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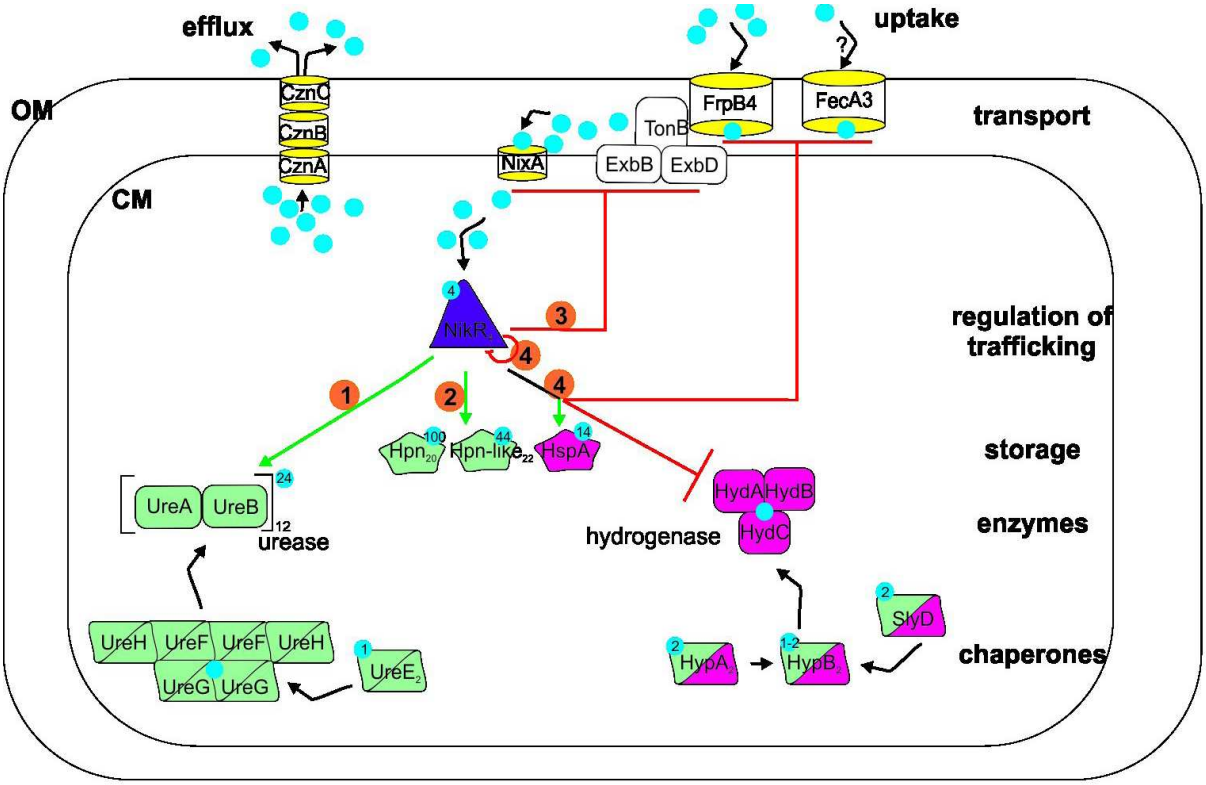

Nickel homeostasis in *Helicobacter pylori* and potential histidine-rich binding sites from various bacterial and fungal pathogens are discussed.

Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Ni^{2+} chemistry in pathogens –a possible target for eradication

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

The survival of all urease and/or hydrogenase containing pathogens depends on the proper homeostasis of nickel. In the scope of this perspectives paper, details of Ni^{2+} metabolism of *Helicobacter pylori*, a widespread stomach-ulcer causing bacteria, are described. Nickel binding proteins and thermodynamics of such metal complexes are discussed in detail and special focus is given to potential nickel binding sequences in this metal's chaperones and regulators. A list of potential Ni^{2+} binding sites in various pathogens is presented, which points out numerous examples of nickel interactions that still need to be understood.

Introduction

The most important features necessary for understanding metal homeostasis are correlations between the protein machinery involved in the uptake, transport, delivery, storage and secretion of the given metal, knowledge about their structures and the understanding of thermodynamics of such metal-protein interactions. In this paper, we focus on all three of these features. First, we introduce the **interactions between nickel dependent proteins in *Helicobacter pylori*** (*H. pylori*), one of the most widespread nickel-dependent pathogens. In the last decade, a reasonable amount of findings have been made about the biological inorganic chemistry of this bacterium. This recent 'Nobel prize winner' is one of the best known examples of the dinickel urease-dependent bacteria; without proper Ni^{2+} nickel homeostasis, it would have been unable to colonize the non-welcoming acidic environment of the stomach. To make an oversimplification, the first chapter could be referred to as 'Ni^{2+} in *H. pylori* seen by a biologist', as it is a detailed discussion about the molecular mechanisms which control the expression of genes encoding nickel-dependent proteins and the interaction between these proteins. Nickel uptake, storage, secretion and the interactions between Ni^{2+} dependent proteins are explained in detail. However inaccurate this approach might seem for a chemist, these findings are in fact the most relevant ones for the biology of *H. pylori*.

In the later parts of the paper, we focus on the **thermodynamics of specific nickel binding sequences**, showing correlation between the type of protein sequence and the stability of metal binding. Again oversimplifying, nickel homeostasis is shown from the point of view of a chemist – however irrelevant this approach might seem for a biologist, in the long run, it is in fact the most accurate one which identifies the most probable sequences capable of binding nickel ions with the highest thermodynamic stability. Understanding thermodynamics of bacterial metal metabolism might give us an idea, which are the 'weak points' of nickel homeostasis that need to be aimed at during therapy, e.g. with a specific chelator.

Finally, we discuss what types of interaction need to be understood in order to get a reasonably full view on bacterial nickel homeostasis. The last part of the paper is the result of an extensive literature, PDB and UniProt database search and can be considered as a 'to-do' list, giving examples of various bacterial and fungal **histidine rich sites that are possibly crucial for nickel binding**, giving an overview and pointing out what still needs to be done in order to understand the correlation between their structure and metal binding abilities. This knowledge would not only make us understand why nature chose such peculiar metal binding sites in nickel chaperones, but would also bring us one step closer towards getting an idea about a more effective eradication of such pathogens.

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Nickel homeostasis in *Helicobacter pylori*

In this chapter, we discuss the correlations between proteins that are involved in nickel homeostasis in one of the most studied nickel-dependent organisms – *H. pylori*, a Gram-negative, microaerophilic, pathogenic bacterium, which infects about 50% of the human population worldwide. It colonizes the human stomach and is usually acquired during childhood. When the patient is not treated, *H. pylori* can persist in this harsh environment (pH of the lumen as low as 1) for a lifetime. Most of the infections are asymptomatic, however 10-15% of individuals suffer from gastric or duodenal ulcers, while 0.5-2.0% develop gastric cancer. It is estimated that 1 million people per year die because of the most severe outcome of *H. pylori* infection -

