UreE Stimulation of GTP-Dependent Urease Activation in the UreD-UreF-UreG-urease Apoprotein Complex†

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ABSTRACT: The activation of metal-containing enzymes often requires the participation of accessory proteins whose roles are poorly understood. In the case of *Klebsiella aerogenes* urease, a nickel-containing enzyme, metallocenter assembly requires UreD, UreF, and UreG acting as a protein chaperone complex and UreE serving as a nickel metallochaperone. Urease apoprotein within the UreD-UreF-UreG-urease apoprotein complex is activated to wild-type enzyme activity levels under physiologically relevant conditions (100 μM bicarbonate and 20 μM Ni^{2+}) in a process that requires GTP and UreE. The GTP concentration needed for optimal activation is greatly reduced in the presence of UreE compared to that required in its absence. The amount of UreE provided is critical, with maximal activation observed at a concentration equal to that of Ni^{2+}. On the basis of its ability to facilitate urease activation in the presence of chelators, UreE is proposed to play an active role in transferring Ni^{2+} to urease apoprotein. Studies involving site-directed variants of UreE provide evidence that His96 has a direct role in metal transfer. The results presented here parallel those obtained from previous in vivo studies, demonstrating the relevance of this in vitro system to the cellular metallocenter assembly process.

Accessory proteins often participate in the synthesis of metal-containing enzymes (*1*); however, their roles in metallocenter assembly are not well understood. The activation of urease requires several accessory proteins and presents a tractable system to address this problem. Urease is a nickel-containing enzyme that acts as a virulence factor in a variety of human pathogens and participates in the metabolism of various nitrogen sources in many microorganisms and plants (*2*, *3*). The crystal structure of the heterotrimeric enzyme from *Klebsiella aerogenes* reveals the presence of a dinuclear metallocenter [i.e., $(2\ Ni/UreA\text{-}UreB\text{-}UreC)_3$], where the nickel ions are 3.6 Å apart and bridged both by a carbamylated lysine residue and a hydroxide (*4*, *5*). Synthesis of the urease metallocenter requires three accessory proteins (UreD, UreF, and UreG) and is facilitated by a fourth (UreE) (*6*). These auxiliary proteins are encoded in the same gene cluster (*ureDABCEFG*) as the structural genes (*6*, *7*). The three required accessory proteins form a complex with urease apoprotein (apourease)[1] that is termed UreD-UreF-UreG-apourease (*8*), abbreviated here as UreDFG-apourease. In contrast, UreE is a nickel-binding protein and is proposed to function as a metallochaperone that delivers nickel to urease apoprotein (*9*−*12*).

In vitro urease activation requires the presence of nickel ions along with carbon dioxide/bicarbonate to form the lysine carbamate metal ligand (*13*). For urease apoprotein in the absence of accessory proteins, optimal activation conditions result in a specific activity of ∼400 U mg^{-1} (*14*) corresponding to ∼15% of that associated with fully active enzyme [2500 units mg^{-1} (*15*)]. The extent of in vitro activation increases significantly for apourease with bound accessory proteins. For example, urease apoprotein present in UreD-apourease, UreDF-apourease, and UreDFG-apourease yields enzyme samples with activities of ∼600−800 units mg^{-1} (*14*), ∼700−900 units mg^{-1} (*16*), and ∼800−1500 units mg^{-1} (*17*), respectively. For the latter complex, activity resulting from incubation with 100 μM nickel and 100 mM bicarbonate approximates half that of the wild-type enzyme. More importantly, nickel-dependent activation of apourease in UreDFG-apourease also occurs at low, physiologically relevant (∼100 μM) bicarbonate levels in a process that requires the hydrolysis of GTP (*17*).

Here, we report that urease apoprotein in UreDFG-apourease can be activated in vitro to achieve wild-type enzyme activity levels. Significantly, the activation process requires UreE while using physiologically relevant concentrations of GTP, Ni^{2+}, and bicarbonate. The ability of UreE to overcome inhibition of the activation process by nickel chelators demonstrates its direct involvement in delivery of Ni^{2+} to the urease active site. Finally, based on analysis of site-directed variants of UreE we show that His96 plays a critical role in the activation process.

