Expression and Characterization of the Histidine-rich Protein, Hpn: Potential for Nickel Storage in *Helicobacter pylori*

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Helicobacter pylori (*H. pylori*) is believed to be the etiological agent of peptic ulcerations and type B gastritis. One feature of *H. pylori* is its ability to colonize host cells under highly acidic conditions. The organism produces a large amount of a nickel-containing enzyme urease (~10%), which is believed to neutralize gastric acid by producing ammonia for the survival of the bacteria [1]. Nickel is taken up into *H. pylori* by two transport systems, ATP-binding cassette (ABC) transporters and permeases such as NixA. In addition to NixA nickel transport protein, the cytoplasm of *H. pylori* also contains Hpn, a protein that has strong metal-binding property. The *hpn* gene encodes a histidine-rich cytoplasmic protein, probably involved in the storage of nickel in *H. pylori*. Bacteria lacking Hpn are 4-fold more sensitive than the wild type to bismuth, a metal which has been widely used to treat *H. pylori* infection [2,3].

We report the biophysical characterization of the conformation, multimeric status, and Ni^{2+} binding properties of purified, recombinant Hpn under physiologically relevant conditions. Ni^{2+} induces conformational changes within the protein, reducing disorder and increasing the α-heilcal and β-sheet content. The protein exists in an equilibrium of multimeric forms in solution: with the 20-mers being the dominant form. By UV-vis spectroscopy and ICP-MS, Hpn was found to bind 5.0 ± 0.2 nickel ions per monomer at pH 7.4. More importantly, nickel binding to Hpn is reversible: metal being released either in the presence of a chelating ligand such as EDTA, or at a slightly acidic pH ($pH_{1/2}$~ 6.3). The uptake of Ni^{2+} in *Escherichia coli* BL21(DE3) cells harboring a pET plasmid-based copy of Hpn was increased four-fold upon the addition of IPTG, to induce Hpn expression, compared with un-induced controls, or control cultures lacking the plasmid. Hpn may therefore serve multiple roles inside the bacterium: storage of a "reservoir" of Ni^{2+}; donation of Ni^{2+} to metalloenzymes such as urease or other proteins; and detoxification via sequestration of excess Ni^{2+} .

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[1] Eaton KA; Brooks CL; Morgan DR; Krakowka S (1991) Infect. Immun. 59, 2470.
[2] Mobley HL; Garner RM; Chippendale GR; et al (1999) Helicobacter 4, 162.
[3] Sun HZ; Zhang L; Szeto KY (2004) Metal Ions Biol Syst. 41, 333.