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Solution NMR structures reveal unique homodimer formation by a winged helix-turn-helix motif and provide first structures for protein domain family PF10771

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Abstract

High-quality NMR structures of the homo-dimeric proteins Bvu3908 (69-residues in monomeric unit) from *Bacteroides vulgatus* and Bt2368 (74-residues) from *Bacteroides thetaiotaomicron* reveal the presence of winged helix-turn-helix (wHTH) motifs mediating tight complex formation. Such homo-dimer formation by winged HTH motifs is otherwise found only in two DNA-binding proteins with known structure: the C-terminal wHTH domain of transcriptional activator FadR from *E. coli* and protein TubR from *B. thuringiensis*, which is involved in plasmid DNA segregation. However, the relative orientation of the wHTH motifs is different and residues involved in DNA-binding are not conserved in Bvu3908 and Bt2368. Hence, the proteins of the present study are not very likely to bind DNA, but are likely to exhibit a function that has thus far not been ascribed to homo-dimers formed by winged HTH motifs. The structures of Bvu3908 and Bt2368 are the first atomic resolution structures for PFAM family PF10771, a family of unknown function (DUF2582) currently containing 128 members.

Keywords

Bvu3908; Bt2368; PF10771; DUF2582; Winged helix-turn-helix; Structural genomics

Introduction

Bvu3908 (UniProtKB ID A6L747) and Bt2368 (Uni-ProtKB ID Q8A574) are 69- and 74-residue proteins from *Bacteroides vulgatus* and *Bacteroides thetaiotaomicron*, respectively. The two proteins share 37% sequence identity (Fig. 1a) and belong to Pfam [1] protein family PF10771 of unknown function (DUF2582), which currently contains 128 members from eubacteria and archaeobacteria. Neither of the two proteins has significant sequence similarity to any protein with known structure. Bvu3908 and Bt2368 were selected by the Northeast Structural Genomics Consortium (NESG; <http://www.nesg.org>; target IDs BvR153 and BtR375, respectively) as part of a Protein Structure Initiative program aimed at determining 3D structures of novel proteins discovered by metagenomic sequencing of the human gut microbiome [2–4]. Here we report the high-quality solution NMR structures of Bvu3908 and Bt2368, which represent the first atomic resolution structures for PF10771.

Materials and methods

Bvu3908 and Bt2368 were cloned, expressed, and purified following protocols [5–7] established by the NESG (see Supplementary Material for details; <http://www.nmr2.buffalo.edu/nesg.wiki>). The proteins included short C-terminal hexaHis tags (LEHHHHHH). The corresponding pET expression vectors (BvR153-21.1 and BtR375-21.2), have been deposited in the PSI Materials Repository (<http://psimr.asu.edu/>).

[U-¹³C,¹⁵N]- and [5% ¹³C; U-¹⁵N]-labeled Bvu3908 NMR samples were prepared at ~1.1-mM concentration in a buffer containing 90% H₂O/10% D₂O, 20 mM ammonium acetate, 100 mM NaCl, 10 mM DTT, 5 mM CaCl₂, and 0.02% NaN₃ at pH 4.5, while Bt2368 NMR samples were prepared at ~1.3 mM concentration in a buffer containing 90% H₂O/10% D₂O, 20 mM MES, 100 mM NaCl, 10 mM DTT, 5 mM CaCl₂, and 0.02% NaN₃ at pH 6.5. The [5% ¹³C; U-¹⁵N]-labeled samples enabled stereospecific assignment of the methyl groups of Val and Leu residues [8]. Isotropic overall rotational correlation times of about 8.0 and 8.4 ns were inferred for Bvu3908 and Bt2368, respectively, from ¹⁵N spin relaxation times, indicating that both proteins form homodimers in solution. This finding was confirmed by analytical gel-filtration (Agilent Technologies) followed by static light scattering (Supplementary Figs. S1, S2).