MATERIALS AND METHODS

Protein Production and Purification. Escherichia coli DH5α cells containing plasmids pKAUG-1 (to express *ureG*) and pKAUD2F+Δ*ureG* (containing the urease gene cluster missing *ureG* and with an extra copy of *ureF*) were used

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[1] Abbreviations: apourease, urease apoprotein; H144*UreE, truncated form of UreE; UreDF-apourease, urease apoprotein in a complex with UreD and UreF; UreDFG-apourease, urease apoprotein in a complex with UreD, UreF, and UreG; IDA, iminodiacetic acid; NTA, nitrilotriacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

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for production of recombinant UreG and UreDF-apourease (*16*, *18*). UreDFG-apourease was produced by overnight incubation of UreDF-apourease with UreG (*17*). *Escherichia coli* HMS-174(DE3) and BL21(DE3) cells carrying derivatives of pET21 (Novagen) were used for production of UreE and recombinant variants of H144*UreE (a truncated and functional version of UreE missing 15 residues at its His-rich carboxyl terminus) (*9*, *11*, *12*). All proteins were purified by using previously published methods (*11*, *16*−*18*).

Urease Activity Assays. Urease activities were measured by quantitating the rate of ammonia release from urea by formation of indophenol, which was monitored at 625 nm (*19*). One unit of urease activity was defined as the amount of enzyme required to hydrolyze 1 μmol of urea/min at 37 °C. The standard assay buffer consisted of 25 mM HEPES (pH 7.75), 0.5 mM EDTA, and 50 mM urea. Protein concentrations were determined by using a commercial assay (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard.

Activation of Urease Apoprotein. Routine apourease activation buffer consisted of 100 mM HEPES (pH 8.3) and 140 mM NaCl (*13*, *14*). The typical UreDFG-apourease concentration was 0.48 μM when measured in terms of potential active sites, i.e., UreA-UreB-UreC units. For most experiments, bicarbonate, Ni^{2+}, GTP, and UreE were present at 100, 20, 200, and 20 μM, respectively. These concentrations were altered in selected studies, and in some cases, $ZnCl_2$, $CuCl_2$, or Ni^{2+} chelators (IDA, NTA) were added to the activation mix. In all studies, the concentration of Mg was twice that of GTP.

Calculation of Free Ni^{2+}, Ni-UreE, and 2Ni-UreE Concentrations. The concentrations of nickel-containing species in selected activation mixtures were estimated by successive approximation. The calculations made use of the previously reported (*11*) values of K_1 (47 μM = $[Ni_{free}][UreE_{free}]/[Ni\text{-}UreE]$) and K_2 (1.5 μM = $[Ni_{free}][Ni\text{-}UreE]/[2Ni\text{-}UreE]$) for cooperative binding of nickel by H144*UreE. The added amounts of Ni^{2+} and UreE are $[Ni_{total}] = [Ni_{free}] + [Ni\text{-}UreE] + 2[2Ni\text{-}UreE]$ and $[UreE_{total}] = [UreE_{free}] + [Ni\text{-}UreE] + [2Ni\text{-}UreE]$.

RESULTS

Activation of Urease Apoprotein using UreDFG-Apourease in the Presence of UreE and GTP. Previous work has shown that urease apoprotein in UreDFG-apourease can be activated to obtain enzyme exhibiting over half the wild-type activity levels (~ 800−1500 units mg^{-1} versus 2500 units mg^{-1}) in the presence of 100 mM bicarbonate and 100 μM Ni^{2+} (*17*). At more physiological concentrations of bicarbonate (100 μM), activation was observed only in the presence of GTP and yielded enzyme with activity levels of ~250−500 units mg^{-1} (*17*). In an effort to improve the efficiency of GTP-dependent activation, we studied the effect of UreE on this process (Figure 1). A truncated form of UreE (H144*UreE that lacks the carboxyl terminal His-rich region, yet still binds two Ni per dimer) was used in nearly all experiments to simplify nickel binding analyses. Whole cell studies previously had demonstrated the ability of H144*UreE to function in urease activation (*9*, *11*, *12*). At a concentration of 100 μM bicarbonate and 20 μM Ni^{2+}, essentially wild-type activity levels (~2300 units mg^{-1}) were generated from

