PROTEIN STRUCTURE REPORT

The solution structure of ribosomal protein S17E from *Methanobacterium thermoautotrophicum*: A structural homolog of the FF domain

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Abstract

The ribosomal protein S17E from the archaeon *Methanobacterium thermoautotrophicum* is a component of the 30S ribosomal subunit. S17E is a 62-residue protein conserved in archaea and eukaryotes and has no counterparts in bacteria. Mammalian S17E is a phosphoprotein component of eukaryotic ribosomes. Archaeal S17E proteins range from 59 to 79 amino acids, and are about half the length of the eukaryotic homologs which have an additional C-terminal region. Here we report the three-dimensional solution structure of S17E. S17E folds into a small three-helix bundle strikingly similar to the FF domain of human HYPB/GBP1, a novel phosphopeptide-binding fold. S17E bears a conserved positively charged surface acting as a robust scaffold for molecular recognition. The structure of *M. thermoautotrophicum* S17E provides a template for homology modeling of eukaryotic S17E proteins in the family.

Keywords: heteronuclear NMR; *Methanobacterium thermoautotrophicum*; ribosomal protein S17E; Northeast Structural Genomics Consortium

The ribosome as the core of the translation machinery is the focus of intensive study (Ramakrishnam 2002). Crystal structures of a bacterial 30S subunit and 50S subunit (Wimberly et al. 2000; Brodersen et al. 2002), a

bacterial 50S subunit (Harm et al. 2001), an intact bacterial 70S ribosome and its complex with tRNA and mRNA (Yusupov et al. 2001; Schuwirth et al. 2005; Korostelev et al. 2006; Selmer et al. 2006), and an archaeal 50S subunit (Ban et al. 2000), together with a yeast 80S ribosome at 15 Å resolution (Spahn et al. 2001) and the bovine 55S mitochondrial ribosome at 13.5 Å (Sharma et al. 2003) by cryo-electron microscopy, have increased our understanding of the structure and activity of the ribosome. However, detailed models for the 30S archaeal subunit and the eukaryotic ribosome are still

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lacking. The archaeal ribosome more closely resembles that of eukaryotes than that of bacteria in terms of ribosomal protein composition (Lecompte et al. 2002). Comparisons of amino acid sequences and sequence patterns in archaeal genomes have identified many putative ribosomal proteins homologous only to eukaryotic proteins. As part of the Northeast Structural Genomics Consortium (www.nesg.org), several archaeal 30S ribosomal proteins have been determined by NMR, including S24E (Jeon et al. 2006), S27E (Herve du Penhoat et al. 2004), and S28E (Aramini et al. 2003; Wu et al. 2003). These proteins increase the database of structural information available to analyze the function of versatile ribosomal proteins in archaea and eukaryotes.

The ribosomal protein S17E, a 62-residue protein from the archaeon *Methanobacterium thermoautotrophicum* and NESG target TT802, is a component of the 30S ribosomal subunit. S17E is conserved in archaea and eukaryotes and has no counterparts in bacteria (Fig. 1). Archaeal S17E proteins range from 59 to 79 amino acids, about half the length of the eukaryotic homologs which have an additional C-terminal region. The *Saccharomyces cerevisiae* and *Homo sapiens* S17E share 50% and 42%

sequence identity with *M. thermoautotrophicum* S17E, respectively. Human S17E (135 residues) can be phosphorylated by p70 S6 kinase both in vitro and in vivo, and is believed to influence the functional properties of the mammalian 40S ribosomal subunit (Patel et al. 1996). Recently, mutations of the gene encoding ribosomal protein S17 were implicated in Diamond-Blackfan anemia (DBA) (Cmejla et al. 2007). In this paper, we report the solution structure of S17E from *M. thermoautotrophicum*. The structure of archaeal S17E provides a template for homology modeling of eukaryotic proteins in this family and a structural basis for future functional studies.