gastric adenocarcinoma [1]. At present, *H. pylori* is treated by complex triple and quadruple therapies composed of proton pump inhibitor (PPI) and two antibiotics (clarithromycin and amoxicillin or metronidazole) or PPI, bismuth salts and two antibiotics (metronidazole and tetracycline), respectively. These antibiotics target basic cellular processes such as protein synthesis (clarithromycin) or DNA integrity (metronidazole) and thus can exert bacteriostatic and bactericidal effects on the natural human microflora. In addition, the level of successful eradication has declined recently due to the increased rate of drug resistance in *H. pylori*, particularly to clarithromycin or metronidazole [2, 3]. Thus, there is a need to reveal novel targets for future drugs against *H. pylori*. Tentative candidates for such targets are proteins involved in nickel homeostasis in this bacterium. The idea for eradication seems to be very simple – Ni^{2+} is toxic for the mammalian host, but crucial for *H. pylori.* If the rules that govern metal homeostasis in the bacterium are well understood, a way to deprive *H. pylori* of nickel ions could also be found. To explain the importance of nickel ions and nickel-binding enzymes for *H. pylori*, it is necessary to realize that a major prerequisite of colonization and persistence of *H. pylori* is the capacity to survive the acidic pH of the human stomach. *H. pylori* physiology does not resemble that of an acidophilic bacteria, which grow optimal at acidic conditions; in contrast, it prefers neutral or close to neutral environment (pH 5.5-7.5), while the pH in the stomach varies between 1 to 7 in response to daily food intake and also fluctuates within gastric compartments [4, 3]. Thus, *H. pylori* needs to sense the environment, to respond to changes in pH and to adapt to alterations by regulation of many cellular processes in order to maintain intracellular pH homeostasis. In all of these processes nickel plays a pivotal role. It is primarily used as a co-factor of two enzymes necessary for colonization of the human stomach: urease - the enzyme required to neutralize H^+ ions, which diffuse into the cytoplasm from the acidic environment [5, 6], and a [Ni-Fe] hydrogenase – the enzyme which provides energy for the cell by oxidation of molecular hydrogen [7]. Moreover, nickel ions are important for a control of many cellular processes via nickel-binding NikR regulator [8], which directly controls the expression of more than 10 genes in response to acidity and/or nickel concentration [9–11]. In addition, *H. pylori* probably uses Ni^{2+} as a sensor for monitoring extracellular pH, because nickel bioavailability and influx greatly increases at lower pH, which changes the nickel balance in the cytoplasm [12, 13]. Thus, nickel is absolutely essential for *H. pylori* to colonize the human stomach. However, excess cytoplasmic nickel is toxic for the cells. Thus stringent nickel homeostasis is maintained by a sophisticated nickel trafficking system composed of proteins exhibiting specialized functions: i) importers acquire Ni^{2+} from the environment, ii) nickel-binding chaperones together with iii) maturation proteins deliver nickel to the active sites of the metalloenzymes, iv) nickel-storage proteins sequester Ni^{2+} from the cytoplasm for its availability during nickel deficiency periods, and, together with V) exporters, maintain nickel below the toxic level (Fig. 1) [14, 15].

Since nickel ions are toxic for humans, they are present in the serum at relatively low concentration (3-10 nM, [16]) and its daily intake depends on the dietary habits [17]. Thus Ni^{2+} must be actively transported into *H. pylori* cells to reach ca. 60 nM concentration [16, 11]. In *H. pylori,* the nickel is imported in a two-step-process, since the bacterium belongs to Gram-negative bacteria and thus contains two cellular membranes: the outer membrane (OM) and the inner cytoplasmic membrane (CM). There are two OM transporters, **FrpB4** [16, 18] and **FecA3** [19, 20], and **NixA -** the so far only known nickel transporter of *H. pylori*, which is located in the cytoplasmic membrane (Fig. 1, Tab. 1). As has been shown experimentally, FrpB4 is a nickel receptor and transporter embedded in the OM, which is able to transport the nickel ions at low pH in a TonB-dependent manner (i.e. the energy from the proton motive force is converted by the TonB/ExbD/ExbB into conformational changes of the FrpB4, which results in nickel transport). Other studies showed that *fecA*3 (HP1400) expression is regulated by **NikR** (see below), which leads led to the proposition of FecA3 to constitute a second nickel receptor/transporter system. Additionally, it was proposed that at high nickel concentration, nickel is transported passively through porins [16] Moreover, it was hypothesized that there might be some additional proteins- **nickelophores** - which might be responsible for nickel chelating, facilitating its import from the environment, similarly to Fe-binding siderophores [16]. Interestingly, it has been recently shown by crystallography that the *H. pylori* **CeuE** protein, previously annotated as the periplasmic component of an ABC-type transporter system responsible of iron uptake, actually binds a Ni-$(L\text{-}His)_2$ complex [22]. Similar studies showed that such molecules also facilitate nickel import in *E. coli* [20–22], however, the presence of the nickelophore, i. e. the molecule synthesized by the bacterium and exported to the environment in purpose to chelate nickel ions, have not been confirmed yet. Thus, it still remains unknown whether *E. coli* and other microorganisms such as *H. pylori* use nickelophores as nickel chelating agents to aid nickel transport. Once nickel is imported to the periplasm, it is transported to the cytoplasm by the NixA protein (37 kDa) – a permease belonging to the family of nickel-cobalt transporters (NiCoTs) characterized by the eight transmembrane helices spanning the CM, which form a channel for nickel ions [26–28]. Upon nickel delivery to the cytoplasm, the ions are either directed to the nickel-dependent enzymes or are bound by storage proteins. In case of excess cytoplasmic nickel, it is exported by the **CznABC** transporter, which additionally exports cadmium and zinc [29, 14]. Relatively little is known about the transporter. It is composed of the three subunits located at the subfractions of the cell membrane: CznA in CM, CznB in the periplasm and CznC in OM. It is known that CznA and especially CznC are important for nickel efflux. The ion selectivity of the CznABC transporter has not been characterized, but it might be used to keep the homeostasis of the transition metals in *H. pylori*, essential for regulation of urease and/or hydrogenase activity (see also below). Urease and [Ni-Fe] hydrogenase are the two enzymes absolutely crucial for *H. pylori* colonization in the human stomach [30, 31]. Interestingly, both enzymes use substrates, which are useless for the host: urea, a waste product of the protein hydrolysis, is used by urease and molecular hydrogen, produced by colonic microflora by hydrogenase. It is worth reminding that nickel is also not known to be a cofactor of any human protein. By choosing this strategy *H. pylori* does not compete with the host for biologically essential

