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Backbone ^1H , ^{13}C and ^{15}N resonance assignments of the 39 kDa staphylococcal hemoglobin receptor IsdH

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Abstract

During infections *Staphylococcus aureus* preferentially uses heme as an iron source, which it captures from human hemoglobin using the Iron regulated surface determinant (Isd) system. On the cell surface two related staphylococcal surface receptors called IsdH and IsdB bind to hemoglobin and extract its heme. Both receptors contain multiple NEAr iron Transporter (NEAT) domains that either bind to hemoglobin, or to heme. All previous structural studies have investigated individual NEAT domains and have not explored how the domains might interact with one another to synergistically extract heme from hemoglobin. Here, we report the near complete ^1H , ^{13}C and ^{15}N backbone resonance assignments of a bi-domain unit from IsdH that contains the N2 and N3 NEAT domains, which bind to hemoglobin and heme, respectively (IsdH^{N2N3}, residues 326–660, 39 kDa). The assigned backbone resonances lay the foundation for future NMR studies that will explore the molecular basis of IsdH function.

Keywords

Staphylococcus aureus; Heme acquisition; Iron uptake; NEAT domain; Secondary structure; NMR resonance assignments

Biological context

Staphylococcus aureus is a leading cause of life-threatening infections in the industrialized world. To successfully establish an infection it needs to acquire the essential nutrient iron from its human host. The Iron regulated surface determinant (Isd) system allows *S. aureus* to actively harvest heme iron from human hemoglobin (Hb) (Maresso and Schneewind 2006). The Isd system consists of nine proteins that work together to harvest heme from Hb. They capture Hb and extract its heme on the cell surface (performed by IsdH and IsdB), transport the heme molecule across the cell wall (IsdA and IsdC), import it into the cytoplasm (IsdD, IsdE and IsdF), and then degrade the heme to release free iron (IsdG and IsdI). As several Isd proteins have been shown to be required for staphylococcal virulence, they are prime targets for the development of new anti-bacterial agents.

Heme and protein capture is mediated by the IsdH, IsdB, IsdA and IsdC proteins which contain NEAr iron Transporter (NEAT) domains (Andrade et al. 2002). The hemebinding proteins IsdA and IsdC contain a single NEAT domain, while the IsdB and IsdH proteins

contain two and three copies of the module, respectively. In IsdH we have recently shown that the domains can have distinct functions, they either bind heme (the N3 domain) or they bind to Hb (the N1 and N2 domains) (Dryla et al. 2003; Pilpa et al. 2009).

The atomic structures of the NEAT domains from IsdA and IsdC as well as isolated NEAT domains from IsdH have been determined by NMR spectroscopy and X-ray crystallography (Grigg et al. 2007; Pilpa et al. 2006; Sharp et al. 2007; Villareal et al. 2008; Watanabe et al. 2008). This work revealed that NEAT domains adopt a related eight-stranded immunoglobulin-like β -sandwich fold. In addition, structures of IsdA, IsdC and IsdH hemoprotein complexes have been determined, as well as structures of the IsdH N1 domain bound to Hb and a transient transfer complex of IsdA and IsdC (Kumar et al. 2011; Villareal et al. 2011). Although this work has revealed how individual NEAT domains bind to their respective ligands, whether the domains function cooperatively within the multi-domain IsdH and IsdB receptors remains unknown. To better understand the mechanism of heme capture by IsdH and to elucidate how NEAT domains within this protein work together to extract heme from Hb, we used NMR spectroscopy to assign the backbone resonances of IsdH^{N2N3}, a 39 kDa bi-domain unit from IsdH that contains NEAT domains N2 and N3, which bind to Hb and heme, respectively. Our data indicates that the two domains form a structured unit and lays the foundation for determining the atomic structure of the receptor.

Methods and experiments

Expression and purification of IsdH^{N2N3}

Plasmid pRM216 was generated that encodes the small ubiquitin-like modifier (SUMO)-tagged IsdH^{N2N3} protein (IsdH residues 326–660 including the N2 and N3 domains with a Y642A mutation to disrupt heme binding) under control of an inducible promoter. [²H, ¹³C, ¹⁵N]IsdH^{N2N3} was expressed in *Escherichia coli* BL21(DE3) cells (New England BioLabs) adapted to ²H₂O based M9 minimal medium containing 2.0 g/L D-[¹³C]glucose, 1.0 g/L ¹⁵NH₄Cl and BME vitamin solution (Sigma-Aldrich). Protein expression was induced with 1 mM IPTG for 20 h at 25°C. The cells were harvested and protein released by sonication. After centrifugation, the supernatant containing the SUMO-tagged protein was initially purified using a Co²⁺ chelating column (Thermo Scientific). The SUMO-tag was cleaved by Ulp1 protease leaving only one additional Ser residue at the N-terminus (Fig. 1a). The protein was reappplied to a Co²⁺ chelating column to remove the cleaved affinity tag and further purified by size exclusion chromatography on a Superdex 75 column (GE Healthcare). To completely exchange backbone amides, IsdH^{N2N3} was incubated in 6 M guanidinium hydrochloride for 30 min at room temperature and refolded in vitro by rapid dilution into 0.5 mM arginine containing phosphate buffer at 4°C with a yield of refolded monomeric IsdH^{N2N3} of 88%. Final protein yield was 28 mg/L with an overall deuteration level of non-exchangeable hydrogens of ~81%.