Results and Discussion

Overall fold

The solution structure of L40E is represented in Figure 1B and C, and the structure parameters are summarized in Table 1. The protein folds into a compact three-helix bundle formed by residues Ser 7-Ile 17 ($\alpha 1$), Phe 28-Glu 36 ($\alpha 2$), and Lys 46-Gln 61 ($\alpha 3$). Linking the helices are two long loops: L1 (Glu 18–Asp 27) and L2 (Glu 37–His

Figure 1. (A) Sequence alignment of S17E from various species. Archaea: *Methanothermobacter thermoautotrophicum* (O26894), *Methanococcus jannaschii* (P54026), *Pyrococcus abyssi* (Q9V0G0), *Methanosarcina Mazei* (Q8PXL8), *Pyrococcus horikoshii* (P58503), *Pyrococcus furiosus* (Q8U0U1). Eukaryote: *Saccharomyces cerevisiae* (P14127), *Neurospora crassa* (P27770), *Drosophila melanogaster* (P17704), *Arabidopsis thaliana* (Q9SQZ1), *Mus musculus* (P06584), *Homo sapiens* (P08708). Identical and similar residues are highlighted in black and gray, respectively. The NMR-derived secondary structural elements of S17E are illustrated above the alignment. (B) Ribbon representation of the lowest energy structure of S17E. (C) Stereoview of an ensemble of 15 refined structures represented in an orientation similar to B.

Table 1. Structural statistics for the ensemble calculated for S17E

	3D ¹⁵ N	3D ¹³ C	
NOESY spectral data			
Total number of peaks	702	1653	
Number of "assignable" peaks	701	1649	
Number of peak assignments	657	1558	
Peak assignment (%)	94	94	
Distance restraints			
All		744	
Intraresidue ($i = j$)		141	
Sequential ($ i - j = 1$)		205	
Medium range ($2 \leq i - j \leq 4$)		219	
Long range ($ i - j > 4$)		135	
Hydrogen bonds		22 × 2	
Dihedral angle restraints			
All		109	
φ		59	
ψ		50	
¹³ C chemical shifts			
All		119	
¹³ Cα		61	
¹³ Cβ		58	
Number of violations			
Distance restraints (>0.3 Å)		0	
Dihedral angle restraints (>5°)		0	
RMSD from experimental restraints			
Distance (Å)		0.012 ± 0.0014	
Dihedral angle (°)		0.10 ± 0.081	
RMSD from idealized covalent geometry			
Bond (Å)		0.0023 ± 0.0001	
Bond angles (°)		0.43 ± 0.011	
Impropers (°)		0.26 ± 0.019	
CNS energy (kcal/mol)			
Total		−2170 ± 92	
Van der Waals		−172 ± 16	
Electrostatic		−2423 ± 102	
Pairwise RMSD			
All residues ^a			
Backbone atoms		1.17 ± 0.12	
All heavy atoms		1.92 ± 0.16	
Ordered regions ^b			
Backbone atoms		0.77 ± 0.12	
All heavy atoms		1.36 ± 0.13	
Ramachandran plot (%) ^b			
Residues in most favored regions		97.9	
Residues in additional allowed regions		2.1	
Residues in generously allowed regions		0.0	
Residues in disallowed regions		0.0	
Global quality scores ^c			
Raw			Z-score
Procheck (phi-psi) ^b		0.54	2.44
Procheck (all) ^b		0.29	1.71
MolProbity clash		21.49	−2.16
RPF scores ^d			
Recall		0.957	
Precision		0.918	
F-measure		0.91	
DP-score		0.71	

S17E consists of an ensemble of the 15 lowest energy structures out of 112 calculated.

^aRMSD values for residues 7–61.

^bOnly residues 7–17, 28–36, and 46–61 are included.

^cCalculated from NESG PSVS program (Bhattacharya et al. 2007).

^dRPF scores were defined and calculated by using RPF program (Huang et al. 2005).

45). The loops are less well defined due to a relatively low number of detectable NOEs. The three helices are stabilized by a hydrophobic core formed by Ala 13, Met 16, Ile 17, the aliphatic portion of Asn 31, Leu 34, Val 35, Val 41, Ile 50, Ala 51, Ile 54, Thr 55, and Ile 58. These residues are well conserved in different species as shown in Figure 1, indicating that the *M. thermoautotrophicum* fold is likely conserved throughout the S17E family.

The extensive hydrophobic packing effectively determines the relative orientations of the helices. The aromatic ring protons of Tyr 53 and side chain of Ile 50 and Ile 54 connect α3 with α1 and α2 through 48 observable long-range ¹H-¹H NOEs. Helix α1 also makes hydrophobic contacts with α2. Therefore, α1 bridges the other two helices at an angle of 92° to α2 and 81° to α3. The helices α2 and α3 are oriented at an angle of 127° to each other.