molecules, which is beneficial for lifetime colonization. Urease is an intracellular enzyme, responsible to buffer the cytoplasm in response to low extracellular pH (for discussions on urease localization and cytoplasmic pH homeostasis see [32, 5]). It catalyses the hydrolysis of urea into CO_2 and ammonia in the cytoplasm. NH_3 buffers H^+ leaking into the cytoplasm, CO_2 interconversion to bicarbonate is probably mediated by the periplasmatic α-carbonic anhydrase. In consequence, the intracellular environment of *H. pylori* is buffered [32, 33, 5]. *H. pylori* urease, composed of 12 UreA and 12 UreB subunits (MW 1.1 MDa in total), exhibits the highest affinity for its substrate ever described for ureases (K_m for urea <0.5 mM) [34, 35]. The urease cluster contains genes for its own nickel-supplying and maturation proteins: **UreE** - a Ni^{2+} chaperone, and the complex-forming proteins **UreG**, **UreF** and **UreH**, which, by a cascade of ordered protein-protein interactions and conformational changes, are responsible for proper incorporation of nickel from UreE into apo-urease [36, 14, 37]. Apo-urease is inactive; for the activity it requires to bind up to 24 nickel ions. The incorporation of nickel is enhanced by its environmental availability, which is higher at lower pH. However, not all of the cellular urease molecules, representing 6-10% of the total proteins, are constantly loaded with nickel ions and the enzyme is far from fully active all the time. In fact as little as 7-25% of the whole apo-urease activated with nickel, present in *H. pylori* grown at neutral pH suffice for acid resistance at extreme low pH in the presence of urea [5]. It is proposed that *H. pylori* urease loads as much nickel as possible at the "nickel prosperity" times to get the potential for fast activation of urease upon pH decrease [38] or to be able to pass sufficient amount of the active urease to the daughter cells that they can survive unfavorable, nickel-deficient conditions by using inherited pool of active urease [5]. Hydrogenase is the second nickel metalloenzyme in *H. pylori*, indispensible for colonization of the human stomach. The enzyme has not yet been purified as recombinant proteins, thus detailed biochemical or structural characteristics are still missing [7]. Unlike most of the bacterial hydrogenases, which are composed of the two subunits, *H. pylori* hydrogenase probably consists of three subunits: a small α-subunit (encoded by *hydB*), a large β-subunit (encoded by *hydA*) and a γ-subunit (encoded by *hydC*). α [Fe-S] and β [Fe-Ni] subunits are required for catalytic activity of the enzyme, while the γ-subunit – the membrane anchored cytochrome b – transfers electrons from H_2 to quinones. In contrast to hydrogenase itself, the accessory proteins required for hydrogenase maturation are better studied, also in the context of competition for nickel ions with urease-maturation proteins [39–43]. There are six proteins HypABCDEF, which are responsible for hydrogenase maturation [14]. Among them, **HypA** and **HypB** bind Ni^{2+} and deliver it into hydrogenase active site. HypA is a dimeric metallochaperone which binds two nickel ions and, upon interaction with HypB, transfers nickel to the latter one [43]. Ni^{2+} binding induces dimerisation of HypB, which is necessary for subsequent nickel transfer into hydrogenase, possibly to [Ni-Fe] center of β-subunit [43]. It has been recently found that there is another nickel chaperone – SlyD – which provides nickel for hydrogenase [44]. SlyD interacts with HypB, transfers nickel to this protein and stimulates its GTPase activity enhancing nickel transfer into hydrogenase [45]. It is not known why *H. pylori* requires another metallochaperone for hydrogenase maturation, but it is suggested that HypA and SlyD might function as backup for each other to ensure nickel incorporation to such crucial *H. pylori* enzyme as hydrogenase [45]. It is also suggested, that SlyD plays a role in hydrogenase maturation by interaction with twin arginine motif (Tat signal, RRxFxK) of a small hydrogenase subunit and thus preventing the translocation of immature hydrogenase into cytoplasmic membrane [44, 46]. More interestingly, HypA, HypB and SlyD are also involved in urease maturation [40, 44, 47]. It has been recently shown that HypA competes with UreG for interaction with UreE nickel chaperone, with the favored HypA-UreE complex formation [42]. However, the UreG-UreE interactions were enhanced and stabilized in the presence of Zn^{2+}, which may suggest a role of zinc ions in regulation of hydrogenase and/or urease maturation, especially in situations when *H. pylori* encounters conditions favoring maturation of only one of the two enzymes [74]. Indeed, urease activity was shown to be stimulated by nickel, while inhibited by Zn^{2+} and other transition metals [48, 49]. Additionally, many other nickel-trafficking proteins were also shown to bind nickel and zinc, but also other transition metal ions, which might modify their function or activity [28]. Thus, the regulation of nickel and zinc concentration, possibly mediated by the before-mentioned CznABC triple-ion exporter [29] and possibly also *via* CadA resistance factor [49], might be important to maintain the balance between the activity of the two nickel-requiring enzymes.

Since *H. pylori* is absolutely dependent on nickel, the bacterium developed a nickel-storage system, which sequesters nickel from the cytoplasm, guaranteeing sufficient nickel when required but also preventing toxicity. The system is composed of three proteins: **Hpn** and **Hpn-like**, binding five and two nickel ions per monomer, respectively [50, 51], and **HspA**, which binds two Ni^{2+} ions [52].

It has been shown that Hpn and Hpn-like are important for urease activity, while HspA constitutes a nickel storage pool for maturation of hydrogenase [53, 54]. Though the molecular mechanisms of nickel transfer from nickel storage proteins are unknown, it is assumed that they donate nickel when increased activity of urease or hydrogenase is required. Indeed, Hpn, Hpn-like and HspA have been shown to release Ni^{2+} at acidic pH, while they sequester the ion at neutral pH [50–52]. It has also been shown that under nickel-starvation conditions *H. pylori* mutants lacking *hpn* and *hpn*-like genes colonized mouse stomachs with severely reduced efficiency compared to the wild type strain [55]. A detailed discussion about metal binding properties of those proteins follows in the later chapters of this paper.

The expression of genes encoding nickel-binding proteins is mainly controlled by the **NikR** regulator; the urease operon has been shown to be additionally regulated by the **Mua** protein, preventing urease overexpression when nickel concentration is high [56]. NikR belongs to the family of nickel dependent regulators commonly present in bacteria, which exclusively control the transcription of genes encoding nickel-binding proteins. However, in contrast to other bacterial NikR proteins, *H. pylori* NikR (*Hp*NikR) plays pleiotropic roles in regulation of expression of genes. First, it directly regulates transcription of many genes involved in nickel trafficking and nickel-dependent

enzymes (Fig. 1), but also in iron homeostasis and acid stress response [9, 19, 12]; in addition it is involved, either directly or indirectly, in control of genes encoding proteins important for several cellular functions such as chemotaxis, respiration or stress response [9]. Second, it acts as activator or repressor of several genes [11]. For a long time, the pleiotropic influence of *Hp*NikR has not been understood. Recent works have shown that probably both differential coordination of nickel by *Hp*NikR and variability in the *Hp*NikR binding sequences in the promoter regions of particular genes are responsible for *Hp*NikR ability to discriminate between low and high affinity sites (K_d of μM and nM, respectively) [57]. *Hp*NikR is a tetramer composed of two domains: a central tetrameric metal binding domain, which coordinates four Ni^{2+} ions/tetramer, and two flanking ribbon-helix-helix DNA-binding domains. There are two classes of nickel binding sites: high affinity (HA) sites and weak binding site (external site, X-site). The HA site can be further subdivided into two coordination geometries: square-planar referred as "4-site" and square pyramidal /octahedral referred as "5/6-site" [58–60]. Although the exact nickel coordination sites are still controversial due to different *Hp*NikR crystallization conditions [60], it is proposed that diverse coordination of nickel ions in these sites affect the overall protein conformation, including the long-range effect on the flexibility of the DNA-binding domains. In the model proposed by West and co-workers the sole 4-site coordination of all four nickel ions results in affinity towards weak binding sites, while mixed coordination of 4-site and 5/6-site might result in affinity towards strong binding sequences [60]. It is also suggested that the mode of coordination of Ni^{2+} by *Hp*NikR might be dependent on pH and the nickel availability (and possibly other metal ions such as Mg^{2+} or Mn^{2+}), thus enabling *Hp*NikR to respond to environmental changes including acid stress [59, 60]. However, nickel coordination mode in *Hp*NikR is not the sole feature enabling to discriminate between low and high affinity sites. The NikR-binding site was determined to be a pseudo-palindromic sequence with a general consensus of 5'-TATTATT-X_{11}-AATAATA-3' [57]. Further studies have shown that the sequence of 3' part of the palindrome might be important in determining the affinity towards *Hp*NikR and proved that nucleotide at position 21st is the main discriminator between weak and strong *Hp*NikR binding: in high-affinity sites thymine is located at position 21, while for weak binding sites there are no preferences for any of the nucleotides. Thus both the structural properties of *Hp*NikR, mainly affected by nickel coordination, and the differences in *Hp*NikR binding sequences of the promoter regions are responsible for the pleiotropic activity of *Hp*NikR and allows this protein to gradually coordinate the response to specific environmental stimuli, in particular to acid stress.