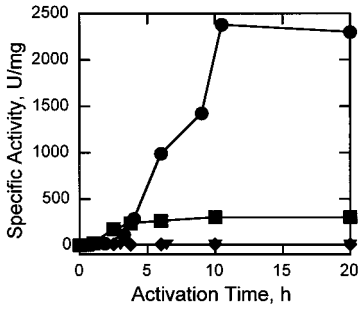


FIGURE 1: Effect of UreE and GTP on the activation of urease apoprotein using UreDFG-apourease at low Ni^{2+} and bicarbonate concentrations. UreDFG-apourease (0.48 μM) was incubated at 37 °C in solutions containing 20 μM Ni^{2+}, 100 μM HCO_3^-, 100 mM HEPES (pH 8.3), and 140 mM NaCl (◆), or similar solutions amended with 20.5 μM H144*UreE dimer (▼); 200 μM Mg_2GTP (■); or both 200 μM Mg_2GTP and 20.5 μM H144*UreE dimer (●). For each sample, aliquots were assayed at the indicated time points.

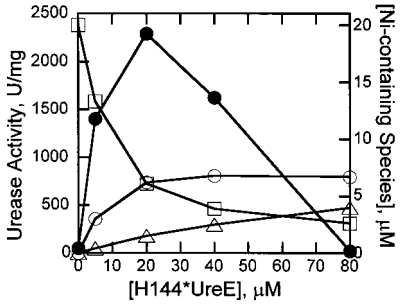


FIGURE 2: UreE dependence of urease apoprotein activation using UreDFG-apourease. UreDFG-apourease (0.48 μM) was incubated in solutions containing 20 μM Ni^{2+}, 100 μM HCO_3^-, 100 mM HEPES (pH 8.3), 140 mM NaCl, 200 μM Mg_2GTP, and the indicated amounts of H144*UreE dimer. Activation was allowed to continue for 10 h at 37 °C. The left axis describes the measured urease activity (●) as a function of added H144*UreE. The right axis reports the concentrations of free nickel ions (□), Ni-H144*UreE (△), and 2Ni-H144*UreE (○), calculated as described in Materials and Methods.

urease apoprotein in UreDFG-apourease when both Mg_2GTP (200 μM) and H144*UreE (20 μM) were present. Substitution of H144*UreE by wild-type UreE yielded similar results (data not shown), but the final urease activities were somewhat reduced. In contrast, no significant activation was observed in the absence of Mg_2GTP. Furthermore, inclusion of 200 μM Mg_2GTP in samples lacking UreE resulted in specific activities of only ~250 units mg^{-1}, as previously described (*17*). The rate of GTP- and H144*UreE-dependent activation was very slow, needing about 8−10 h for completion. The ability of UreE to enhance GTP-dependent urease activation at low concentrations of Ni^{2+} and bicarbonate points to its importance in urease metallocenter assembly under physiological conditions.

Effect of UreE, Bicarbonate, and GTP Concentrations on Urease Activation using UreDFG-Apourease. The degree of urease activation using UreDFG-apourease at 20 μM nickel, 100 μM bicarbonate, and 200 μM Mg_2GTP was shown to be critically dependent on the ratio of H144*UreE to nickel (Figure 2). For samples activated with increasing concentrations of H144*UreE, the enzyme specific activity increased up to the point at which ~20 μM H144*UreE was added (a 1:1 ratio with nickel). We note that the urease activation competence increased in parallel with the calculated con-

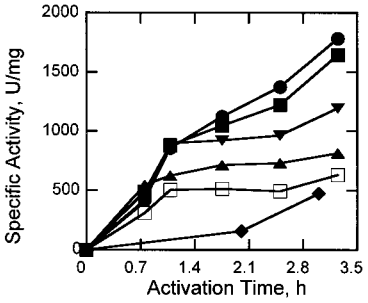


FIGURE 3: Bicarbonate dependence of urease apoprotein activation using UreDFG-apourease in the presence of UreE and GTP. UreDFG-apourease (0.48 μM) was incubated in solutions containing 20 μM Ni^{2+}, 200 μM Mg_2GTP, 20 μM H144*UreE, 100 mM HEPES, 140 mM NaCl, and 1 mM (◆) 5 mM (■), 10 mM (●), 20 mM (▼), or 50 mM (▲) HCO_3^-. A control solution without H144*UreE and containing 10 mM HCO_3^- (□) was also prepared. All samples were at pH 8.3 and 37 °C. Aliquots were assayed at the indicated time points.

