

Metal binding profiles of *H. pylori* metallochaperones HypA and HspA in cells

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Metal ions play either catalytic or structural roles in a quarter to one-third of all proteins in biological systems. Bacterial metalloproteins have evolved an elaborate mechanism acting in concert to maintain cellular metal homeostasis^[1]. The bacterial pathogen *Helicobacter pylori* is the leading risk factor for the development of human gastric cancer. Its infectious capacity relies heavily on two Ni-enzymes urease and [Ni, Fe]-hydrogenase, which are dependent on the intracellular Ni²⁺ that is tightly controlled by a battery of metallochaperones. Bi-based antiulcer drugs have long been used for the treatment of *H. pylori* infection. Proteins are commonly believed to be the targets of the metallodrug and disruption of certain metalloproteins/enzymes may account for its mechanism of action^[2].

In the present study, we examined metal specificity of metallochaperones HypA and HspA that regulate Ni homeostasis in *H. pylori*, as well as the effect of Bi on the binding in cells. Using the established GE-ICP-MS system^[3], metal-binding stoichiometries were determined. HypA binds stoichiometric Zn and substoichiometric Ni when heterologously overexpressed in *E. coli*, suggested a transient HypA-Ni binding. HypA showed a high fidelity towards its structural metal Zn, even in the presence of the highly competitive Cu and Bi ions. In contrast, HspA selectively associates with Co, Ni, Cu and Zn from an essential metal pool, with Zn being the largest portion. In the presence of Bi, binding of Ni to HypA and HspA were significantly suppressed, indicating that Bi competes with Ni and inhibits Ni delivery in cells. This work provides a direct insight into the intricate metal-protein partnership in cells.

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References

- [1] a) N. J. Robinson, K. J. Waldron, *Nat. Rev. Microbiol.* **2009**, 6, 25-35. b) A. Cvetkovic, A. L. Menon, M. P. Thorgersen, et al., *Nature* **2010**, 466, 779-782.
- [2] H. Li, H. Sun, *Curr. Opin. Chem. Biol.* **2012**, 16, 74-83.
- [3] L. Hu, T. Cheng, B. He, L. Li, Y. Wang, Y. Lai, G. Jiang, H. Sun, *Angew. Chem. Int. Ed.* **2013**, 52, 4916-4920.