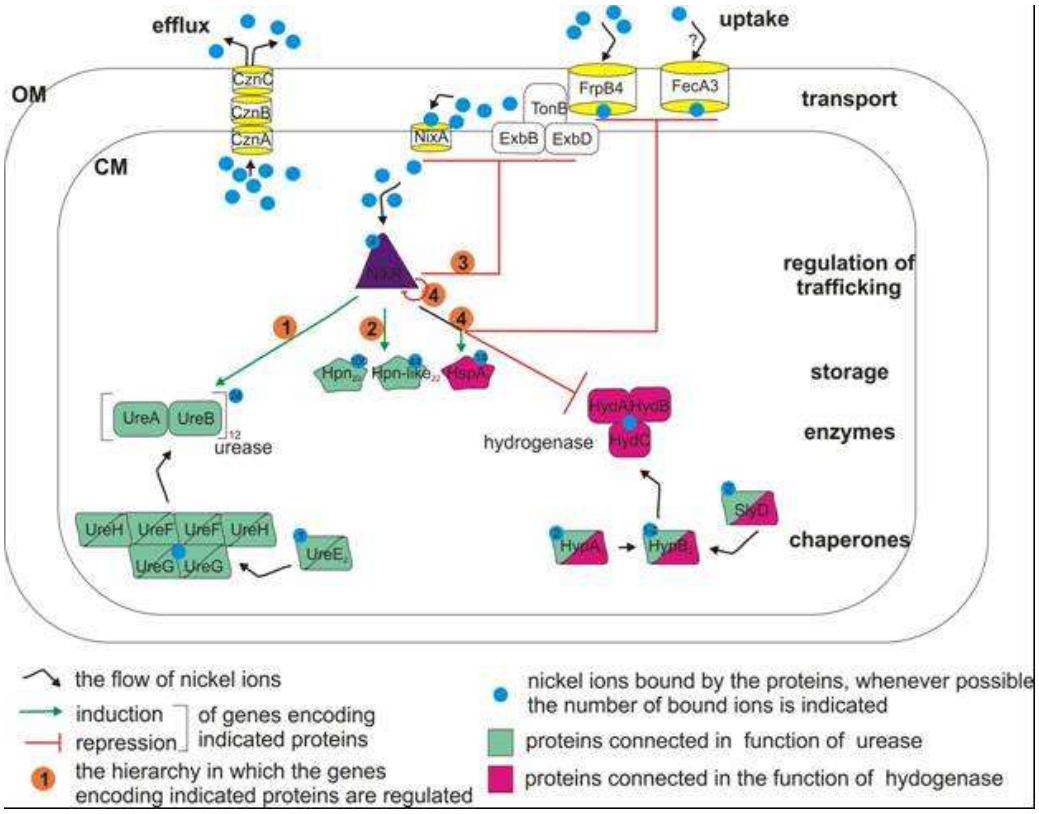

Figure 1. Regulation of nickel homeostasis in *H. pylori*. The detailed description can be found in the text. CM, cellular membrane, OM, outer membrane

Table 1. Nickel binding enzymes and proteins involved in nickel trafficking in *H. pylori*. The protein gene symbols are according to annotation of 26695 strain.

Protein	Function/properties	Ni^{2+} bound per functional unit	Biochemical characteristics	References
Transporters				
FrpB4 (HP1512) 97 kDa, pI 9.2	Importer of Ni^{2+} from the environment to the periplasm; located in the OM, transfers Ni^{2+} in TonB-dependant manner	ND	ND	[16, 18]
FecA3 (HP1400) 95 kDa, pI 9.2	Putative importer of Ni^{2+} from the environment to the cytoplasm	ND	ND	[19, 21, 20]
NixA (HP1077) 37 kDa, pI 9.2	Importer of the Ni^{2+} from periplasm to cytoplasm- located in the CM, belongs to the family of nickel-cobalt transporters (NiCoTs)	ND, it acts as a monomer	K_m 11.3±2.4 nM; V_{max} 1750±220 pmol Ni^{2+} $min^{-1}10^{-8}$ cells	[26, 27, 70]
CznABC: CznA (HP0969) 111 kDa, pI 8.4 CznB (HP0970) 39.5 kDa, pI 8.8 CznC (HP0971) 47.5 kDa, pI 8.6	Exporter of Ni^{2+}, Cd^{2+} and Zn^{2+} from cytoplasm to environment; composed of three subunits (Fig. 1); CznB and CznC bind Ni^{2+}, CznB also binds Cd^{2+} and Zn^{2+}	ND	ND	[29]
Regulators				
NikR (HP1338) 17 kDa, pI 5.0	Transcriptional regulator of wide range of *H. pylori* genes	4-8* Ni^{2+}/tetramer	K_d* 3.5×10^{-12} M or 1.2×10^{-8} M for high affinity sites and 0.5×10^{-6} M for low affinity sites	[11, 57–60, 71, 72]
Mua (HP0868) 18.3 kDa, pI 9.7	Secondary regulator of urease's genes expression- does not act by direct binding to urease promoter	2 Ni^{2+}/dimer	K_d ~2.0×10^{-5} M	[56]
Urease and urease chaperones				
Urease: UreA (HP0073) 26.4 kDa, pI 8.7 UreB (HP0072) 61.5 kDa, pI 5.6	Enzyme: responsible for acid acclimation via buffering the intracellular environment caused by the influx of the H^+- composed of two subunits: UreA and UreB forming a dodecameric complex (four trimers of UreA-UreB dimers)	24 Ni^{2+}/dodecameric complex	K_m 0.3 ± 0.1 mM and a V_{max} 1,100 ± 200 µmols of urea hydrolyzed/min/mg of protein	[5,32, 34, 73]
UreE (HP0070) 19.2 kDa, pI 8.7	Chaperone: promotes Ni^{2+} insertion into the apo- urease; binds also zinc (1 Zn^{2+}/dimer), which stabilizes UreE-UreG complex	1 Ni^{2+}/dimer	K_d 0.15×10^{-6} M	[36, 74–76]
UreF (HP0069) 28.5 kDa, pI 7.2	Chaperone: involved in the insertion of Ni^{2+} in the urease active site	2 Ni^{2+}/dimer	K_d 6.4×10^{-6} M	[36, 77]
UreG (HP0067) 21.8 kDa, pI 4.8	Chaperone: involved in the insertion of Ni^{2+} in the urease active site- binds GTP, which together with Ni^{2+} stimulates UreG dimerisation; GTP hydrolysis releases nickel from UreG; binds also zinc (1 Zn^{2+}/dimer),	0 Ni^{2+}/ $UreG_{apo}$ monomer 1.2 Ni^{2+}/ $UreG_{GTP}$	K_d 1×10^{-5} M	[36, 42, 78]

	which stabilizes UreE-UreG complex; competes with HypA for UreE	dimer		
UreH (HP0066) 29.6 kDa, pI 5.8	Chaperone: involved in the insertion of Ni^{2+} in the urease active site	none	ND	[36]
Hydrogenase and hydrogenase chaperones				
Hydrogenase: HydA (HP0631) 42.2 kDa, pI 6.4 HydB (HP0632) 64 kDa, pI 6.8 HydC (HP0633) 25.7 Kda, pI 9.8	Enzyme: responsible for utilization of H_2 as an energy source, HydA is an [Fe-S] α-subunit of hydrogenase, HydB is large [Fe-Ni] β-subunit while HydC constitutes γ-subunit (membrane anchored cytochrome b). The enzyme has not been purified in the recombinant form so far	ND	ND	[7, 79]
HypA (HP0869) 13 kDa, pI 5.0	Chaperone: delivers Ni^{2+} to hydrogenase *via* interaction with HypB and to urease *via* interaction with UreE; also binds zinc (1 Zn^{2+}/dimer) which is involved in nickel and pH sensing	2 Ni^{2+}/dimer at pH 7.3 1 Ni^{2+}/dimer at pH 6.3	K_d 0.13-5.8 × 10^{-5} M	[13, 40, 42, 80]
HypB (HP0900) 27.2 kDa, pI 5.3	Chaperone: delivers Ni^{2+} to hydrogenase/nickel stimulates HypB dimerisation, HypB binds GDP/GTP which modulates nickel binding; GTP hydrolysis stimulates nickel delivery to hydrogenase; HypB binds also zinc (1 Zn^{2+}/monomer), which inhibits GTP hydrolysis; HypB interacts with HypA and SlyD	2 Ni^{2+}/ $HypB_{apo}$ dimer 1 Ni^{2+}/ $HypB_{GTP}$ dimer 0 -1^/$HypB_{GDP}$ monomer	K_d $HypB_{apo}$ 0.32× 10^{-6} M K_d $HypB_{GTP}$ 0.11× 10^{-6} M K_d $HypB_{GDP}$ 0.7× 10^{-6} M	[45, 46, 81]
SlyD (HP1123) 19.8 kDa, pI 4.7	Chaperone: delivers nickel to hydrogenase *via* interaction with HypB; binds zinc; stimulates GTPase activity of HypB facilitating nickel delivery to hydrogenase	2 Ni^{2+}/monomer	K_d 2.74 × 10^{-6} M	[46, 45, 81]
Storage proteins				
Hpn (HP1427) 6.9 kDa, pI 6.3	Stores nickel- histidine rich (28 out of the total 60 aa,); releases nickel upon acidic pH and EDTA	100 Ni^{2+}/20-mer	K_d 7.1 × 10^{-6} M	[50, 55]
Hpn-like (HP1432) 8.6 kDa, pI 6.7	Stores nickel- histidine and glutamine rich (18 and 30 out of the total 72 aa, respectively); releases nickel upon acidic pH and EDTA	44 Ni^{2+}/22-mer	K_d 3.8 × 10^{-6} M	[51, 53]
HspA (HP011) 12.6 kDa, pI 6.1	Stores nickel- characterized by a unique histidine- and cysteine-rich domain at the C terminus; releases nickel upon acidic pH and EDTA	14 Ni^{2+}/heptamer	K_d 1.1 × 10^{-6} M	[52, 54]

