necessary to modulate the HAS of HypB, several apo SlyD variants were incubated with holo HypB in the presence of EGTA, followed by ESI-MS analysis. Very little apo HypB was detected when mixed with EGTA alone, but the addition of wildtype SlyD or the triple mutant (has reduced capacity and affinity for Ni(II) due to the removal of all three pairs of Cys residues[9]) lead to acceleration of Ni(II) release from HypB (Figure S5). The application of ESI-MS revealed that Ni(II)-bound SlyD was not detected at any time point, indicating that the nickel released from HypB was not transferred to SlyD. As expected,[12] $SlyD_{1–146}$ (lacks MBD), Δflap SlyD, (deletion of a loop in the IF-domain abrogates interaction with HypB[12]) and SlyD-MBD (only contains residues 147–196) had no effect on holo HypB (Figure S5), confirming that complex formation and the MBD are both necessary to accelerate Ni(II) release from HypB.

To assess whether nucleotide could modulate the high-affinity Ni(II) site of HypB, metal release from HypB to EDTA was monitored in the presence of GDP or the GTP-analog, GMP-PNP using electronic absorption spectroscopy. Ni(II) coordination to the Cys ligands of the HAS of HypB results in a ligand to metal charge transfer band at 320 nm (ε_{320} 7300 M^{-1} cm^{-1}).[7] Taking advantage of the observation that the nickel from holo-HypB is not transferred into SlyD (discussed above), the decrease in signal at 320 nm should correlate with Ni(II) release from HypB into the solution containing EDTA (K_D of 2.7×10^{-16} M at pH 7.5).[17] Addition of apo SlyD stimulates nickel release from HypB-GMP-PNP to the same extent as from HypB in the absence of nucleotide, whereas metal release is further enhanced when SlyD is mixed with HypB-GDP (Figure 2). Fits of the metal release profiles to a 1st order decay event yields a $t_{1/2}$ ~24 h and 73 min for holo HypB in the absence and

presence of SlyD, respectively. The half-life for the GMP-PNP-loaded HypB with SlyD is unaffected ($t_{1/2}$ ~75 min) whereas that of the GDP-bound form decreases to $t_{1/2}$ ~33 min. Similar nucleotide dependent enhancement in interactions has been observed between MeaB[20] and UreG[19] GTPases and their partner proteins.

GTP hydrolysis is mandatory for maturation of the hydrogenase enzyme,[6] but it is not clear how energy derived from this reaction is utilized for assembling the [NiFe]-centre. The results presented here provide evidence that GTP hydrolysis can regulate the loss of Ni(II) from the HAS of HypB. The enhanced metal release from the holo HypB-SlyD heterodimer upon GDP binding is consistent with a model in which GTP hydrolysis precedes Ni(II) delivery to the precursor enzyme (Figure S6), and may be due to a more favorable interaction between SlyD and GDP-bound HypB. Nickel insertion to the hydrogenase precursor appears to be gated by GTP hydrolysis and this link between hydrolysis and metal release is achieved through SlyD.

In summary, SlyD functions as a reservoir of Ni(II) for hydrogenase biosynthesis under nickel-limiting conditions, but its role extends to optimal biogenesis of the [NiFe]-hydrogenase by modulating the activities of HypB (Figure S6). These discoveries provide insight as to how protein-protein interactions as well as the GTPase function of HypB can be exploited to ensure the timely delivery of the metal ion. Furthermore, the protein-protein mediated effects highlight how the coordinated actions of multiple protein factors can overcome the barriers for efficiently inserting the nickel ion into a partially assembled hydrogenase active site.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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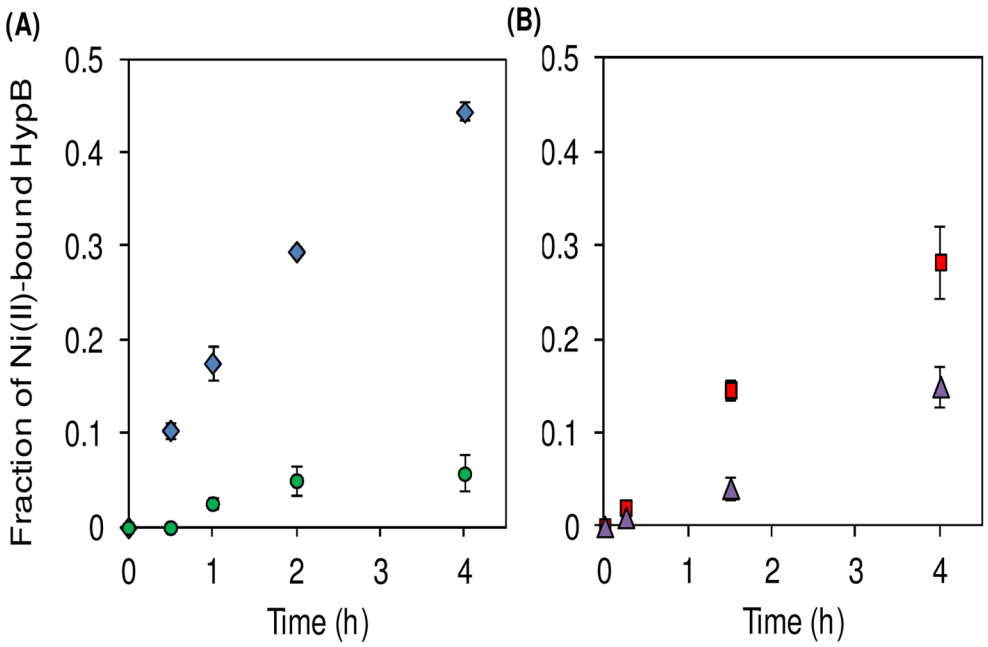

Figure 1.