NMR data were acquired on Varian INOVA 600 and 750 MHz spectrometers equipped with cryogenic ¹H{¹³C, ¹⁵N} probes, a Bruker AVANCE 600 MHz spectrometer equipped with a cryogenic ¹H{¹³C, ¹⁵N} probe, and a Varian INOVA 500 MHz spectrometer equipped with conventional ¹H{¹³C, ¹⁵N} probe. Virtually complete sequence-specific ¹H, ¹⁵N and ¹³C resonance assignments (Table 1; Supplementary Figs. S3, S4) were obtained from conventional triple-resonance NMR experiments (Supplementary Material) using the program AutoAssign 2.3.0 [9, 10], followed by manual assignment of side-chain resonances. Assignments were validated using the AVS software suite [11]. Backbone ¹⁵N-¹H residual dipolar couplings (RDCs) were measured for two alignment media in 2D *J*-modulated spectra (Supplementary Material). Chemical shifts, NOESY peak lists, RDCs, and time-domain data have been deposited in the BioMagResBank on 6/29/2010 (BMRB IDs: 17025 for Bvu3908; 17026 for Bt2368).

Structure calculations were performed using standardized methods of the NESG consortium [12, 13] using the program CYANA 3.0 [14, 15] based on ¹H-¹H NOE derived upper limit distance constraints, backbone dihedral angle constraints that were derived from chemical shifts using the program TALOS+ [16] for residues located in well-defined regular structure elements, and orientational constraints derived from polypeptide backbone ¹⁵N-¹H RDCs. Stereospecific assignment of methylene protons were performed using the GLOMSA module of CYANA and the final structure calculation was performed with CYANA followed by refinement of selected conformers in an ‘explicit water bath’ [17] using the program CNS 1.2 [18]. Validation of the resulting 20 refined conformers was performed with the Protein Structure Validation Software (PSVS) server 1.4 [19], and the agreement of structures and NOESY peak lists was verified using the AutoStructure/RPF 2.2.1 package [12].

The structural coverage provided by the structures of Bvu3908 and Bt2368 within PF10771 was calculated for the 87 unique sequences of this family using the program PSI-BLAST [20] with parameters published previously [21], i.e. *E*-value upper and lower cutoffs of 10⁻¹⁰ and 0.0005, respectively.

A search for protein domains exhibiting structurally similar dimerization by a winged HTH motif was performed as follows. Structural neighbors of Bvu3908 were identified by searching a non-redundant structure database (60% sequence identity cutoff) with the program SKAN [22] using a protein structural distance (PSD) cutoff [23] of 0.8. For each structural neighbor, we identified interfacial residues that aligned to the interfacial helix of Bvu3908 (residues 39–52) using the structure-based sequence alignment based on a heavy-atom distance cutoff of 4.0 Å.

Results and discussion

High-quality NMR structures of Bvu3908 and Bt2368 (Fig. 1b, c) were obtained (Table 1) and the coordinates were deposited in the Protein Data Bank [24] on 6/29/2010 (accession codes 2L01 and 2L02, respectively). The monomeric units of Bvu3908 and Bt2368 adopt a winged helix-turn-helix (wHTH) fold [25, 26], which consists of a three-stranded anti-parallel β -sheet with topology A($\uparrow$)C($\downarrow$)B($\uparrow$), and three α -helices packed against one side of the sheet (Fig. 1d, e). The locations of the regular secondary structure elements are: β -strands A (residues 25–26 in Bvu3908/23–24 in Bt2368), B (55–60/52–57), C (63–67/60–65), and α -helices I (4–19/3–19), II (27–34/25–31) and III (40–51/36–47), with α -helices II and III participating in the HTH motif. The subunits form tight binding homodimeric proteins resulting in buried surface areas of 1,800 Å² for Bvu3908 and 1,980 Å² for Bt2368. The interfaces of the homodimers are formed primarily by hydrophobic side-chains (Leu 4, Ala 8, Phe 43, Leu 44, Leu 46, Trp 48, Leu 50 in Bvu3908; Val 6, Ile 25, Ala 40, Leu 41, Ala 47, Ile 54, Ile 61, Ile 63 in Bt2368). Five of the hydrophobic residues belong to α -helix III, which thus promotes dimerization by forming a coiled-coil like arrangement between the α -helices III of the two subunits. The monomeric units of Bvu3908 and Bt2368 are very similar, with the root mean square deviation (RMSD) calculated for the mean coordinates of the backbone heavy atoms N, C $^{\alpha}$ and C' of regular secondary structure elements to be only 1.1 Å. The relative orientation of the monomeric units is, however, somewhat different as reflected by the corresponding RMSD of 2.9 Å calculated for the homodimeric proteins. The major difference is observed for the relative orientations of α -helix II as well as the tip of the 'wing' comprised of the β -strands B and C and the intervening turn: in adjacent subunits these substructures are positioned closer in the Bt2368 homodimer which results in a more compact shape when compared with Bvu3908.