*There is still a debate for the number and the mode of coordinated nickel ions, which may be the result of different nickel purification and crystallization conditions (for a discussion see [60]). The nickel binding to *Hp*NikR is strongly dependent on pH and thus may affect the measured dissociation constant; ^number of Ni^{2+} ions changes upon UreG and HypB cycling. HypBapo/GTP and UreGapo/GTP refer to GTP binding. Kd, dissociation constant; V_{max}, the maximum reaction rate; Km, Michaelis-Menten constant (substrate concentration at which the reaction rate is half of V_{max})

Cite this: DOI: 10.1039/c0xx00000x

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As has been presented, nickel trafficking pathways are crucial for *H. pylori* ability to colonize human stomach. In addition, few of the nickel-binding proteins have also been suggested to directly contribute to gastric pathogenicity in *H. pylori*-associated diseases. For example, urease was shown to be involved in bacterial adhesion to gastric epithelial cells, disruption of cellular junctions leading to loss of the integrity in the epithelium and affecting the signaling pathway in eukaryotic cells leading to cell apoptosis [61–66]; the SlyD protein was proved to promote proliferation and transformation of AGS cells, to improve invasion and to resist apoptosis *in vitro* [67]. Taking into account the pivotal role of nickel-binding proteins for *H. pylori* survival and pathogenicity, it is rational to consider them as perfect targets for development of drugs inhibiting *H. pylori* ability to colonize the human stomach, especially that there are no known nickel enzymes in humans. However, a deeper understanding of nickel homeostasis and the mechanisms of nickel-dependent activation of urease and hydrogenase is still needed. It is especially important to understand the binding mode and thermodynamic stability of nickel coordination to each of *H. pylori* nickel chaperones, together with the role of binding and the way of discriminating other metal ions by nickel-binding proteins (e.g. zinc to HypA and HypB). Such ions might play regulatory role of nickel binding-proteins, but might also inhibit the proteins by blocking the metal-binding site thus disturb nickel trafficking in *H. pylori* cells. The example of such competitive interactions is the binding of bismuth ions to urease, HspA, HspB, which probably affects the delivery of Ni^{2+} and/or activity of the two major enzymes, urease and hydrogenase [68, 69].

In the next chapters, we will focus on the specificity of nickel binding to its various coordination sites in bacterial Ni^{2+} homeostasis proteins. Except for being a fascinating issue from the point of view of bioinorganic chemistry, further studies on this topic might actually lead to a real progress in the eradication of nickel-dependent pathogens. But, before we discuss that point, we briefly focus on something more basic:

Nickel's bioinorganic preferences

The coordination chemistry of nickel is rather well understood; complexes of the divalent d^8 Ni^{2+} are usually four-coordinated, square planar and diamagnetic; tetrahedral, paramagnetic Ni^{2+} complexes are less common. Five-coordinated Ni^{2+} species can have either trigonal-bipyramidal, square pyramidal or distorted geometries and be either high or low spin. Six-coordinated Ni^{2+} complexes are octahedral and have two unpaired electrons.

What makes the biological inorganic chemistry of this element special, is the fact that, like copper, it is able to induce deprotonation of protein amide functions at neutral or basic pH [82]. Simple nickel – oligoglycine complexes are good examples of a Ni^{2+} -peptide complexes. The peptide GG forms octahedral, paramagnetic Ni^{2+} complexes with a $[NiH_{-2}L_2]$ stoichiometry. The metal binding sites are the terminal amino nitrogen, the deprotonated amide nitrogen and the carboxylate oxygen groups [83,84]. When more amide nitrogens are available, e.g. in GGG or longer peptides, Ni^{2+} complexes are diamagnetic and square planar at basic pH [85]. The deprotonation of the two or three amide functions is highly cooperative. At basic pH, uncoordinated nickel precipitates in the form of nickel hydroxide.

For mammals, nickel is toxic and causes mutagenic effects after binding to DNA [86,87]. However, for several hundreds of organisms, mostly bacterial and fungal, this element is one of the crucial factors necessary for survival. Ni^{2+} is essential for the proper functioning of nine nickel-dependent enzymes: urease, hydrogenase, carbon monoxide dehydrogenases, methyl coenzyme M reductase, acetyl-coenzyme A decarbonylase/ synthase, superoxide dismutases and glyoxylases from lower organisms, aci-reductone dioxygenase and methylenediurease [88]. To those proteins, Ni^{2+} can coordinate *via* a variety of donors; the most tempting coordination sites for nickel are cysteine sulfurs and nitrogens from histidine imidazoles, amide and terminal amine groups [89]. Trying to understand or even predict the geometries and thermodynamic stabilities of such complexes is quite a challenging task for bioinorganic chemists. Often, such studies are performed not on large protein assemblies, but, provided that the metal binding site does not have a particular, clearly defined structure and all of its side chains are available for metal binding, the work is carried out on protein fragments. This kind of approach both facilitates the study and makes the thermodynamic results more exact and easier to compare to similar data. Such studies can be performed by potentiometry, calorimetry and several spectroscopic techniques in order to understand the thermodynamics of the formed complexes in detail (for a discussion on this approach, see [90]).

In the next chapter, we discuss the coordination of nickel to parts of proteins that originate from *H. pylori* and later give an overview of phenomena that still need to be understood, discussing various possible coordination modes and showing probable sequences from various pathogens that are, from the chemical point of view, likely to coordinate Ni^{2+} ions.

Tempting binding sites in *Helicobacter pylori*

Histidine-rich sequences are potential binding sites for several so-called "intermediate" metal ions, according to the Person's classification. Their imidazoles, with a pK value of about 6.5 are partially deprotonated at physiological pH - they exert a buffer action and are available for metal ion binding, willing to donate a nitrogen lone electron pair to a nickel ion.

Sequences with consecutive histidines have been chosen not only by nature, by also by molecular biologists as effective metal binders and are commercially used in immobilized metal affinity chromatography (IMAC). The $(His)_6$ tag serves as a molecular

'anchor' that bind to a metal ion (usually nickel), chelated by a nitrilotriacetic acid (NTA) bound to a solid support. In nature, polyhistidine sequences are found in chaperones of urease and hydrogenase utilizing species (on which we focus in this chapter), but also in Zn^{2+} transporters, prion proteins, in histidine-rich glycoproteins, which posses a massive amount of functions, in some snake venoms and antimicrobial peptides (for a recent review, please refer to [91] and references therein).