Ni(II) transfer from SlyD to HypB monitored by ESI-MS. (A) Incubating SlyD (100 μM) bound to 1 equivalent of Ni(II) with apo HypB (100 μM) leads to an increase in the amount of Ni(II)-bound HypB (diamonds). Slower metal transfer was observed if the proteins were separated by a membrane (circles). See Supplemental Information for a discussion on the protein concentrations. (B) Ni(II)-bound SlyD (100 μM) was incubated with apo HypB (100 μM) in the presence of 1 mM EGTA (squares). Less holo HypB was obtained when 100 μM Ni(II) was added to apo HypB (100 μM) and 1 mM EGTA (triangles). Each data point is an average from at least 3 independent experiments and the error bars represent one standard deviation.

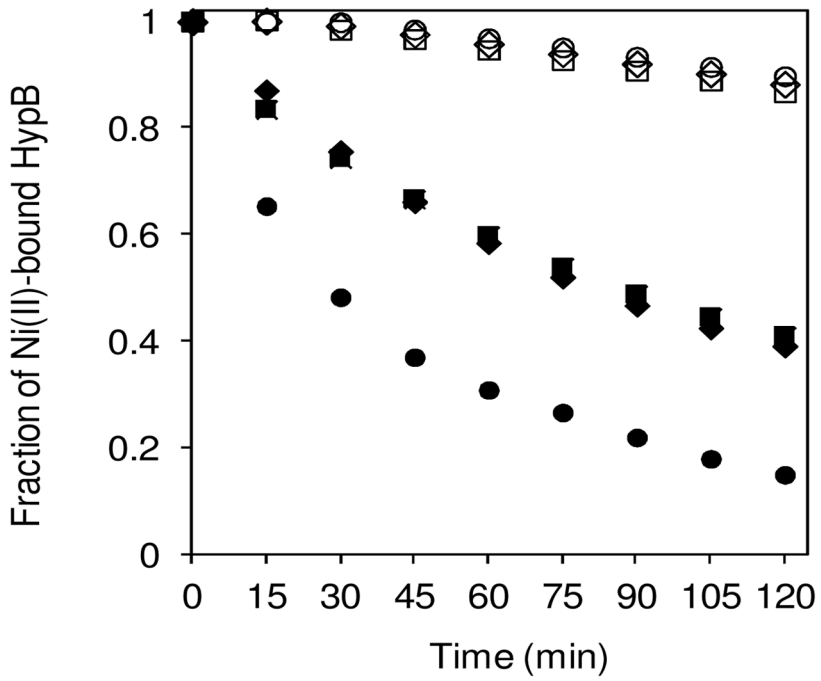

Figure 2.

Metal release from HypB to EDTA monitored by electronic absorption spectroscopy. The decrease in the fraction of 100 μM Ni(II)-bound HypB in the presence of 1 mM EDTA (empty symbols) was monitored at 320 nm. Metal release from HypB on its own (empty squares) is not affected upon addition of GDP (empty circles) or GTP analog (unfilled diamonds). Including 100 μM SlyD (filled symbols) results in an increase in the rate of metal release. Metal release from HypB bound to GTP analog is not effected (filled diamonds) whereas SlyD further accelerates metal release from HypB-GDP (filled circles).

Table 1

GTP hydrolysis rates of HypB under saturating conditions.*

Metal bound at the G-domain	$HypB_{WT}$	$HypB_{WT}$ + $SlyD_{WT}$	$HypB_{WT}$ + $SlyD_{1–146}$	$HypB_{C2,5,7A}$	$HypB_{C2,5,7A}$ + $SlyD_{WT}$
--	0.27 ± 0.06	0.82 ± 0.04	0.36 ± 0.08	0.21 ± 0.05	0.75 ± 0.08
Ni(II)	0.13 ± 0.03	0.87 ± 0.01	0.12 ± 0.03	0.13 ± 0.02	0.78 ± 0.09
Zn(II)	0.07 ± 0.04	0.91 ± 0.09	0.06± 0.04	0.06 ± 0.02	0.88 ± 0.05

*The values reported are k_{cat} (min^{-1}). Assays were performed using 1 μM holo HypB with nickel in the high-affinity site, 450 μM GTP and, when required, SlyD at a final concentration of 50 μM. The k_{cat} are averages and standard deviations from more than three independent experiments. To load the G-domain metal site, HypB was incubated with 10 molar excess Ni(II) or 1.5 molar excess Zn(II).