Crystal structure of histone-like protein from *Streptococcus mutans* refined to 1.9 Å resolution

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Nucleoid-associated proteins (NAPs) in prokaryotes play an important architectural role in DNA bending, supercoiling and DNA compaction. In addition to architectural roles, some NAPs also play regulatory roles in DNA replication and repair, and act as global transcriptional regulators in many bacteria. Bacteria encode multiple NAPs and some of them are even essential for survival. *Streptococcus mutans*, a dental pathogen, encodes one such essential NAP called histone-like protein (HLP). Here, the three-dimensional structure of *S. mutans* HLP has been determined to 1.9 Å resolution. The HLP structure is a dimer and shares a high degree of similarity with other bacterial NAPs, including HU. Since HLPs are essential for the survival of pathogenic streptococci, this structure determination is potentially beneficial for future drug development against these pathogens.

1. Introduction

In bacteria, chromosomes are efficiently condensed by a group of small basic proteins called nucleoid-associated proteins (NAPs; for a recent review, see Dillon & Dorman, 2010). Since these proteins are functionally similar to eukaryotic histone proteins, they are often referred to as histone-like proteins (HLPs). In general, NAPs can be grouped into two categories: those that compact the DNA by wrapping or bending and those that bridge large segments of the DNA (Dillon & Dorman, 2010; Luijsterburg *et al.*, 2006). Although bacteria encode numerous NAPs, only a few of them are generally present in high quantities. For example, *Escherichia coli* encodes about 12 NAPs, but only five are present in high abundance (Azam & Ishihama, 1999). These abundant proteins include Dps, Fis, H-NS, HU and IHF. Because of their increased abundance and high DNA-binding properties, these NAPs are considered to be the most important players in chromosome organization and compaction (Luijsterburg *et al.*, 2006; Travers & Muskheishvili, 2005). However, among these proteins, HU is considered to be the most abundant and highly conserved in bacteria (Drlica & Rouviere-Yaniv, 1987).

HU is a small, basic and thermostable protein originally identified in *E. coli* as a factor that stimulates the expression of phage lambda genes (Rouviere-Yaniv & Kjeldgaard, 1979; Rouviere-Yaniv & Gros, 1975). Apart from in *E. coli* and its close relatives, HU exists as an 18 kDa homodimer encoded by a single gene. However, the *E. coli* forms of HU mainly exist as a heterodimer containing two homologous subunits, HU α and

HU β (encoded by two different genes), but occasionally form homodimers of each subunit depending on the growth stage (Claret & Rouviere-Yaniv, 1997). The *E. coli* HU heterodimer binds nonspecifically to double-stranded DNA and with higher affinity to structurally distorted DNA, which leads to significant DNA bending, DNA compaction and negative supercoiling (Pontiggia *et al.*, 1993; Kamashev & Rouviere-Yaniv, 2000; Bonnefoy *et al.*, 1994). *E. coli* HU proteins are not essential for viability; however, cells lacking one or the other of the HU-encoding genes show perturbed cell division and increased sensitivity to γ and UV irradiation (Huisman *et al.*, 1989; Boubrik & Rouviere-Yaniv, 1995). *E. coli* HU is also involved in DNA repair and recombination, and preferentially binds to DNA containing nicks, gaps and/or abasic sites (Kamashev & Rouviere-Yaniv, 2000). Furthermore, *E. coli* HU plays regulatory roles in replication as well as acting as a global regulator of gene transcription (Oberto *et al.*, 2009).

HU proteins from different species share a high degree of sequence similarity. For example, the HU protein from *Bacillus* spp. displays 52–57% sequence identity to the *E. coli* HU subunits (Swinger & Rice, 2004). Crystal structures of HU in the absence of DNA have been determined for *E. coli* homodimeric or heterodimeric HU, *Bacillus stearothermophilus* HU and *Thermotoga maritima* HU. Furthermore, a few co-crystal structures of HU bound to DNA have also been determined (White *et al.*, 1999; Christodoulou & Vorgias, 1998; Swinger *et al.*, 2003). Based on these studies, it appears that HU proteins adopt a conserved, compact core of intertwined monomers. Two helical segments from each monomeric subunit constitute an α -helical ‘body’ with two protruding β -ribbon ‘arms’, which extend to bind the DNA helix. These DNA-binding β -ribbons are largely disordered in the absence of DNA; however, they adopt a folded structure upon DNA binding. The helix packing within the body is influenced by the sequence of the proteins. A highly conserved proline residue at the tips of the DNA-binding β -ribbons mediates two sharp DNA kinks, and the DNA bend derives from the proline residues partially intercalating through the minor groove (Swinger *et al.*, 2003). The DNA-intercalating proline residue

Figure 1
Crystals of SmuHLP obtained from Wizard 1 condition A5.