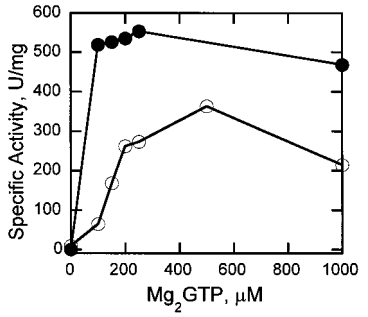


FIGURE 4: GTP dependence of urease apoprotein activation using UreDFG-apourease. UreDFG-apourease (0.48 μM) was incubated in solutions containing 20 μM Ni^{2+}, 100 μM HCO_3^-, 100 mM HEPES (pH 8.3), 140 mM NaCl, the indicated concentrations of Mg_2GTP, and the absence (○) or presence (●) of 3.8 μM H144*UreE. Samples were assayed after 6 h incubation at 37 °C.

centrations of the 2Ni-H144*UreE species. Further additions of H144*UreE led to reductions in activation competence, even though the concentration of 2Ni-H144*UreE was little changed. The increasing concentrations of metal-free H144*UreE accompanying the decrease in activation efficiency may indicate a competition between the free and metal-coordinated forms of H144*UreE.

The bicarbonate dependence of apourease activation in UreDFG-apourease was studied in the presence of 20 μM H144*UreE, 20 μM Ni^{2+}, and 200 μM Mg_2GTP (Figure 3). Under these conditions, the fastest rate of apourease activation occurred at approximately 5−10 mM bicarbonate. As shown in Figure 1, however, fully active enzyme was obtained with 0.1 mM bicarbonate after longer time periods. Inhibition occurs at high (>10 mM) bicarbonate concentrations, presumably due to coordination of free Ni^{2+} by HCO_3^-.

The effect of GTP concentration on urease apoprotein activation in UreDFG-apourease was compared in the presence or absence of H144*UreE. When H144*UreE was absent (Figure 4, open circles) optimal activation required ~0.5 mM GTP resulting in a urease specific activity of 380 units mg^{-1}. Further addition of GTP led to a depression in activation competence, arising from chelation of nickel by the nucleotide (*17*). In the presence of 3.8 μM H144*UreE, a distinct pattern of UreDFG-apourease activation was observed (Figure 4, closed circles). Only very low concentrations of GTP were required to yield an increase in urease

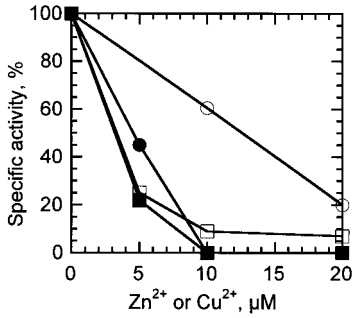


FIGURE 5: Metal ion inhibition of urease apoprotein activation using UreDFG-apourease. UreDFG-apourease (0.48 μM) was incubated in solutions containing 20 μM Ni^{2+}, 100 μM HCO_3^-, 200 μM Mg_2-GTP, 100 mM HEPES (pH 8.3), 140 mM NaCl, the indicated concentrations of Zn^{2+} (circles) or Cu^{2+} (squares), and the presence (open symbols) or absence (closed symbols) of 20 μM UreE. Samples were assayed after 10 h incubation at 37 °C.

activity to ~500 units mg^{-1}. Furthermore, high GTP levels did not lead to inhibition of activation competence. Inclusion of a GTP recycling system had no effect on the results (data not shown).