S17E is structurally homologous to the FF domain, a novel phosphopeptide-binding fold

The three-helix bundle is a common fold found in many other proteins either as a distinct domain or as part of a larger domain. A search on the DALI server (Holm and Sander 1993) for structures similar to S17E yielded a number of weakly related proteins. The maximal Z score of 4.4 is found for the FF domain from human HYPA/FBP11 (PDB code 1UZC; Allen et al. 2002), which has only 9% sequence identity in the structurally homologous region to S17E. Superposition of 36 Cα coordinates of the two proteins, corresponding primarily to three helices in S17E, yielded an RMSD of 3.1 Å. The C-terminal domain (residues 298–366) of human protein serine/threonine phosphatase 2C (PDB code 1A6Q) (Das et al. 1996) has a Z score of 3.9 and an RMSD of 3.2 Å over 57 equivalent residues with 7% sequence identity. Figure 2 shows similarities in both topology and electrostatic surface between S17E and the FF domain.

S17E is a basic protein with calculated pI of 10.70. Twelve basic residues (4 Arg and 8 Lys) and seven acid residues (3 Asp and 4 Glu) contribute to the surface charge distribution. A positively charged surface is created by a cluster of highly conserved Arg and Lys residues in three helices encompassing Lys 10, Arg 11, Lys 14, Lys 32, Lys 33, Arg 47, and Lys 49. As shown in Figure 2B, S17E possesses surface properties similar to those of the FF domain. The conserved residues Lys 10 and Lys 49 of S17E are aligned at the same positions as residues Lys 393 and Lys 433 that engage in the phosphor-peptide binding on the FF domain surface.

Ribosomal protein S17E is a phosphoprotein of eukaryotic ribosomes. Immunoprecipitation and in vitro kinase assays showed that human S17E is the target of the p70 S6 kinase and phosphorylation of human S17E has been

Figure 2. Comparison of S17E and the FF domain of HYPA/FBP11. (A) Ribbon superposition of S17E (red, 1RQ6, residues 6–62) and the FF domain of HYPA/FBP11 (blue, 1UZC, residues 13–71). The C α atoms of S17E residues S7–I17, F28–E36, and L46–Q61 were superimposed on the C α atoms of the FF domain residues K14–L24, W36–I44, and K54–T69. (B) Surface electrostatic potential of S17E and the FF domain as calculated by MOLMOL. Red and blue colors represent negative and positive electrostatic potential, respectively. The view is in the same orientation as in A. Residues Lys393 and Lys433 of the FF domain are labeled.

shown to be inhibited by the immunosuppressant rapamycin (Patel et al. 1996). Unlike 40S ribosomal protein S6 whose phosphorylation has been studied in greater detail (Meyuhas 2000), nothing is known about the role that phosphorylation may have in the function of S17E. S17E contains conserved Ser and Thr residues that may serve as the site of phosphorylation. FF domains are potential protein–protein interaction modules, and are present in a number of eukaryotic proteins, such as the transcription elongation factor CA150 and the splicing factor PRP40 and HYPA/FBP11. The FF domains of CA150 appear to bind the phosphorylated C-terminal domain of RNA polymerase II (Carty et al. 2000). The structural and surface similarities of S17E and the FF domain suggest a possible conserved function of S17E

family proteins with that of the FF domain, including phosphor-peptide binding. S17E in the eukaryotes is larger than S17E in archaea with an additional C-terminal region. Alternatively, it is possible that the positively charged surface on this domain may mediate interactions with RNA and other binding partners as is found in many ribosomal proteins and the added C terminus uniquely found in eukaryotes are likely the interacting target of p70 S6 kinase.

Materials and Methods

Protein purification

The gene *mth803* coding for ribosome protein S17E (62 amino acids) from *M. thermoautotrophicum* was subcloned into the pET-15b expression vector with an N-terminal His tag. It was expressed in *Escherichia coli* strain BL21(DE3) growing in M9-minimal medium supplemented with ^{15}N ammonium chloride (1 g/L) and ^{13}C glucose (2 g/L). The protein was purified to homogeneity using metal affinity chromatography as described previously (Yee et al. 2002). The purified protein contained the complete sequence of S17E plus His₆ affinity tag (MGTSHHHHHHSSGRENLYFQGH) at the N terminus of the protein. The concentration of protein samples ranged from 1.0 mM to 1.5 mM in an aqueous solution containing 25 mM sodium phosphate (pH 6.5), 450 mM NaCl, 1 mM DTT, 95% H₂O/5% D₂O.