Table 1

Crystallographic data for SmuHLP refined to 1.9 Å resolution.

Values in parentheses are for the highest resolution shell.

Data collection	
Unit-cell parameters (Å, °)	$a = 32.46, b = 76.33,$ $c = 36.93, \beta = 107.2$
Space group	$P2_1$
Resolution (Å)	38.17–1.90 (1.94–1.90)
Wavelength (Å)	1.0000
Temperature (K)	100
Observed reflections	45407
Unique reflections	13437
$\langle I/\sigma(I) \rangle$	13.8 (1.9)
Completeness (%)	98.8 (99.4)
Multiplicity	3.4 (3.4)
$R_{\text{merge}}^{\dagger}$ (%)	5.1 (71.4)
$R_{\text{meas}}^{\ddagger}$ (%)	6.1 (84.8)
$R_{\text{p.i.m.}}^{\S}$ (%)	3.3 (45.4)
$CC_{1/2}^{\parallel}$	0.999 (0.710)
Refinement	
Resolution (Å)	38.17–1.90
Reflections (working/test)	12752/664
$R_{\text{work}}/R_{\text{free}}^{\dagger\dagger}$ (%)	17.7/21.7
No. of atoms (protein/water)	1189/60
Model quality	
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	0.962
Average B factor (Å ²)	
All atoms	41.2
Chain A	40.7
Chain B	41.5
Water	43.8
Coordinate error, maximum likelihood (Å)	0.24
Ramachandran plot	
Most favored (%)	98.7
Additionally allowed (%)	1.3

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity measured for the i th reflection and $\langle I(hkl) \rangle$ is the average intensity of all reflections with indices hkl . $\ddagger R_{\text{meas}}$ is the redundancy-independent (multiplicity-weighted) R_{merge} (Evans, 2006, 2011). $\S R_{\text{p.i.m.}}$ is the precision-indicating (multiplicity-weighted) R_{merge} (Diederichs & Karplus, 1997; Weiss, 2001). $\parallel CC_{1/2}$ is the correlation coefficient of the mean intensities between two random half sets of data (Karplus & Diederichs, 2012; Evans, 2012). $\dagger\dagger R_{\text{work}} = \sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|$; R_{free} is calculated in an identical manner using a randomly selected 5% of reflections that were not included in the refinement.

is conserved in HU and other homologous proteins, and the replacement of this proline in IHF leads to significantly altered DNA binding (Lee *et al.*, 1992).

In pathogenic streptococci, HLP, an NAP protein closely related to HU, plays an important role in virulence. Surprisingly, streptococcal HLP has been shown to bind to heart and kidney basement membranes (Choi & Stinson, 1989; Bergey & Stinson, 1988; Winters *et al.*, 1993). Streptococcal HLP also appears to be secreted by unknown mechanisms and is highly immunogenic (Liu, Yumoto, Hirota *et al.*, 2008; Boleij *et al.*, 2009). In contrast to *E. coli* HU, streptococcal HLP is essential for survival (Bugrysheva *et al.*, 2011; Liu, Yumoto, Murakami *et al.*, 2008); therefore, it is a potential target for drug development. Streptococcal HLP bind to DNA nonspecifically, although certain streptococcal HLPs have a preference for AT-rich DNA (Liu, Yumoto, Murakami *et al.*, 2008; Biswas & Mohapatra, 2012). We and other researchers have also shown that streptococcal HLP also participates in gene transcription by binding to the target DNA (Liu, Yumoto, Murakami *et al.*, 2008; Biswas & Mohapatra, 2012).

In this communication, we report the crystal structure of apo HLP from *Streptococcus mutans*, a dental pathogen. Our data suggest that *S. mutans* HLP (SmuHLP) is also a homodimer with the characteristic α -helical 'body' and two protruding β -ribbon 'arms' that are highly disordered in the absence of DNA.