Helix-turn-helix motifs are characterized by the presence of two conserved sequence motifs in which polar residues ('p'), hydrophobic residues ('h') and small residues ('s') form a tripeptide segment with consensus signatures 'phs' in α -helix II and 'shs' in the turn connecting α -helices II and III [25]. In Bt2368, these segments are 'Glu 27–Leu 28–Ala 29' in helix II and 'Asn 32–Leu 33–Ser 34' in the turn. In Bt2368, deviations are observed from this consensus signature: in 'Gln 29–Ile30–Lys 31' and 'Lys35–Leu 36–Lys 37' small residues are replaced by Lys residues.

Both dimeric proteins exhibit quite similar conserved surface regions formed by residues located in α -helix I, the C-terminal segment of α -helix II, and the turn connecting this helix with β -strand B (Fig. 1a; Supplementary Fig. S5). Analysis of the structures using the programs ProFunc [27] and Mark-Us [28] revealed that these conserved surface regions contain three cavities, one centrally located at the interface and one located within each of the subunit. The central cavity is formed primarily by two conserved Trp and Arg residues (Trp 48 and Arg 51 in Bvu3908; Trp 45 and Arg 48 in Bt2368), and the other two cavities likewise involve conserved Trp and Arg residues along with conserved Gly, Glu and Lys residues (Gly 9, Gly 13, Trp 16, Trp 48, Arg 51, Glu 52 and Lys 54 in Bvu3908; Gly 7, Gly 11, Trp 14, Trp 45, Arg 48, Glu 49 and Lys 51 in Bt2368). The opposite face of both proteins shows high variability, with the exception of Lys 31 and Lys 35 of Bvu3908. The resulting electrostatic surface potential of the two proteins is thus of mixed positive and negative charge (with the opposite face being more positively charged in Bvu3908 and more negatively charged in Bt2368; Supplementary Fig. S6).

As expected for a structure comprising a HTH motif, a search of the PDB [24] for structurally similar proteins using the DALI server [29] yielded a very large number of structurally quite similar significant hits spanning a quasi-continuum of *Z*-scores > 4.0 (i.e., more than 700 for both Bvu3908 and Bt2368). The highest *Z*-scores resulted for both

proteins for the HTH motif of the ArsR-type DNA-binding domain of protein MJ1563 from *Methanococcus janashii* (PDB code 1KU9; Z-scores 9.4/10.3; RMSD of C $^{\alpha}$ atoms: 2.9/2.4 Å; 64/63 aligned residues with 17%/11% sequence identity to Bvu3908/Bt2368). The vast majority of the remaining hits are, likewise as expected, nucleic acid binding proteins in which the ‘recognition α -helix III’ conveys nucleic acid sequence specific binding. In Bvu3908 and Bt2368 the α -helix III is buried in the interface of the homodimer. In stark contrast, a specific search for HTH domain structures exhibiting dimerization via α -helix III yielded only two proteins, FadR from *E. coli* [30, 31] (PDB codes 1E2X, 1H9G, 1H9T, 1HW1 and 1HW2) and TubR from *B. thuringiensis* [32] (PDB code 3M8E), both actually ranking in the lower half of the list of significant DALI hits. FadR is a transcriptional repressor/activator involved in fatty acid metabolism and comprised of an N-terminal acyl-CoA binding domain and a C-terminal wHTH domain [30, 31]. TubR is a 104-residue protein encoded by the *B. thuringiensis* pBtoxis plasmid responsible for plasmid DNA segregation [32].