Below, several examples of well-studied histidine-rich nickel chaperones and regulators are discussed.

Bacterial His-rich sequences

The most striking example of a His-rich protein from *H. pylori* is definitely **Hpn** – 47% out of its 60 amino acids are histidine residues (Tab. 2). This small protein is involved in the storage and detoxification of nickel ions and it accounts for 2% of all proteins synthesized in the cell [92]. Hpn is multimeric, with 20-mers being the predominant species. At pH 7.4, it binds five Ni^{2+} ions per monomer with a K_d = 7.1 μM. The binding of Ni^{2+} induces conformational changes in the protein, which was monitored both by CD-spectroscopy [93] and FRET (fluorescence resonance energy transfer) [94].

In vivo protection of E. coli cells grown in metal ion supplemented media by recombinantly expressed Hpn (as deduced from bacterial growths rates) decreases in the order: Ni^{2+}>Bi^{3+}>Cu^{2+}≈Zn^{2+} [95,96].

Hpn is rich in potential nickel binding sites; it has an albumin-like N-terminal sequence (MAH), several His-rich motifs (HHH) and two CC motifs [97]. The affinities of those typical sites were recently compared [91], showing that the CC sequence from the C-terminal part of Hpn forms the most stable complexes with nickel ions. Ni^{2+} binds to two cysteine sulfurs and an amide nitrogen between the two residues, forming thermodynamically stable five membered chelate rings (a {$2S^-$,N^-} binding mode). Nickel complexes of both MAH and HHH are less stable than the Ni-CC one. The albumin-like Ni-MAH binding (a {NH_2, $2N^-$, N_{im}} donor set) is thermodynamically stronger than the Ni-HHH one (imidazoles-bound only) above pH 6.4; below this pH, the contrary is observed. The mentioned binding modes are depicted on Fig. 2.

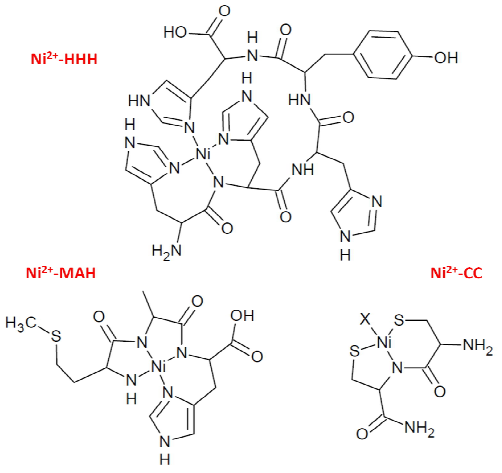

Figure 2. Possible Ni^{2+} binding modes in Hpn.

Another histidine-rich protein, which was found in *H. pylori*, **Hpn-like** (Hpnl or Hpn2), has a similar sequence to Hpn, and possesses two additional glutamine-rich motifs (25% of its sequence are histidines, 42% are glutamines) [98,99]. Hpn-like is a 22-mer in its native state, binding two nickel ions per monomer with a K_d of 3.8 μM [100].

Recently, the formation of Ni^{2+} complexes with N-terminal domains of Hpn and Hpnl [101] were studied, confirming that the N-terminal albumin-like sequence (MAH-) is a very efficient coordination site for both metals. Although the coordination geometry and the donor-atom set is the same for the two peptides corresponding to the N-terminal domains of Hpn and Hpnl ({NH_2, N^-, N_{im}} [102]), Hpnl binds nickel with higher affinity than Hpn, which is in agreement with the K_d values measured for the whole proteins. The explanation of this difference lies in the subsequent poly-glutamine amino acid sequence; details of the thermodynamics of such interactions were revealed in another study, where Ni^{2+} complexes of Hpn and Hpn-like model peptides (MAHHE-NH_2, MAHHEEQ-NH_2, MAHHEEQHG-NH_2, MAHHEQQ-NH_2, MAHHEQQHQA–NH_2 and MAHHEQQQQQQA-NH_2) were studied by experimental and molecular modelling techniques [101]. This study shows that the complex stabilities distinctly increase with the number of glutamine residues present in the peptide, although glutamine side-chains do not directly take part in coordination; they form a set of hydrogen bonds around the metal-binding site, disfavoring the binding of water molecules to the central nickel ion. The number of intramolecular hydrogen bonds directly depends on the number of Q side chains.

Zeng et al. suggest that histidine residues in the N terminus are not involved in metal binding and that both of the Ni^{2+}-binding sites are localized in the His-rich domain of Hpnl [103]. In an alanine-substitution study, His-29 and His-31 were proved to be crucial residues for the Ni^{2+} binding. The same work showed that Hpn-like also binds Cu^{2+}, Co^{2+} and Zn^{2+} with moderate affinities, but have a higher affinity towards nickel ions *in vivo*.

Sequences of consecutive histidines are both fascinating and challenging to study. We recently tried to understand the coordination of nickel to a polyhistidyl fragment of Hpn (Ac-THHHHYHGG-NH_2). To show, which His is involved in binding, we studied six analogues of this wild-type sequence, in which consecutive residues (His or Tyr) were replaced by Ala. The fourth His residue is critical for Ni^{2+} binding and the stability of coordination varies even if the substituted amino acid does not directly bind. Moreover, nickel did not bind to four consecutive histidines [104].

Recently, synthetic Hpn was shown to completely inhibit the urease activity of *H. pylori* in liquid cultures, indicating that nickel uptake was effectively blocked by Hpn [105]. Thus, Hpn can be considered interesting not only because of unique coordination chemistry or biological significance, but also because of a potential therapeutic use.

In the UniProt database, there are over 250 proteins, which share more than 40% homology with Hpn, and more than half of them (135) are bacterial proteins. Most of them are not characterized, but the ones that are can functionally be divided

into: i) accessory proteins required for the maturation of the nickel active site in enzymes, ii) metal cation membrane transporters and iii) GTP-ases involved in cobalamin synthesis.

An example of a His-rich bacterial protein, which coordination chemistry is not fully understood is **HypB**, a GTPase important for hydrogenase maturation. It is rich in histidines in most bacteria, however not in *H. pylori* and *E. coli* (in both cases they are required for hydrogenase maturation and supported by HypA in *H. pylori* [106] and by the histidine-rich **SlyD** in *E. coli* [107]). The latter protein has an unstructured, His-rich C-terminus and is an activator of nickel release from *E. coli* HypB [108]. Coordination abilities of two histidine-rich peptides from the C-terminus of SlyD (Ac-GHGHDHGHEHG-NH_2 and Ac-AHGHVHGAHDHHHD-NH_2) showed that only imidazole side chains of histidine residues are involved in metal binding at physiological pH [109]. The latter peptide is able to bind 2 metal ions at pH range 5 to 7. At higher pH, one of the metal ions is released and the other one binds to subsequent amide nitrogens. This might explain the mechanism in which nickel ions are forwarded to other proteins when pH changes.

Studies on a truncated SlyD in *E. coli* (His substituted with Ala in position 155) showed that SlyD155 could interact with HypB, but nickel release from HypB was slower than in the presence of full-length SlyD [110]. Moreover, SlyD155 was able to bind only one Ni^{2+} ion to at least two histidine ligands [111].

UreE (facilitates the insertion of nickel into urease) from *Klebsiella aerogenes* has a 15 amino acid long C terminus with 10 His residues. UreE binds up to six nickel ions per dimer with a K_d of 9.6±1.3 μM. Cells without the histidine-rich region in UreE showed only 73% of urease activity [112].

H. pylori UreE does not have a poly-His motif. Its dimer is able to bind only one Ni^{2+} ion, but if a $(His)_6$-tag is added to the C-terminus of *H. pylori's* UreE, it is possible to bind more nickel ions and increase urease activity [113].