2. Materials and methods

2.1. Protein expression and purification

Plasmid pIB-B39 contains the *hlp* gene from *S. mutans*, which was cloned into NdeI and XhoI sites to produce C-terminally His-tagged HLP (Biswas & Mohapatra, 2012). The plasmid was transformed into *E. coli* BL21(DE3)/pLysS cells and the production of HLP-His was induced by the addition of isopropyl β -D-1-thiogalactopyranoside (IPTG). A sample of SmuHLP was purified on a nickel column followed by cation-exchange chromatography on a HiTrap SP column using buffer A (20 mM Tris-HCl pH 7.4, 100 mM NaCl) and elution buffer B (20 mM Tris-HCl pH 7.4, 1 M NaCl). Fractions were pooled and concentrated to 8.0 mg ml⁻¹ in 20 mM Tris-HCl pH 7.4, 100 mM NaCl, 2.5% glycerol for crystallization. The protein appeared to be greater than 95% pure as judged by SDS-PAGE analysis with Coomassie staining.

2.2. Crystallization and data collection

Crystallization screening was conducted in Compact Junior (Rigaku Reagents) sitting-drop vapor-diffusion plates at 20°C using equal volumes of crystallization solution and protein solution equilibrated against 75 μ l crystallization solution. Crystals that displayed a prismatic morphology (~50 μ m)

were observed after 1–2 d from condition A5 of the Wizard 1 screen (30% PEG 400, 0.1 M CAPS pH 10.5) as shown in Fig. 1. Samples were transferred to a fresh drop of crystallant, which also served as the cryoprotectant, and cooled in liquid nitrogen for data collection. Data were collected on beamline 17-ID at the Advanced Photon Source using a Pilatus 6M pixel-array detector (Dectris).

2.3. Structure solution and refinement

Intensities were integrated using *XDS* (Kabsch, 2010), and the Laue class was checked and the data were scaled using *AIMLESS* (Evans, 2011; Evans & Murshudov, 2013). The highest probability Laue class was $2/m$ with space group $P2_1$. Based on the Matthews coefficient (Matthews, 1968), the asymmetric unit contained two molecules ($V_M = 2.0 \text{ \AA}^3 \text{ Da}^{-1}$, 39.2% solvent content). Structure solution was conducted by molecular replacement with *Phaser* (McCoy *et al.*, 2007) using the DNA-binding protein HU from *B. anthracis* (PDB entry 3rhi; Center for Structural Genomics of Infectious Diseases, unpublished work) as the search model. Space groups $P2$ and $P2_1$ were both tested and the top solution was obtained in $P2_1$. Initial refinement with *PHENIX* (Adams *et al.*, 2010) converged at an R_{work} and R_{free} of 36 and 41%, respectively, and the model was improved using automated model building in *ARP/wARP* (Langer *et al.*, 2008). Subsequent refinement and manual model building were conducted with *PHENIX* and *Coot* (Emsley *et al.*, 2010), respectively. Disordered side-chain atoms were truncated to the point where electron density could be observed. TLS (Painter & Merritt, 2006; Winn *et al.*, 2001) parameters were incorporated in the later stages of refinement to model anisotropic atomic displacement parameters. Structure validation was conducted with

Figure 2

Overall three-dimensional structure of SmuHLP. (a) NCS dimer colored by subunit (subunit A, magenta; subunit B, cyan). The dashed lines indicate the disordered regions. (b) SmuHLP colored by secondary structure. The α -helices and β -sheet secondary-structure elements are indicated for subunit A.

MolProbity (Chen *et al.*, 2010) and figures were prepared with *CCP4mg* (McNicholas *et al.*, 2011). Relevant crystallographic data are provided in Table 1. Coordinates and structure factors have been deposited in the Worldwide Protein Data Bank (wwPDB) with accession code 5fbm.

