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Solution NMR structure of VF0530 from *Vibrio fischeri* reveals a nucleic acid binding function

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Abstract

Protein domain family PF09905 (DUF2132) is a family of small domains of unknown function that are conserved in a wide range of bacteria. Here we describe the solution NMR structure of the 80-residue VF0530 protein from *Vibrio fischeri*, the first structural representative from this protein domain family. We demonstrate that the structure of VF0530 adopts a unique four-helix motif that shows some similarity to the C-terminal double-stranded DNA (dsDNA) binding domain of RecA, as well as other nucleic acid binding domains. Moreover, gel shift binding data indicate a potential dsDNA binding role for VF0530.

Keywords

DUF2132; PF09905; nucleic acid binding domain; structural genomics

INTRODUCTION

We present the solution NMR structure of the small (80-residue), basic (pI 9.4) VF0530 protein from *Vibrio fischeri* (UniProtKB/TrEMBL ID, Q5E7H1_VIBF1; NESG ID, Vfr117), a member of a conserved prokaryotic Pfam protein domain family PF09905 of unknown function (DUF2132). The structure of VF0530 was determined as part of the efforts of the Protein Structure Initiative to obtain experimental structures of representatives from broadly conserved protein domain families. Our structural and biochemical analyses of VF0530 reveal a potential nucleic acid binding function.

MATERIALS AND METHODS

Isotopically enriched samples of VF0530 ($[U-^{13}\text{C}, ^{15}\text{N}]$ - and $[U-5\%-^{13}\text{C}, 100\%-^{15}\text{N}]$ -VF0530) for NMR spectroscopy were cloned, expressed, and purified following standard

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Additional Supporting Information may be found in the online version of this article.

protocols of the Northeast Structural Genomics Consortium (NESG);¹ see Supplementary Material for a detailed description of the methods used in this work. VF0530 is a monomer under the conditions used in the NMR studies (20 mM ammonium acetate, 100 mM NaCl, 10 mM DTT, 5 mM CaCl₂, pH 4.5, 20°C) based on analytical gel filtration chromatography, static light scattering (Supplementary Fig. S1) and one-dimensional ¹⁵N *T*₁ and *T*₂ relaxation data (Supplementary Fig. S2). Backbone ¹H, ¹³C, and ¹⁵N resonance assignments were made using AutoAssign 2.4.0 (Supplementary Fig. S3 and S4);² side chain assignments were completed manually. The solution NMR structure of VF0530 was calculated using CYANA 2.1,^{3,4} followed by refinement in explicit water using CNS 1.2.^{5,6} NMR resonance assignment validation and structure quality analyses were performed using the AVS,⁷ PSVS,⁸ MolProbity,⁹ and RPF¹⁰ software packages. The final ensemble of 20 models (excluding the C-terminal His₆ tag) and NMR resonance assignments for VF0530 were deposited into the Protein Data Bank (PDB ID, 2JVW) and BioMagResDB (BMRB accession number, 15491), respectively. The pET expression vector for VF0530, (NESG Vfr117-21.1), has been deposited in the PSI Materials Repository (<http://psimr.asu.edu/>).

RESULTS AND DISCUSSION

The solution NMR structure of VF0530 consists of a unique four α -helix bundle (α 1, L17-Y28; α 2, W30-M36; α 3, I48-K57; α 4, D59-H75), comprised of two interleaved antiparallel helix-hairpin-helix motifs tethered by a 10-residue loop, and flanked by disordered N- and C-terminal tails (Fig. 1A,B). Structural statistics for VF0530 are presented in Table 1. The well-defined core and not-well-defined terminal polypeptide segments of the structure correlate well with ¹H-¹⁵N heteronuclear NOE data for VF0530 (Supplementary Fig. S4). Electrostatic surface potential¹¹ (Fig. 1C) and ConSurf¹² (Fig. 1D) analyses of the VF0530 structure reveal a largely positively charged face featuring several highly conserved residues clustered in helices α 3 and α 4 and in the loop between helices α 2 and α 3, strongly suggesting that this is a functionally important region of the molecule. A stretch of conserved residues is also present in the disordered N-terminal tail of the protein.