According to DALI, the monomeric units of Bvu3908/Bt2368 are structurally somewhat more similar to chain B of TubR (3M8E; Z-scores 5.9/6.6; RMSD of C $^{\alpha}$ atoms: 2.4/1.9 Å; 60/60 aligned residues with 13%/15% sequence identity) than to chain A of FadR in its most similar structure (1HW1; Z-scores 4.7/5.3; RMSD of C $^{\alpha}$ atoms: 2.7/2.7 Å; 58/59 aligned residues with 14%/14% sequence identity). However, the relative orientation of monomeric units in Bvu3908/Bt2368 is actually significantly more similar to the FadR than the TubR wHTH domain. Visual inspection reveals that the polypeptide backbone of the Bvu3908 dimer is overall structurally very similar to the one of FadR wHTH domain dimer, with the exception of the longer and more twisted ‘wings’ and a different orientation of α -helices I relative to the rest of the molecule (Supplementary Fig. S7A). The structure of the Bt2368 dimer exhibits the same deviations in its ‘wings’ and α -helices I, but exhibits, in addition, a different relative orientation of α -helices III at the dimer interface, coming along with a much closer location of α -helices II (Supplementary Fig. S7B). In contrast, the monomeric units of TubR are tilted significantly more relative to each other when compared with both Bvu3908 and Bt2368 (Supplementary Fig. S8), which is primarily due to the fact that α -helix I is much shorter in TubR than in Bvu3908 and Bt2368.

Both FadR and TubR wHTH domains were shown to bind to their specific double-stranded DNA regions in essentially the same ‘non-canonical’ manner, such that the tips of the α -helices III interact with the major groove of the DNA while the ‘wings’ are inserted into the adjacent minor grooves [30–32]. The DNA-binding interfaces of FadR and TubR form conserved positively charged bands on their surfaces (Supplementary Fig. S9). The dimer interfaces between FadR and TubR wHTH domains are formed mainly through interactions between α -helices III resulting in ‘dumbbell’ shapes of the dimers (Fig S9). The β -hairpin ‘wings’ of FadR and TubR are thus left as being exposed, allowing them to interact with the minor grooves of the DNA, while the sugar-phosphate backbone is accommodated in the protein grooves. In contrast, the β -hairpin ‘wings’ of Bvu3908 and Bt2368 form part of the homo-dimer interface. Furthermore, Bt2368 lacks a positively charged surface patch (Fig S6B), and though Bvu3908 does exhibit one (Fig S6A), it has a high sequence variability among Bvu3908 homologs, and runs nearly perpendicular to the bands in FadR and TubR. Of the nine residues in FadR that form the DNA-binding interface (Glu 34, Arg 35, Thr 44, Arg 45, Thr 46, Thr 47, Arg 49, Glu 50, His 65) none is conserved in Bvu3908 and Bt2368, and only Lys 28 of Bvu3908 and Arg 56 of Bt2368 can be considered equivalent to Arg 35 and His 65, respectively. The key basic residues of TubR required for interaction with DNA (Lys 43, Arg 74, Arg 77 and Lys 79) are not conserved in Bvu3908, and only Arg 74 matches Arg 56 in Bt2368. Taken together, it is very unlikely that Bt2368 binds to DNA and while this might be the case for Bvu3908, complex formation would have to be quite different from that observed for FadR and TubR. Hence, we conclude that the members of

PF10771 function in way that has not yet been described for proteins forming homo-dimers via a winged HTH motif.

Finally, the novel structural leverage, that is, the number of new protein structures that can be reliably modeled, [21, 33] provided by the structures of Bvu3908 and Bt2368 within PF10771 is 17 each. Since the modeling families for the two structures do not overlap, the two atomic resolution structures yield a total structural coverage of 39% for the 87 unique sequences of PF10771.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DSS	4,4-Dimethyl-4-silapentane-1-sulfonate sodium salt
DTT	Dithiothreitol
HTH	Helix-turn-helix
MES	2-(<i>N</i> -Morpholino)ethanesulfonic acid
NESG	Northeast Structural Genomics Consortium
NOE	Nuclear Overhauser effect
PDB	Protein Data Bank
RDC	Residual dipolar coupling
RMSD	Root mean square deviation
wHTH	Winged helix-turn-helix

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Fig. 1.