3. Results and discussion

The structure of SmuHLP dimer spanning residues Ala2–Lys91 was traced in the electron-density maps for each subunit. Portions of the DNA-binding region between Lys60 and Glu69 (chain *A*) and between Lys60 and Glu68 (chain *B*) were disordered (Fig. 2*a*). SmuHLP adopts a fold consisting of an N-terminal helix–turn–helix motif (α 1– α 2) followed by three antiparallel β -sheets (β 1– β 2– β 3) and a short C-terminal helix (α 3), as depicted in Fig. 2(*b*), which is very similar to that

observed for other HU-type proteins. A search for homologous structures was conducted using the *DALI* server (Holm & Rosenström, 2010) and the top hits for similar apo structures consisted of the following: (i) the HU protein from *B. stearothermophilus* (PDB entry 1huu; White *et al.*, 1989), (ii) HU α 2 protein from *E. coli* (PDB entry 1mul; Ramstein *et al.*, 2003), (iii) DNA-binding protein HU from *B. anthracis* (PDB entry 3rhi; Center for Structural Genomics of Infectious Diseases, unpublished work) and (iv) HU from *Staphylococcus aureus* (PDB entry 4qjn; Kim *et al.*, 2014). The amino-acid and secondary-structure alignment results from the *DALI* search are shown in Fig. 3. Superposition of the dimeric forms of homologous structures on the SmuHLP dimer was conducted with *GESAMT* (Krissinel, 2012), which yielded root-mean-square (r.m.s.) deviations of 1.09 Å (156 residues, PDB entry 1huu), 0.87 Å (150 residues, PDB entry 1mul),

Figure 3
Comparison of SmuHLP with homologs found from a *DALI* search showing the amino-acid alignment and secondary-structure alignment. The numbering is relative to the HLP construct used for crystallization. Positively charged residues (Arg/Lys) located in the DNA-binding region are indicated by asterisks.

Figure 4
(*a*) Superposition of homologous HU-type proteins with SmuHLP (magenta). Homologs are colored as follows: PDB entry 1huu, blue; PDB entry 1mul, green; PDB entry 3rhi, cyan; PDB entry 4qjn, tan. (*b*) Superposition of the lowest frequency normal-mode conformations of HLP computed using *eINemo*. DQ = –100 to 100 in steps of 20. The asterisk indicates the conformation that corresponds to the HLP crystal structure.

1.44 Å (152 residues, PDB entry 3rhi) and 1.36 Å (159 residues, PDB entry 4qjn). Overall the structures are quite similar apart from the DNA-binding region, as shown in Fig. 4(a).

Not surprisingly, the major differences were observed in the DNA-binding region, as each of these structures were determined in their apo forms and are flexible in the absence of DNA. This was investigated further by normal-mode analysis (NMA) using the *elNémo* server (Suhre & Sanejouand, 2004). As depicted in Fig. 4(b), the lowest frequency normal mode of SmuHLP shows the largest motion in the DNA-binding region. The largest normalized mean-square displacement (R^2) for the C^α atoms was observed at Glu69 (0.1319) and Glu68 (0.1575) for subunits *A* and *B*, respectively.

Examination of the DNA-binding region of HLP revealed that this area is primarily composed of positively charged residues that form a pocket to accommodate DNA binding (Fig. 5a). The specific residues that comprise the DNA-binding pocket are depicted in Fig. 5(b). It should be noted that the side-chain electron density for Arg56, Lys60, Lys71 and Lys73 of subunit *A* and Arg54, Lys76 and Lys81 of subunit *B* was

partially disordered and could not be traced in the electron-density maps. Therefore, the side chains were modeled in idealized rotamer positions for the electrostatic surface calculations.

Based on the structural similarity to other HU-like proteins, we believe that SmuHLP is also likely to be involved in an architectural role to overcome the torsional rigidity of the B-DNA. However, how exactly SmuHLP binds to the DNA or the extent of DNA bending induced by SmuHLP remains to be explored. Since the streptococcal HLPs are essential for survival, they are ideal targets for drug development to control infections caused by these organisms. Indeed, derivatives of stilbene compounds have been used as inhibitors of *Mycobacterium tuberculosis* HU (MtbHU; Bhowmick *et al.*, 2014). These small molecules specifically interfered with the binding of DNA by MtbHU. Since the MtbHU structure (PDB entry 4pt4) is similar to that of SmuHLP, these stilbene compounds might be effective in inhibiting streptococcal HLP as well, and experiments are under way to evaluate this possibility.

Figure 5

(a) Two views of the electrostatic surface of SmuHLP. (b) Positively charged residues that reside in the DNA-binding region. Residues are colored magenta and labeled for subunit *A*. The corresponding residues in subunit *B* are colored cyan.

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