Effect of UreE on Inhibition of UreDFG-Apourease Activation by Non-Ni Metal Ions and Chelators. Several divalent metal ions have been shown to inhibit nickel- and bicarbonate-dependent activation of urease apoprotein (*13*, *14*). Similar results were observed for urease activation using UreD-apourease (*14*), UreDF-apourease (*16*), and UreDFG-apourease (*17*). To assess whether the presence of UreE provides any protection against inhibition by non-nickel metal ions (or assists in selection of the proper metal ion), activation was carried out in the presence of zinc and copper (Figure 5). In the case of zinc, the presence of UreE provided significant protection against inhibition as shown by the higher levels of activation achieved for samples containing the metallochaperone. For example, the presence of 10 μM zinc led to a 90% reduction in final urease activity in the sample lacking UreE, but the same concentration of zinc resulted in only a 40% reduction of urease activity after activation in its presence. A more limited protection by UreE was observed for samples amended with copper ions.

To determine if UreE is involved in the direct transfer of Ni^{2+} to UreDFG-apourease, chelators with a high binding affinity for this metal were added to the activation solution and allowed to compete with H144*UreE. Figure 6 shows the effect of increasing amounts of IDA (panel A) and NTA (panel B) on the activation of apourease in UreDFG-apourease. In the absence of UreE, increasing concentrations of each chelator were added until activation was completely inhibited (200 μM for IDA and 40 μM for NTA). This inhibition likely arises from the decrease in free nickel ion concentration in solution. H144*UreE prevents inhibition of urease activation by the nickel chelators.

*Effect of H144*UreE Mutations on Urease Activation.* To further elucidate the mechanism of Ni^{2+} transfer by H144*UreE, we compared the effect of several variants of this protein on activation of apourease in UreDFG-apourease. The chosen variants were known to have altered metal binding properties and/or were shown to alter the whole cell urease activation efficiency (*11*, *12*). The rates and extents of activation were measured at 20 μM nickel and 10 mM bicarbonate concentrations, with ~20 μM H144*UreE vari-

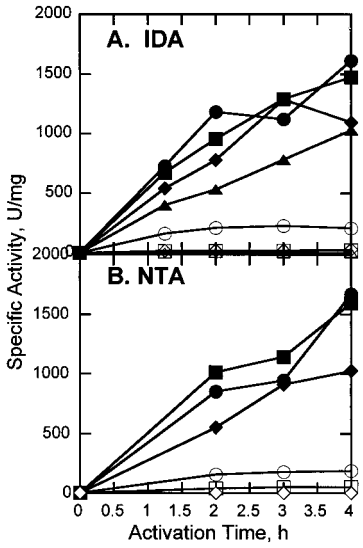


FIGURE 6: Effect of UreE on activation of urease apoprotein in UreDFG-apourease in the presence of nickel chelators. UreDFG-apourease (0.48 μM) was incubated in solutions containing 20 μM Ni^{2+}, 10 mM HCO_3^-, 100 mM HEPES, 140 mM NaCl, and 200 μM Mg_2GTP. In addition, samples either lacked (open symbols) or included (closed symbols) 20 μM H144*UreE and contained (A) 0 μM (circles), 40 μM (squares), 100 μM (diamonds), and 200 μM (triangles) IDA or (B) 0 μM (circles), 20 μM (squares), and 40 μM (diamonds) NTA. All samples were incubated at pH 8.3 and 37 °C. Aliquots were assayed at the indicated time points.

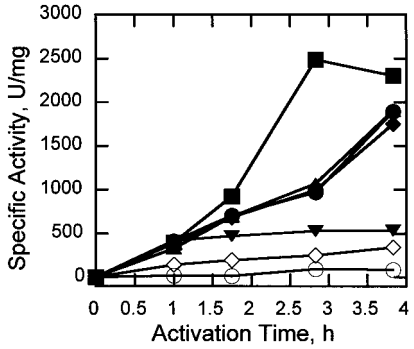


FIGURE 7: Activation of apourease in UreDFG-apourease at low Ni^{2+} concentration in the presence of UreE or its variants. UreDFG-apourease (0.48 μM) was incubated in solutions containing 20 μM Ni^{2+}, 10 mM HCO_3^-, 100 mM HEPES, 140 mM NaCl, 200 μM Mg_2GTP, and 20.5 μM H144*UreE (■), 20. 4 μM C79A/H144*UreE (▲), 19.8 μM H110A/H144*UreE (●), 20.7 μM D111A/H144*UreE (◆), 19.6 μM H112A/H144*UreE (▼), 20.1 μM H96A/H144*UreE (○), and a control lacking H144*UreE (◇). All samples were incubated at pH 8.3 and 37 °C. Aliquots were assayed at the indicated time points.