a Multiple sequence alignment of a representative subset of members of PF10771, including Bvu3908, Bt2368 along with homologues from *Bacteroides ovatus*, *Bacteroides caccae*, *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*. The location of the regular secondary structure elements observed in Bvu3908 are indicated above the alignment. The figure was generated with the program Chroma v1.0 using the default color scheme: negative (red), positive (dark blue), charged (magenta), polar (light blue), Ser/Thr (cyan), tiny (light green), small (dark green), aliphatic (gray on yellow), aromatic (blue on yellow), big (violet on pale yellow), hydrophobic (black on yellow). **b** Stereoview of the 20 conformers representing the solution structure of Bvu3908 obtained after superposition of the C α atoms of the regular secondary structure elements for minimal root-mean-square deviation (RMSD). Residues 70–77 of the disordered C-terminal polypeptide segment were omitted for clarity, and the N- and C-termini are labeled as “N” and “C”. **c** Same as in B but for Bt2368. Residues 68–75 of the disordered C-terminal polypeptide segment were omitted for clarity. **d** Stereoview of a ribbon diagram of the lowest-energy conformer of Bvu3908: α -helices are shown in red and yellow, β -strands are depicted in cyan, other polypeptide segments are in gray. **e** Same as in **d** for Bt2368

Table 1

Statistics for structures of Bvu3908 and Bt2368

	Bvu3908	Bt2368
Completeness of resonance assignments ^a (%)		
Backbone/side-chain	99/99	100/95
Completeness of stereospecific assignments ^b		
Val and Leu isopropyl/ ^β CH ₂ / ^α CH ₂ of Gly	90/0/8	92/6/40
Conformation-restricting distance constraints ^c		
Intraresidue [<i>i</i> = <i>j</i>]	248	199
Sequential [<i>i</i> − <i>j</i> = 1]	297	270
Medium range [<i>i</i> < <i>i</i> − <i>j</i> < 5]	313	293
Long range [<i>i</i> − <i>j</i> ≥ 5]	360	288
Intermolecular	21	20
Total	1,218	1,050
Dihedral angle constraints (φ/ψ)	56/56	62/62
Residual dipolar coupling constraints (¹⁵ N- ¹ H)	97	94
Distance constraints per residue (of those, long-range)	18.5 (5.0)	15.4 (3.8)
CYANA target function (Å ²)	1.02 ± 0.21	1.88 ± 0.21
Average number of distance constraint violations per conformer		
0.2–0.5 Å	5.8	5.6
>0.5 Å	0.0	0.1
Average number of dihedral angle constraint violations per conformer		
>10°	0.0	0.0
Average RMSD from mean coordinates (Å)		
Backbone heavy atoms (all heavy atoms) ^d	0.8 (1.1)	0.6 (1.1)
Backbone heavy atoms (all heavy atoms) ^e	0.8 (1.2)	0.7 (1.1)
Global quality scores ^c (raw/Z-score)		
PROCHECK [34] G-factor (φ and ψ)	0.19/1.06	0.19/1.06
PROCHECK [34] G-factor (all dihedral angles)	0.11/0.65	0.11/0.65
Molprobity [35] clash score	12.58/−0.63	12.34/−0.59
Verify3D [36]	0.42/−0.64	0.34/−1.93
ProsaII [37]	0.76/0.45	0.65/0.00
RPF scores [12]		
Recall/precision/F-measure	0.98/0.84/0.90	0.98/0.83/0.90
DP-score	0.87	0.83
Molprobity [35] Ramachandran summary ^e (%)		
Most favored regions	99.6	98.8
Additionally allowed regions	0.4	1.2
Disallowed regions	0.0	0.0

^a Calculated with AVS suite [11] excluding eight non-native residues of the C-terminal tag, N-terminal and Lys and Arg side chain amino groups, hydroxyl of Ser, Thr and Tyr, carboxyls of Asp and Glu, and non-protonated aromatic carbons

^bRelative to pairs with non-degenerate chemical shifts

^cCalculated with PSVS 1.4 [19]

^dResidues in regular secondary structure elements: 4–19, 25–34, 40–51, 55–60, 63–67 (Bvu3908), 3–19, 23–31, 36–47, 52–57, 60–65 (Bt2368)

^eOrdered residues: 3–35, 36–67 (Bvu3908), 3–65 (Bt2368)