RcnA, a resistance to cobalt and nickel protein and most probably both a nickel chelator and exporter [114,115] from *E. coli* has an extremely histidine-rich region, with 17 histidines in one cytoplasmic loop (Tab. 2) [116]; also RcnA from *Salmonella enterica* has a remarkable His- rich region - 26 His residues are present in a 50 amino acid fragment [117]. Table 2 shows endless examples of His-rich sequences from bacterial and fungal nickel chaperones and regulators, whose interactions with Ni^{2+} ions still need to be understood.

Other His-rich sequences from proteins and peptides, such as those from nuclear speckles (up to ten consecutive histidines in HOXA1), His-rich glycoproteins**,** His-rich antimicrobial peptides, His-rich malaria proteins and His-rich venoms have recently been reviewed [91].

Histidines – some useful statistics

Usually, the repeat of one single amino acid has a biological reason; proteins rich in cysteines often protect against heavy metals and oxidant damage [118]; Gln-rich proteins mediate protein–protein interactions and such repeats are often overexpressed in several neurodegenerative diseases [119]; Gly and Ala-rich stretches are considered as structural elements of proteins. His-rich proteins remain, to some extent, a puzzle. The average occurrence of His residues in proteins is relatively low (approximately 2.3% of all amino acids on average) [120] and thus makes His-rich sequences even more noticeable. It might also be interesting to keep in mind, that two amino acid residues are most likely to interrupt a His-rich sequence – aspartic acid and glycine, with their average contents being 14.5% and 9.8%, respectively, in a His-rich sequence. Aspartic acid most probably compensates for the positive charge of histidine residues and also provides an additional metal-binding site; glycines might increase the flexibility of His-rich domains. Quite often, also alanine, glutamic acid and serine interrupted His-rich motifs [121]

In the last few years, bacterial His-rich proteins have gained a reasonable amount of attention, it still remains unclear why nature chose to accumulate so many histidines in close sequential proximity. To get an overview how complicated this matter is, and what still needs to be understood, we present several His-rich sequences from Ni^{2+} homeostasis proteins and debate on their possible metal binding affinities.

To best of our knowledge, up to this date, no structures of His-rich motifs are deposited in the Protein Data Bank. Moreover, even if a partial structure of a protein with a His-rich sequence is available, the structure of the histidine rich motif is not solved. This is most probably due to the flexibility of His-rich sequences [122]. As an example, we show the structure of SlyD from *E. coli* (Fig.3) – the poly-His region, unlike the rest of the protein, remains unstructured.

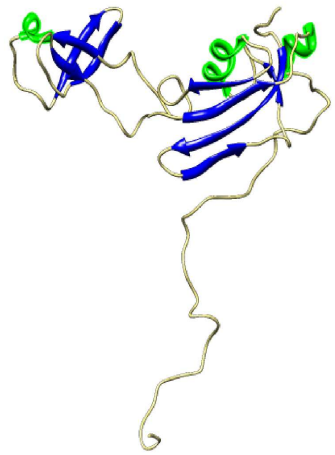

Figure 3. SlyD from *E. coli* (pdb 2KFW)– the C-terminal unstructured His-rich region is shown in white [123].

Recently, Cheng et al. analyzed the proteomes of 675 prokaryotes, looking for histidine-rich motifs and showed that most His-rich proteins are directly related to metal homeostasis, most commonly being transmembrane metal transporters, G3E family GTPase proteins, nickel accessory proteins or proteins involved in cobalamin biosynthesis [121]. Examples of proteins with the highest His content are COG0523s, CzcDs, HypBs, RcnAs and UreEs.

The record holder for the highest histidine content was found in a cation diffusion facilitator transporter from an opportunistic pathogen, *Acinetobacter baumannii* - it contained 25 histidines in a 30-residue motif and among it, 19 histidines were consecutive.

The longest stretch with consecutive histidines was found in the non-redundant database of NCBI: DDB_G0274557 (XP_644032) from a soil-living amoeba *Dictyostelium discoideum* AX4 has a fragment with 44 uninterrupted histidines [121].

Nickel-binding sites are eradication targets not only in the case of *H. pylori*; urease is a virulence factor in numerous pathogenic organisms.

Recently, the nickel-dependent urease complex from an opportunistic fungal pathogen *Cryptococcus neoformans* was named as the 'Achilles heel' of the pathogen [124]. *C. neoformans* utilizes urease as a virulence factor to invade the central nervous system (CNS) via the blood-brain barrier and cause life-threatening meningoencephalitis; as other urease containing organisms, it also requires a variety of accessory proteins for its activation. An Ala-substitution study showed that His's-rich regions of those proteins are crucial for urease activity [125].

Cun et. al. applied a bioinformatics approach to study His-rich clusters [120]; those do not necessarily involve His-rich sequences, but can also be formed by inconsecutive histidines in primary sequences, which form a local histidine-rich surrounding, ready to coordinate a metal ion. Such clusters were analyzed according to their metal preference and the participation of other coordinating groups. It was shown, that they are most tempting for zinc (51%), followed by nickel (19%), copper (18%), iron (7%), manganese (3%) and others (2%). Statistically, half of such binding sites are His-only ones (often together with a water molecule), 40% involve an acidic amino acid residue (Asp or Glu) and in 7%, a Cys is present. The latter binding mode ([3His, 1Cys]) is preferential for Ni^{2+}; an example of such is NikR, a nickel-responsive transcription factor which binds DNA in the presence of nickel [126]. The geometry of this site is square planar (PDB ID: 1Q5Y). Recently, the correlations between different types of sequence motifs and their structure, function and metal binding abilities are receiving an increasing amount of attention. As an example – a recent study showed, that the most common Ni-binding motifs in *Streptococcus pneumoniae* are H(X)nH, M(X)nH and H(X)nM [127]. Another histidine rich motif, HXHXXHXXHXH was found to occur in most membrane-bound hydrogenases in the purple sulphur photosynthetic bacterium *Thiocapsa roseopersicina* [128]. Other examples of intriguingly His-rich motifs are presented in Table 2.

Table 2. Potential Ni^{2+}-binding sequences from various bacterial and fungal species

species		protein (accession number)	potential Ni^{2+}-binding sequence
Helicobacter pylori	stomach ulcer causing bacteria	Hpn (P0A0V6)	MAHHEEQHGG HHHHHHHTHH HHYHGGEHHH HHHSSHHEEG CCSTSDSHHQ EEGCCHGHHE
Helicobacter pylori	stomach ulcer causing bacteria	Hpnl (A3RDS2)	MAHHEQQQQA QQQQQQQANS QHHHHHHAHH HHYYGGEHHH HNAEQHAEQQ AEQQAQQQQA HQQQQQKAQQ QNQQY
Cryptococcus neoformans	basidiomycete yeasts that cause infection of the central nervous system, lungs and skin of the hosts	UreG (J9VEN6)	TGTDSHHAHH HHTPSGASSA ISHTHDNMPH DHGQFHDHGP GLWTPEEHGH THEHLEHAGK
Dickeya dadantii	anaerobic plant pathogen	HypB (D2BS19)	HSHHHPHTHD HHHEHSHSHD HAHSHPHHHD HDHGHDHHHD DVAAAPSVII HHHHYYYHHG ZVHHHYHGTS RPLAVRHAHE HHAHTHDSHD HHDHDNHDHD NHDHHHEHAH HSHAHHGHDH HAHSHEHRQQ
Proteus	causes urinary	HypB	SHDHHHHSHD HDHHDHGHHH HGHHHHHGHH HGHDHHHEHN