ant added (Figure 7). Results for each variant showed a decrease in activation competence relative to the H144*UreE control. The H110A, C79A, and D111A variants were capable of facilitating the activation of urease apoprotein in UreDFG-apourease at a slightly reduced rate in comparison to H144*UreE. The H112A variant, on the other hand, resulted in the production of urease with only 20−25% of full activity. The most dramatic effect came from mutation of His96 to alanine, which gave a significantly lower extent of activation than the control experiment without added H144*UreE (Figure 7). In contrast to the two-phase concentration dependence observed for H144*UreE with maximal activation observed at 20 μM protein (Figure 2), increasing concentrations of H96A/H144*UreE in the activation mixture resulted in continuously decreasing levels of apourease activation (data not shown).

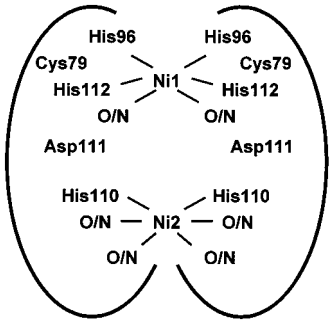
DISCUSSION

We have developed an in vitro activation system that for the first time allows us to convert urease apoprotein into fully active enzyme. As found in the case of cellular urease activation (*6*, *20*), the in vitro system requires four urease accessory proteins and a source of energy. UreE, a nickel-binding protein, is proposed to serve as the nickel donor to urease apoprotein within the UreDFG-apourease complex in a process that requires Mg_2GTP. On the basis of the high specific activity generated in the presence of only 100 μM bicarbonate and 20 μM Ni^{2+}, we propose that this in vitro system has physiological relevance to the in vivo urease activation process.

Our data described here and reported previously (*17*) are consistent with the presence of two distinct activation mechanisms for urease apoprotein in UreDFG-apourease. At high Ni^{2+} and bicarbonate concentrations, urease activation proceeds by a GTP-independent free diffusion mechanism whereby the apoprotein reacts with carbon dioxide (forming the carbamate of Lys217) and nickel ions. Alternatively, activation occurs by a GTP-dependent mechanism that requires much lower concentrations of bicarbonate. UreE stimulates activation by the second mechanism, but not by the first, and allows activation to proceed at lower Ni^{2+} concentrations. We propose that cellular activation of urease apoprotein closely parallels the in vitro UreE- and GTP-dependent activation system examined here. The presence of UreE facilitates activation at low total nickel concentrations where much of the nickel present is bound to the metallochaperone. These conditions greatly reduce the diffusional mechanism of activation, minimize the formation of nonproductive metallocenters formed during the diffusional process (*14*, *21*), and eliminate the nickel-dependent inhibition of the enzyme (*5*). The rate of apourease activation is very slow, but the overall extent of activation is greatly enhanced. This slow rate may be attributed to nickel being incorporated into prefolded protein or to the absence of still unidentified cellular factors; however, we note that our published in vivo studies also exhibit a slow rate of urease activation (*12*, *20*). The UreE concentration, or the ratio of UreE to Ni^{2+}, must be carefully controlled to allow maximal activation. The highest levels of activity are generated in samples where the UreE and Ni^{2+} concentrations are approximately identical, whereas elevated UreE levels lead to a depression in activation competence (Figure 2). The results are consistent with the 2Ni-UreE species, not free Ni^{2+}, participating in urease apoprotein activation. In addition, the data are compatible with metal-free UreE (and perhaps the 1Ni-UreE species) competing with 2Ni-UreE for a metallochaperone-binding site on UreDFG-apourease where metal transfer takes place.