NMR spectroscopy

NMR spectra were acquired at 37°C on back-exchanged triple labeled IsdH^{N2N3} samples (1.1 mM in 50 mM NaCl, 20 mM sodium phosphate buffer, pH 6.0, 0.01% NaN₃, 0.01% protease inhibitor cocktail (Roche), 8% ²H₂O (v/v) in a 5 mm Shigemi tube). NMR experiments were performed on cryoprobe-equipped Bruker Avance-600 or 800 MHz spectrometers. Sequence-specific backbone assignments of IsdH^{N2N3} were obtained from 2D and 3D TROSY-based versions of ¹⁵N-¹H HSQC, HNCACB, HN(CO)CACB, HNCO and HN(CA)CO experiments. A 3D ¹⁵N-¹H NOESY-HSQC experiment with 120 ms mixing time was used to verify the sequential backbone assignments. ¹⁵N-¹H 2D TROSY-HSQC spectra were recorded for 3 h digitizing 256 points in the indirect ¹⁵N dimension. The triple resonance 3D experiments were measured for 12–48 h. Spectral data was processed

using NMRDraw and NMRPipe (Delaglio et al. 1995) and analyzed using Cara (Keller 2005).

Assignments and data deposition

Despite its size of 39 kDa, the NMR spectra of IsdH^{N2N3} are of high quality. The well dispersed ¹⁵N-¹H TROSY-HSQC spectrum of IsdH^{N2N3} with assigned residues is shown in Fig. 1b. The level of completeness for the assignment was 91% for backbone ¹H_N and ¹⁵N (292/320), 86% of ¹³C' (287/335), 90% of ¹³C_α (300/335) and 87% of ¹³C_β (292/335). All of the unassigned backbone amides are part of the N2 domain of IsdH^{N2N3}. Most of these unassigned amide resonances are located at the extreme N-terminus or are clustered around the hemoglobin binding site. As only two unassigned peaks are present in the HSQC spectrum, most of the unassigned residues are likely broadened as a result of intermediate exchange, which has been observed for residues in the binding site of the functionally homologous NEAT domain IsdH-N1 (Pilpa et al. 2006). The chemical shift assignments have been deposited at the BioMagRes-Bank (<http://www.bmrb.wisc.edu>) under accession number 17820.

To assess the secondary structure of IsdH^{N2N3}, deviations of C_α and C_β chemical shifts from random coil chemical shifts were determined (Fig. 2a). Further, backbone ϕ and ψ dihedral angles were determined using TALOS+ (Fig. 2b) (Shen et al. 2009). TALOS-predicted secondary structure elements of IsdH^{N2N3} are presented in Fig. 2c. Seven β strands are predicted for both NEAT domains of IsdH^{N2N3}. The secondary structure predictions for the N3 domain are in good agreement with the secondary structure elements present in the crystal structure of the isolated NEAT domain IsdH^{N3}, which contains eight β strands (Watanabe et al. 2008). The β sheet fold predicted for the N2 domain supports the assumption that it adopts a typical NEAT domain fold. The results further predict that the previously uncharacterized linker region between the two NEAT domains is structured.

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Fig. 1.

Resonance assignments of IsdH^{N2N3}. **a** Amino acid primary sequence of IsdH^{N2N3}.

b ¹⁵N–¹H 2D TROSY-HSQC NMR spectrum of [U–²H,¹³C,¹⁵N]IsdH^{N2N3} acquired at 800 MHz. An enlarged view of the most crowded region is shown in the *top-left corner*. The assigned backbone resonances are labeled with sequential numbers. Two tryptophan side chain signals are marked with “Wsc”. The position of peaks being too weak for observation at the current intensity level are marked with a “+”. Signals arising from unassigned arginine epsilon protons are marked with an *asterisk*

Fig. 2. Summary of secondary structure predictions of IsdH^{N2N3}. **a** Plot of the secondary chemical shifts. Chemical shift deviations with respect to corresponding random coil values are plotted versus amino acid residue number, after multiplication with a 1:2:1 weighing function for residues $i-1:i:i+1$. **b** Backbone dihedral angles calculated using TALOS+ (Shen et al. 2009). Phi (ϕ) and psi (ψ) angles are shown as *black* and *grey circles*, respectively. **c** Location of secondary structure elements of IsdH^{N2N3} identified by TALOS+. Predicted secondary structure elements are indicated using *arrows* for sheets and *rectangles* for helices. Secondary structures determined for the crystal structure of isolated IsdH^{N3} are also shown as a comparison (Watanabe et al. 2008)