The interaction of metallochaperones HypA and UreE facilitates nickel transfer: a "cross-talk" between hydrogenase and urease

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As a pathogen bacterium found in nearly half of the population, *Helicobacter pylori* relies heavily on proper function of urease and hydrogenase for its survival and successful pathogenesis[1-3]. Maturation of these two metalloenzymes requires a series of metallochaperones and accessory proteins. Among these, HypA and SlyD have been shown to form complexes with HypB and subsequently to help nickel insertion into hydrogenase[4-7]. Previous study showed that HypA was involved in the maturation of both hydrogenase and urease; unexpectedly, it was also suggested to interact with urease metallochaperone UreE[8,9].

In this work, we showed the Ni^{2+}-induced tetramerization of UreE in solution and investigated the interaction of HypA and UreE. By chemical cross-linking and static light scattering, we showed that one HypA binds to one UreE dimer to form a hetero-complex (i.e. HypA-$(UreE)_2$), with the dissociation constant (K_d) of 4 μM in the absence of nickel ions. Upon the binding of Ni^{2+} on UreE, the stability of the complex decreased. The putative residues involving the binding on HypA are mainly located in the cleft between α1 and β1/β6 mapped by NMR chemical shift perturbation. The role of the flexible C-terminus (residues 158-170) of UreE in the discrimination of HypA was demonstrated by cross-linking, size exclusion chromatography and isothermal titration calorimetry. Deletion of C-terminus (residues 158-170) of UreE leads to the significant decrease of urease activity as well as nickel transfer from HypA to UreE. The HypA-UreE complex was also observed intracellularly by using GFP-fragment reassembly, and a model was proposed on the interaction.

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