Structure
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Promiscuous Nickel Import in Human Pathogens: Structure, Thermodynamics, and Evolution of Extracytoplasmic Nickel-Binding Proteins

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SUMMARY

In human pathogenic bacteria, nickel is required for the activation of two enzymes, urease and [NiFe]-hydrogenase, necessary for host infection. Acquisition of Ni(II) is mediated by either permeases or ABC-importers, the latter including a subclass that involves an extracytoplasmic nickel-binding protein, Ni-BP. This study reports on the structure of three Ni-BPs from a diversity of human pathogens and on the existence of three new nickel-binding motifs. These are different from that previously described for *Escherichia coli* Ni-BP NikA, known to bind nickel via a nickelophore, and indicate a variegated ligand selectivity for Ni-BPs. The structures are consistent with ligand affinities measured in solution by calorimetry and challenge the hypothesis of a general requirement of nickelophores for nickel uptake by canonical ABC importers. Phylogenetic analyses showed that Ni-BPs have different evolutionary origins and emerged independently from peptide-binding proteins, possibly explaining the promiscuous behavior of this class of Ni(II) carriers.

INTRODUCTION

The discovery of nickel in the active site of enzymes dates back to 1975, with the characterization of urease (Dixon et al., 1975). Since then, eight additional redox and nonredox nickel enzymes have been identified (Boer et al., 2014), (Maroney and Ciurli, 2014). Among them, a novel type of nickel-dependent enzyme, lactate racemase, has been recently reported (Desguin et al., 2014). Several important human pathogens (Corbel and Hendry, 1985; De Koning-Ward and Robins-Browne, 1995; Ha et al., 2001; Jose et al., 1991; Lam and Yeo, 1980; Olson and Maier, 2002) express urease and/or [NiFe] hydrogenase, which are often essential for in vivo colonization of the host organism. In any case, the challenge for these bacterial pathogens is to provide enough soluble Ni(II) to these enzymes, especially considering the low availability of Ni(II) in the human body (≈0.5 nM) (Zambelli and Ciurli, 2014).

In bacteria, Ni(II) is transported through the cytoplasmic membrane by two different high-affinity uptake systems: one type is represented by secondary Ni/Co transporters (Eitinger et al., 2005; Zhang et al., 2009), while the other type is represented by ATP-binding cassette (ABC)-type transporters. The latter are generally divided into two subclasses: the well-represented class of energy-coupling factor (ECF) transporters (Rodionov et al., 2006) and the canonical ABC-type importers. ECF transporters are identified as an unusual type of ABC-type transporters in prokaryotes and display extremely high affinities for Ni(II) (in the picomolar to nanomolar range). They are composed of ABC ATPase subunits (A components), a conserved transmembrane protein (T component), and a transmembrane substrate-capture protein (S component) (Eitinger et al., 2011). Canonical ABC-type importers, with affinity in the submicromolar for Ni(II), are made of five components: two channel-forming transmembrane proteins, two nucleotide-binding proteins, and an extracytoplasmic solute-binding protein (SBP) (Cui and Davidson, 2011), the latter being absent in ECF-type transporters. In Gram-negative bacteria, the SBP is located in the periplasm, while in Gram-positive bacteria, it is anchored to the membrane (Wu, 1996). SBPs are the major determinants of the transporter specificity and constitute a huge superfamily capable of importing very diverse ligands (Cui and Davidson, 2011) through a conserved "Venus Flytrap" mechanism (Mao et al., 1982). The reported Ni(II) ABC-type importers belong to the same family as the peptide ABC importers, constituting the peptide/opine/nickel uptake transporter (PepT) family within the transporter classification system (Saier

et al., 2006). Recently, Berntsson et al. have proposed a classification of SBPs in six different clusters (A–F), based on structural features (Berntsson et al., 2010). Among these, nickel-binding proteins (Ni-BPs) belong to cluster C, which comprises proteins with a variety of different ligand specificities (di- and oligopeptides, cellobiose, arginine, and nickel), in agreement with a previous classification based on sequence alignments (Tam and Saier, 1993). In this cluster, SBPs are larger than other ABC-type transporter receptors (from 55 to 70 kDa) because of the presence of an extradomain, supposed to be required to accommodate large ligands such as oligopeptides. Attempts failed to divide cluster C into subclasses according to substrate specificity. Indeed, (1) Ni-BPs do not contain any standard Ni-binding motif, (2) most of the operons encoding the Ni(II) ABC importers are difficult to identify because they are not systematically adjacent to Ni-enzyme gene clusters on genomes, and (3) little functional information is available because only few ABC-type importers have been experimentally shown to import Ni(II); namely, NikABCDE from *E. coli* (Navarro et al., 1993), *Brucella suis* (Jubier-Maurin et al., 2001), and *Vibrio parahaemolyticus* (Park et al., 2000); NikABDE from *Helicobacter hepaticus* (Benoit et al., 2013); NikZYXWV from *Campylobacter jejuni* (Howlett et al., 2012); NikBCDE/NikA (Hiron et al., 2010) and CntABCDE (Remy et al., 2013) from *Staphylococcus aureus*; and YntABCDE from *Yersiniae* (Sebbane et al., 2002). In addition, a Ni(II)/Co(II) ABC-type importer, CeuE, has been described in *Helicobacter* (Stoof et al., 2010). Recently, structural studies showed that CeuE from *H. pylori* can bind a $Ni(L\text{-}His)_2$ complex, like *Ec*NikA (Shaik et al., 2014). However, CeuE does not belong to cluster C, its in vivo function has not been demonstrated, and its homolog in *C. jejuni* has been described as a siderophore importer (Raines et al., 2013).