The solution NMR structure of VF0530 reported here corresponds to the first structural representative from the PF09905 (DUF2132) protein domain family of conserved bacterial proteins with currently unknown function, and exhibits very low sequence similarity (< 20%) with any other structure in the PDB. Dali¹⁴ and Skan¹⁵ structural alignment analyses reveal that the structure of VF0530 shows some similarity to several reported structures of the C-terminal domain of RecA [i.e., PDB ID, 1XP8;¹³ Dali Z-score, 5.1; C α RMSD, 2.6 Å (residues 15 to 75)]. Three of the helices within VF0530 (α 1, α 3, and α 4) overlay well with corresponding helices in this domain, but helix α 2 in VF0530 is replaced by two short antiparallel β -strands in RecA (Fig. 1E). This domain of RecA is thought to bind double-stranded DNA (dsDNA) via residues in the loop preceding its second helix.¹⁶ In the structure of VF0530 this loop flanks the highly basic face formed by helices α 3 and α 4, suggesting that VF0530 may also be a nucleic acid binding protein. Moreover, the VF0530 structure is remotely similar to structures of several protein:dsDNA complexes in the PDB, including that of the MH1 domain of the Smad3 transcription factor (PDB ID, 1OZJ).¹⁷ Interestingly, several of the basic residues in helices 3 and 4 of VF0530 map well to residues in the positively charged dsDNA-binding surface of this Smad3 domain (Supplementary Fig. S5). Indeed, gel shift binding experiments demonstrate an interaction between VF0530 and dsDNA (Supplementary Fig. S6). Finally, the structure of VF0530 also resembles structures of the six-helix bundle pyrin domain (i.e., PDB ID, 2HM2),¹⁸ which plays a role in inflammation and apoptosis.

In summary, we have determined the solution NMR structure of VF0530 from *Vibrio fischeri*, the first structure of a member of the conserved PF09905 (DUF2132) bacterial

protein domain family. The all-helical structure of VF0530 shows similarity to the structures of several dsDNA-binding proteins, suggestive of distant homologous relationships and a potential dsDNA-binding function. Elucidation of the exact function of VF0530 in this symbiotic marine bacterium awaits further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Superposition of the final ensemble of 20 conformers from the solution NMR structure of VF0530 (PDB ID, 2JVW). Secondary structural elements are labeled; α -helices are shown in red and loops are colored grey. Note the disorder in the N- and C-terminal tails. For clarity, residues 1 to 8 have been omitted. (B) Stereoview of the lowest energy (CNS) conformer (residues 9 to 80) of the final solution NMR ensemble of VF0530. Helices are colored and loops are in grey. (C) APBS¹¹ electrostatic surface potential of VF0530 (residues 9 to 80) showing negative (red), neutral (white), and positive (blue) charges. Selected basic residues are indicated. (D) ConSurf¹² image showing the conserved residues in VF0530 (residues 9 to 80). Residue coloring, reflecting the degree of residue conservation over the entire PF09905 protein domain family (Pfam 24.0), ranges from magenta (highly conserved) to cyan (variable). Selected highly conserved residues are labeled. (E) Overlay of the ordered residues (15 to 75) from the solution NMR structure of VF0530 (red) and the C-terminal domain (residues 282 to 341) from the X-ray crystal structure of RecA from *Deinococcus radiodurans* (PDB ID, 1XP8;¹³ green). All structure figures were rendered using PyMOL (www.pymol.org).

Table ISummary of NMR and structural statistics for VF0530^a

Completeness of resonance assignments ^b		
Backbone (%)		98.8
Side chain (%)		95.3
Aromatic (%)		100
Stereospecific methyl (%)		100
Conformationally-restricting constraints ^c		
Distance constraints		
Total		1424
intra-residue ($i = j$)		489
sequential ($ i - j = 1$)		380
medium range ($1 < i - j < 5$)		285
long range ($ i - j \geq 5$)		270
Dihedral angle constraints		88
Hydrogen bond constraints		46
Number of constraints per residue		19.2
Number of long range constraints per residue		3.3
Residual constraint violations ^c		
Average number of distance violations per structure		
0.1 – 0.2 Å		1.2
0.2 – 0.5 Å		0.05
> 0.5 Å		0
Average number of dihedral angle violations per structure		
1 – 10°		0.1
> 10°		0
RMSD from average coordinates (Å) ^{c,d}		
backbone atoms		0.5
heavy atoms		1.1
MolProbity Ramachandran statistics ^{c,d}		
most favored regions (%)		96.1
additional allowed regions (%)		3.7
disallowed regions (%)		0.2
Global quality scores (Raw/Z-score) ^c		
Verify3D	0.33	−2.09
ProsaII	0.69	0.17
Procheck(phi-psi) ^d	0.21	1.14
Procheck(all) ^d	0.21	1.24
Molprobity clash	14.65	−0.99
RPF Scores ^e		
Recall/Precision	0.989	0.927

F-measure/DP-score	0.967	0.808
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^a Structural statistics were computed for the ensemble of 20 deposited structures (PDB ID, 2JVW).

^b Computed using AVS software⁷ from the expected number of peaks, excluding: highly exchangeable protons (N-terminal, Lys, and Arg amino groups, hydroxyls of Ser, Thr, Tyr), carboxyls of Asp and Glu, non-protonated aromatic carbons, and the C-terminal His₆ tag.

^c Calculated using PSVS 1.4 program.⁸ Average distance violations were calculated using the sum over r^{-6} .

^d Ordered residue ranges [$S(\phi) + S(\psi) > 1.8$] : 15–37, 46–75.

^e RPF scores¹⁰ reflecting the goodness-of-fit of the final ensemble of structures (including disordered residues) to the NOESY data and resonance assignments.