mirabilis	tract infection	(B4F2X3)	IHHHHYHNSG DVHLHFYHDA HHHGHDDHHA HSHEHTHSHE HEHSHDHEHS HEHEEQ
Proteus mirabilis	causes urinary tract infection	Nickel/cobalt efflux system RcnA (K1HT30)	QENEHHHHDE GHRGHVHDY DVAFAEHDHD HDH
Bradyrhizobium japonicum	legume-root nodulating, microsymbiotic bacterium	HypB (Q45257)	HAHDHHHDHG HDHDHGHDGH HHHHHGHDQD HHHHHDHAH
Bradyrhizobium japonicum	legume-root nodulating, microsymbiotic bacterium	UreE (G7DIL8)	GGHHHHHDHH H
Vibrio halioticoli	non-motile alginolytic marine bacterium	HypB (V5F373)	QIEGHQHDHH SHSHDHNHDH AHPHDHNHGH SHNNDSGAQP VSVVHHHYHN SGDVHLHIYH APNAVGSEQI TPNSNASQPT HSHAHEHHNA PVHHSHEHHS HQNAEHHHHH EHEHEHSHEH TPHHAGHTLD
Pseudomonas aeruginosa	causes lungs, the urinary tract, and kidney infection, often leads to sepsis	RcnA (U9IGH7)	EEEDEHQHDH HHHDETHRID TGHGRIELSI PEPHEFT ARLSLGHAGH SHDYDLEFHE HDHGHDHSEL
Salmonella enterica	causes salmonellosis	RcnA (V1SUD0)	HHHDHDHDHD HDHDHDHDHD HDHDHDHDHD HDHDHDHDHD HDHHGHIHPE
Coccidioides immitis	fungal pathogen, colonizes the lungs causing San Joaquin Valley fever	UreG (J3KFJ5)	MATQHSHSHS HAHDAGSHGH THE
Mycobacterium tuberculosis	causes tuberculosis	transcriptional regulator NmtR (O69711)	DTHVAQLLDE AIYHSEHLHL
Klebsiella pneumoniae	can lead to pneumonia, urinary tract infections, septicemia, and soft tissue infection	NikR (B5XTJ9)	LVATQHHHHD LSVATLHVHI SHEDCLEIAV
Shigella sonnei	responsible for shigellosis (digestive track); often resistant to antibiotics	RcnA (Q3Z0A4)	HEYDYEHHHH DHEDHHDHGH HHHHEHGEYQ DAHARAH
Salmonella	causes typhoid fever,	RcnA (Q5PEQ4)	HHHDHDHDHD HDHDHHGHIH

paratyphi A	paratyphoid fever and food poisoning		
Eschrichia coli	usually found in the lower intestine; may cause food poisoning	RcnA (N1SJF0)	HGHDYEHHHH DHEHHHDHGH HHHHEH
Acinetobacter baumannii	an opportunistic bacteria in humans	AYE (W3L1H7)	HHHHHHHHHH HHHHHHHHHE HGHGHHHHDL

Cysteine rich sequences in *Helicobacter pylori* - an anchoring site for nickel ions

Bacterial nickel chaperones from *H. pylori* and other pathogens are also rich in cysteines - another amino acid residue, which is tempting for metal ions. Those sequences are not as 'spectacular' as the His-rich ones – the cysteine repeats are simply not as numerous as e.g. in metallothioneins (responsible for the sequestration of toxic heavy metal ions and homeostatic regulation of essential ones; about one third of the residues are cysteines, arranged in conserved CXC, CXXC and CXCC motifs [129]), CRISPs - cysteine-rich secretory proteins (glycoproteins, necessary for a variety of functions, ranging from the proper functioning of the reproductive system [130] to the inhibition of smooth muscle contraction) or chordins (which regulate dorsoventral patterning during gastrulation).

Nevertheless, in some of nickels chaperones, cysteines are more abundant than in others, and below, we discuss their most well known nickel binding motifs – CC, CXC and CXXC.

The **CC binding motif** is present in several chaperones, e.g. Hpn or HspA. The latter one (Heat Shock Protein A), in *H. pylori*, in addition to its usual function as a GroES homolog, also facilitates nickel acquisition by donating it to appropriate proteins in a Ni^{2+}-deficient environment and takes part in the detoxification through the sequestration of excess nickel. The additional function of this protein in *H. pylori* is reflected by an additional C-terminal domain, which is not present in other GroES-like proteins [131]. This 27 amino acid long C-terminal domain has 8 His and 4 Cys residues (GSCCHTGNHDHKHAKEHEACCHDHKKH) and is the protein's metal binding site [52]. However, a mutated HspA without this sequence is also able to chelate zinc and bismuth ions [132]. Studies of the coordination of nickel to a peptide sequence derived from the C terminus, Ac-ACCHDHKKH-NH_2, showed that the nickel complex is square planar, with Ni^{2+} being bound to two sulfurs of neighbouring cysteine residues and to the amide between them [133]. This type of nickel coordination is often found in Ni^{2+} complexes with peptides with a CC sequence, it is also the case for two fragments of Hpn, Ac-CCSTSDSHHQ-NH_2 and Ac-EEGCCHGHHE-NH_2 [134].

Ni^{2+} binds to **CXC type sequences** with a {2S-,2N-} coordination mode, with two cysteines and two amides between them being involved in the binding.

Examples of a **CXXC metal binding motif** can be found in HypA, in a loop region (ELECKDCSHVFKPNALDYGVCEKCHS). Those motifs usually bind zinc, which has a structural role in the protein, while nickel is anchored to the N-terminal MHE motif (His2, Glu3 and Asp40). It is worth noting that zinc ions may also displace nickel ions from their binding sites (provided that no amides are involved in nickel binding) [135].

Another metal ion, which is likely to displace nickel from its binding sites (in nickel chaperones or in urease itself) is bismuth. Bi^{3+} has a very high affinity towards thiolate ligands [136]. In bismuth- peptide complexes, the binding of this metal to cysteine sulfurs is extremely effective and starts to take place already at pH below 1 [137]. As explained in the first chapter, bismuth salts are a part of the quadruple therapy, which usually reaches a fairly good eradication rate [138]. Bismuth's impact on HspA is quite well understood; it induces changes in quaternary structure of the protein, making it refold from native heptamer to a dimer [52]. Thermodynamic studies of Ni^{2+} and Bi^{3+} with the C-terminal sequence, Ac-ACCHDHKKH-NH_2, revealed a surprising stability of the Bi^{3+}- peptide complex (several orders of magnitude stronger than the binding of nickel) and showed that Bi^{3+} is able to displace the Ni^{2+} ions from the complex [139], just like it is assumed to do in the native HspA protein in *H. pylori*.

Bi^{3+} also had an inhibitory effect on *H. pylori*'s HypB, binding to it with a dissociation constant of 0.94×10^{-17} μM, it causes its oligomerisation and loss of function [140].

Conclusions

A variety of bacteria and fungi, which rely on urease and hydrogenase for their survival and pathogenicity, could not exist without a balanced nickel distribution. In the last decade, a reasonable amount of progress was made on understanding the biology and chemistry of Ni^{2+} homeostasis in most common bacteria, elucidating the complicated regulation of uptake, distribution and storage of this metal. Histidine-rich sequences, significantly more abundant in bacterial nickel chaperones than in other proteins, seem to be the tempting sites for Ni^{2+} coordination.

Understanding thermodynamics of the coordination of nickel to its potential binding sites is a step forward towards finding the weakest point of Ni^{2+} homeostasis. Again, using idioms – if indeed nickel metabolism is the Achilles foot of urease

containing pathogens, then the disruption of proper nickel distribution could be Ariadne's thread for their eradication. In this work, we showed numerous examples of possible nickel binding sequences. Understanding their binding properties seems to be a chemically fascinating, but also challenging and time consuming task. Coming back to our idioms - Rome was not built in a day.

Acknowledgements

MRZ would like to acknowledge the support of a scholarship from the Foundation for Polish Science. AZP and JZC gratefully acknowledge financial support received from the WCB EIT+ (POIG 1.1. EIT+, Project 3.1. BioMed) (http://www.eitplus.pl/language/en/).

We wish to thank Kerstin Stingl for critical remarks concerning the manuscript.

Notes and references

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