The only Ni-BP belonging to cluster C that has been structurally characterized so far is NikA from *E. coli* (*Ec*NikA) (Heddle et al., 2003). Like other SBPs, *Ec*NikA is composed of two different lobes connected by a hinge that closes on ligand binding, and its overall structure resembles that of the oligopeptide-binding protein OppA (Tame et al., 1995) and the dipeptide-binding protein DppA (Nickitenko et al., 1995). Several *Ec*NikA crystal structures have been solved, providing valuable information on the nickel-binding mode (Heddle et al., 2003; Cherrier et al., 2005, 2008, 2012). These structures suggested that the protein is not able to bind free Ni(II) because the only direct contact between the protein and the metal ion is formed via a single histidine residue (His416) (Cavazza et al., 2011), implying that a metal-chelating ligand is required to complete the coordination environment of the metal ion (Cherrier et al., 2008). *Ec*NikA is able to bind nonphysiological complexes made of metal ions and carboxylated ligands in vitro, such as Fe(III)-EDTA (Cherrier et al., 2005), suggesting that the natural nickel chelator, designated "nickelophore," contains carboxylate groups. So far, two models of nickelophores have been proposed that correspond to either a tricarboxylated molecule (Cherrier et al., 2008) or to two free histidines (Chivers et al., 2012; Lebrette et al., 2013), but their physiological relevance has not been yet established. Recently, Howlett et al. showed that NikZ from *C. jejuni* is able to bind free Ni(II) in solution but cannot bind a Ni-EDTA complex (Howlett et al., 2012). The question then arose about the nickel-binding modes in other Ni-BPs and the putative general requirement of nickelophores in bacteria. This prompted us to investigate several Ni-BPs from diverse bacteria in order to get information about the mechanisms possibly developed to bind nickel.

In this paper, the structural and biochemical studies of NikA from *B. suis* (*Bs*NikA), YntA from *Yersiniae pestis* (*Yp*YntA), and NikZ from *C. jejuni* (*Cj*NikZ) are described with the aim to analyze and discuss their nickel-binding modes. This study revealed the structural details of the protein framework involved in nickel coordination. Together with calorimetric studies in solution that provided the thermodynamics of ligand binding and with phylogenetic analyses that indicated the evolutionary relationships among Ni-BPs, we identified a diversity of nickel-binding strategies adopted to uptake Ni(II), essential for the survival of human bacterial pathogens.

RESULTS

Crystal Structures of Ni-BPs

*Bs*NikA, *Yp*YntA, and *Cj*NikZ were crystallized or cocrystallized with either $NiCl_2$ or previously described ligands for *Ec*NikA; namely, a mixture of $NiCl_2$ and L-histidine or Fe(III)-EDTA. Crystal structures of the apo-forms of *Bs*NikA, *Yp*YntA, and *Cj*NikZ, corresponding to an open unliganded form, were obtained. The structures of *Bs*NikA in complex with Fe(III)-EDTA, the structures of *Yp*YntA and *Cj*NikZ in complex with Ni(II) + L-histidine, and the structure of *Cj*NikZ in complex with Ni(II) were also determined. Crystallographic statistics are summarized in Table 1.

As expected from the high similarity of their amino acid sequence (Figure 1A), the structures of apo-*Bs*NikA alone and in complex with Fe(III)-EDTA are similar to the previously determined structures of *Ec*NikA (Figure S1 available online) (Heddle et al., 2003; Cherrier et al., 2005). The corresponding global root-mean-square-deviation (rmsd) values are 2.3 Å for apo-forms (this value is higher than expected because of the different opening degrees between the two lobes in the two proteins) and 1.19 Å for Fe(III)-EDTA-bound forms. The metal-binding sites in *Bs*NikA and *Ec*NikA are essentially identical, although Arg95 (Arg97 in *Ec*NikA) is not involved in the binding of Fe(III)-EDTA (Figure S1). On the other hand, the structure of apo-*Yp*YntA revealed the presence of an extra loop between residue 237 and residue 249, not observed in any other structure of Ni-BPs. A solvent-exposed histidine is present at the edge of this loop, but it is far away from the putative nickel-binding site, thus rendering its physiological role unclear.

The structures of *Bs*NikA and *Yp*YntA cocrystallized with $NiCl_2$ corresponded to the apo-proteins with an open conformation of the binding pocket (Figures S1 and S2), suggesting that neither of them was able to bind free Ni(II). On the other hand, the structure of *Cj*NikZ obtained in the presence of $NiCl_2$ revealed a closed conformation of the binding pocket (Figure S2) and featured a site with electron density corresponding to a Ni(II) ion, while the structure of apo-*Cj*NikZ was obtained when the protein was cocrystallized with Fe(III)-EDTA. This is in agreement with recently published data indicating that *Cj*NikZ is able to bind free Ni(II) ion in solution but cannot bind a Ni-EDTA complex (Howlett et al., 2012).

In all cases, the overall structures featured an α/β fold, common to peptide-binding proteins (peptide-BPs), and the conserved extradomain, a signature of SBPs belonging to cluster