The UreE-dependent enhancement of urease activation using UreDFG-apourease only occurs in the presence of bicarbonate and GTP. The low levels of bicarbonate needed for activation of this complex (maximal rate at 5−10 mM, but capable of generating fully active enzyme at 100 μM) contrast with the 100 mM bicarbonate required for achieving maximal activation of apourease not associated with acces-

Scheme 1



sory proteins (*13*). This process resembles the bicarbonate concentration dependence observed for UreDF-apourease activation; however, that activation requires much greater Ni^{2+} concentrations (500 μM) (*16*). Significantly, urease apoprotein activation in UreDFG-apourease occurs at much lower GTP concentrations in the presence of UreE. These results suggest a possible coupling of bicarbonate incorporation and GTP hydrolysis by UreDFG-apourease in the presence of UreE.

We propose that UreE actively transfers Ni^{2+} to urease apoprotein within UreDFG-apourease in a GTP-dependent activation process. This contrasts with an alternative view that UreE functions only to reversibly bind Ni^{2+} and make it available in solution for apourease binding. Supporting our proposal are studies that show UreE stimulates activation in the presence of Ni^{2+} chelators. For example, H144*UreE exhibits an average K_d for Ni^{2+} of 8.6 μM (*11*), yet it overcomes the inhibition caused by IDA or NTA which possess lower dissociation constants (K_d of 7.4 nM and 3.16 pM, respectively) (*22*). Elimination of the chelator inhibition argues for direct Ni^{2+} transfer from UreE to apourease when the latter is bound to the UreD, UreF, and UreG accessory proteins. We suspect that the slow dissociation rates (3×10^{-3} s^{-1} and 10^{-4} s^{-1}) observed for the two nickel sites in isolated H144*UreE (*12*) may increase when the metallochaperone associates with UreDFG-apourease in the presence of GTP. Alternatively, the slow rate of dissociation may be important for increasing the efficiency of transfer to apourease.

Studies involving UreE variants also are compatible with a direct metal transfer role for the metallochaperone. Variants used here were shown in previous work to exhibit changes in cellular urease activation and/or metal ion binding properties (*11*, *12*). Our working model for Ni^{2+} binding to H144*UreE is shown in Scheme 1. The two metal binding sites lie at the interface of the dimer, each exhibiting 6-coordinate geometry (*12*). H144*UreE variants that possess changes at each of the illustrated residues diminish the GTP-dependent activation process. The most significant effects involve H112A/H144*UreE and H96A/H144*UreE, each affecting the same metal site. The H112A/H144*UreE variant binds a single Ni^{2+} per dimer and possesses a significantly lower affinity for the metal. The H96A/H144*UreE protein binds two Ni^{2+} per dimer with essentially unchanged affinity compared to the control protein (*11*). GTP-dependent activation of urease apoprotein in the UreDFG-apourease is nearly abolished in the latter case, consistent with a critical role for His96 in the metal transfer event. The in vitro effects of the UreE variants on UreDFG-apourease activation show strong parallels to the effects of these variants observed in whole cells (*12*). Coupled with earlier studies suggesting an energy dependence to in vivo urease activation (*20*), this work again demonstrates the physiological significance of this GTP-dependent activation process.

The UreE and UreG accessory proteins have counterparts that function in the activation of two other nickel-containing enzymes, carbon monoxide dehydrogenase and hydrogenase. Highly analogous to UreE (although unrelated in overall sequence), CooJ of *Rhodospirillum rubrum* contains a carboxyl terminal His-rich region and binds 4 Ni^{2+} per monomer for incorporation into CO dehydrogenase (*23*, *24*). Also needed for *R. rubrum* CO dehydrogenase activation is CooC, a nucleotide-binding protein related in sequence to UreG. It is reasonable to suspect that the CooJ and CooC CO dehydrogenase accessory proteins carry out roles similar to those associated with UreE and UreG in urease activation. For hydrogenase activation, however, HypB may perform both functions. HypB from *Bradyrhizobium japonicum* possesses a His-rich sequence (in this case at the amino terminus), binds 18 Ni^{2+} per dimer, and is proposed to have a dual role of storing and delivering the metal to Ni-free hydrogenase (*25*, *26*) similar to the proposed role of UreE. In addition, HypB exhibits nearly 25% sequence identity to UreG and, as isolated from *B. japonicum* and *E. coli*, catalyzes the hydrolysis of GTP (*25*, *27*). These comparisons imply that study of the urease metallocenter assembly mechanism may serve as a general model for incorporation of nickel into proteins.

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