NMR spectroscopy

All NMR spectra were collected at 25°C on Varian Inova 600 MHz and 750 MHz spectrometers equipped with pulsed field gradient triple-resonance probes. Chemical shifts were referenced to external DSS. Spectra were processed using the program NMRPipe (Delaglio et al. 1995) and analyzed with the program SPARKY (<http://cgl.ucsf.edu/home/sparky>). The backbone assignments were obtained using HNCOC, CBCA(CO)NH, HNCACB, HNCA, HN(CO)CA, and ^{15}N -edited NOESY-HSQC spectra. Assignments were made initially with the automated program AutoAssign 1.9 (Zimmerman et al. 1997; Moseley et al. 2001) and followed by manual analysis. Aliphatic side chain assignments relied on (H)CC(CO)NH-TOCSY, H(CC)(CO)NH-TOCSY, H(C)CH-COSY, and H(C)CH-TOCSY spectra (Bax et al. 1994; Kay 1997). Aromatic ring resonances were assigned using 2D spectra correlating C β s with H δ s or H ϵ s, (HB)CB(CGCD)HD and (HB)CB(CGCDCE)HE (Yamazaki et al. 1993), and 3D ^{13}C -edited NOESY spectra.

Structure calculation

Distance restraints for structure calculations were derived from cross-peaks in ^{15}N -edited NOESY-HSQC ($\tau_m = 100$ ms), ^{13}C -edited aliphatic and aromatic NOESY-HSQC ($\tau_m = 80$ ms). Slowly exchanging amide protons were monitored by dissolving the protein in D₂O and acquiring a series of ^{15}N -HSQC spectra. A 4D ^{13}C - ^{13}C -HMQC-NOESY-HMQC (D₂O, $\tau_m = 80$ ms) was recorded in D₂O (Vuister et al. 1993). $^3\text{J}(\text{H}^{\text{N}}, \text{H}^{\alpha})$ coupling constants were calculated from cross-peak to diagonal peak intensity in the HNHA spectrum (Vuister and Bax 1993). The

program AutoStructure 2.0beta (Huang et al. 2006), interfaced with Xplor-NIH 1.1.2 (Schwieters et al. 2003) was used to perform automated structure calculation. AutoStructure uses a topology-constrained distance network algorithm for protein structure determination from NOESY data. The program generates an initial fold in cycle1 based on pattern discovery of standard secondary structure geometry and identification of unique connections supported by a large number of potential contacts. Successive cycles automatically generate distance (NOE), dihedral angle and hydrogen bond restraints, and submit parallel structure calculations with the program Xplor-NIH. The input for AutoStructure included the chemical shift list, peak lists from ^{15}N -edited NOESY and ^{13}C -edited NOESY, $^3\text{J}(\text{H}^{\text{N}}-\text{H}^{\alpha})$ scalar coupling constants and dihedral angle restraints derived from the program TALOS (Cornilescu et al. 1999). NOE peaks were picked with intensities using the program SPARKY. The tolerance range between NOESY peaks and resonance assignment was set to ± 0.05 ppm for ^1H and ± 0.5 ppm for ^{15}N and ^{13}C . Ten iterative cycles of AutoStructure assignment and Xplor-NIH structure calculation were performed. A total of 94% of the NOE cross-peaks from ^{13}C -edited NOESY and 94% from ^{15}N -edited NOESY were assigned in cycle10. The NMR-derived experimental restraints contained 700 NOEs (141 intraresidue, 205 sequential, 219 medium-range [$2 \leq |i-j| \leq 4$] and 135 long-range [$|i-j| > 4$] interproton restraint), 44 distance restraints for 22 backbone hydrogen bonds and 109 dihedral angle restraints. The best 20 of 112 Xplor-NIH structures from cycle10 were selected and subsequently subjected to molecular dynamics simulation in explicit water by the program CNS (Brunger et al. 1998). The structures were soaked in a 8 Å layer of TIP3P water molecules (Linge et al. 2003). The 15 structures with lowest energies were retained and assessed by NMR structure quality assessment scores (NMR RPF scores) (Huang et al. 2005). The quality of the structure was further inspected by PROCHECK (Laskowski et al. 1996) and MolProbity (Lovell et al. 2003) using NESG validation software package PSVS (Bhattacharya et al. 2007). The validation report is accessible at www.nesg.org. S17E from *M. thermoautotrophicum* is target TT802 of the Northeast Structural Genomics Consortium. Structures were visualized using the program MOLMOL (Koradi et al. 1996).

Accession numbers

The chemical shifts have been submitted to the BioMagResDB (BMRB accession number 6028), and the structure ensemble and restraint files have been deposited to the Protein Data Bank (PDB ID 1RQ6).

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