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Solution Structure and Characterization of the DNA-Binding Activity of the B3BP–Smr Domain

Tammo Diercks†, Eiso AB†, Mark A. Daniels, Rob N. de Jong, Rogier Besseling, Robert Kaptein and Gert E. Folkers*

Bijvoet Center for Biomolecular Research, Department of NMR Spectroscopy, Faculty of Chemistry, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

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The MutS1 protein recognizes unpaired bases and initiates mismatch repair, which are essential for high-fidelity DNA replication. The homologous MutS2 protein does not contribute to mismatch repair, but suppresses homologous recombination. MutS2 lacks the damage-recognition domain of MutS1, but contains an additional C-terminal extension: the small MutS-related (Smr) domain. This domain, which is present in both prokaryotes and eukaryotes, has previously been reported to bind to DNA and to possess nicking endonuclease activity. We determine here the solution structure of the functionally active Smr domain of the Bcl3-binding protein (also known as Nedd4-binding protein 2), a protein with unknown function that lacks other domains present in MutS proteins. The Smr domain adopts a two-layer α–β sandwich fold, which has a structural similarity to the C-terminal domain of IF3, the R3H domain, and the N-terminal domain of DNase I. The most conserved residues are located in three loops that form a contiguous, exposed, and positively charged surface with distinct sequence identity for prokaryotic and eukaryotic Smr domains. NMR titration experiments and DNA binding studies using Bcl3-binding protein–Smr domain mutants suggested that these most conserved loop regions participate in DNA binding to single-stranded/double-stranded DNA junctions. Based on the observed DNA-binding-induced multimerization, the structural similarity with both subdomains of DNase I, and the experimentally identified DNA-binding surface, we propose a model for DNA recognition by the Smr domain.

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Keywords: DNA repair; IF3C fold; NMR; DNA-binding domain; DNase I

Introduction

The small MutS-related (Smr) domain has previously been identified as the highly conserved C-terminal domain of MutS2 proteins.[1] MutS2 represents a subfamily of MutS homologues,[2] named after the DNA-mismatch recognition and binding component of the ternary *Escherichia coli* MutSLH complex involved in mismatch repair. MutS recognizes double-stranded DNA (dsDNA) mismatches ranging from single nucleotides to extended loops of unpaired bases.[3] MutH acts as a nicking endonuclease that exclusively targets the damaged unmethylated DNA daughter strand, while MutL provides the linking scaffold between MutH and MutS.[4] While MutS and MutL are widely conserved, the endonuclease MutH has no eukaryotic homolog; instead, eukaryotes often contain a MutS2 family member.[5]

*Corresponding author. E-mail address: g.e.folkers@uu.nl.
† T.D. and E.AB contributed equally to this work.
Present addresses: T. Diercks, CiC bioGUNE, Parque Tecnologico de Bizkaia, Ed. 800, 48160 Derio, Spain; E. AB, Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands; M. A. Daniels, Amsterdam University, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands; R. N. de Jong, Genmab BV, Yalelaan 60, 3584 CM Utrecht, The Netherlands.

Abbreviations used: Smr, small MutS-related; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; B3BP, Bcl3-binding protein; N4BP2, Nedd4-binding protein 2; EMSA, electrophoretic mobility shift assay; GST, glutathione *S*-transferase; EDTA, ethylenediaminetetraacetic acid; HSQC, heteronuclear single quantum coherence; NOE, nuclear Overhauser enhancement.

Like MutS1, MutS2 proteins recognize transitions between single-stranded DNA (ssDNA) and dsDNA that occur during recombination (e.g., at replication forks or Holliday junctions).[6,7] While MutS1 proteins recognize and remove heterologous mismatches, MutS2 proteins might interfere with both homeologous and homologous recombinations. Thereby, MutS2 proteins can regulate the rearrangement of endogenous DNA in meiotic crossover and chromosome segregation, as well as the incorporation of exogenous DNA.[6,7]

This functional difference between MutS1 and MutS2 is reflected in their distinct domain architecture[2] (Fig. 1a). Both share a high sequence conservation only for three central domains: the dimerization domain (III), the DNA-binding domain (IV), and an ATPase domain (V). The smaller MutS2 proteins, however, lack the N-terminal mismatch-recognition domain (I) and the connector domain (II).[9,10] In addition, MutS2 has the highly conserved Smr domain located at the C-terminus connected to the conserved core via a putative linker region.[4] This arrangement was suggested to emulate the structure of the bacterial ternary MutSLH complex, where the linker and the Smr domain would play the role of the MutL and MutH components, respectively, implying a MutH-like nicking endonuclease activity for Smr.[4,5] This hypothetical biochemical function was first verified for the C-terminal Smr domain of the human BCL-3 binding protein, Bcl3-binding protein (B3BP), which converts supercoiled plasmid DNA into nicked open circular DNA, confirming nicking endonuclease activity.[11] DNA binding and incision were also shown for the prokaryotic Smr domain in *Thermus thermophilus* MutS2.[12] The "resolving endonuclease" repair activity[4] of the Smr domain in MutS2 proteins can then explain their cellular function, namely, the reversion of DNA strand exchange reactions that initiate homologous recombination, as shown for *Helicobacter pylori* HpMutS2.[6,7]

In humans, the Smr domain is only present in the B3BP/Nedd4-binding protein 2 (N4BP2) protein, which was isolated in a yeast two-hybrid screen by its ability to interact with the E3 ubiquitin ligase Nedd4.[13] Although the exact function for this protein remains unclear, it was postulated to be involved in transcription, recombination, or DNA repair.[11] The recent suggestion that this protein might contribute to sporadic nasopharyngeal carcinoma in the Southern Chinese population underscores its importance.[14] Furthermore, the proteins shown to interact with B3BP, such as Nedd4 and Bcl3, are frequently linked to various cancer types.[15,16]

In this study, we present the NMR structure of the Smr domain—the most conserved domain of B3BP. We confirm DNA binding to mixed single-stranded/double-stranded DNA sequences. Both NMR chemical shift changes upon DNA addition and DNA binding experiments with B3BP–Smr mutants show that the most conserved residues, located in loop regions, form a contiguous exposed DNA-binding surface. Based on these interaction studies and structural homology with DNase I, we propose a model for DNA binding by the B3BP–Smr domain.

Results and Discussion

The Smr domain was identified through BLAST searches using the MutS2-specific C-terminal domain[1] that was subsequently shown to be present in bacteria and eukaryotes.[11] In the bacterial kingdom, this domain can be present either in isolation or in combination with MutS core domains III–V (Fig. 1a). Sequence analysis further revealed significant differences between the two lineages. An amino acid sequence of a representative subset of the eukaryotic Smr domains is shown in Fig. 1b. To determine the solution structure of the Smr domain, we expressed the Smr domain of B3BP (1688–1770) or an N-terminally extended domain (1657–1770). Only with the latter were we able to obtain sufficient soluble protein for structural analysis.

Since our Smr domain contains an N-terminal extension in comparison with the previously characterized Smr domain of B3BP, we first confirmed that this protein retains the ability to nick supercoiled DNA (Fig. 2a).[11] Surprisingly, linear DNA was observed at elevated Smr concentrations, suggesting additional (endo)nuclease activity. To exclude that the observed catalysis was mediated by impurities, a bacterial culture containing an empty vector was expressed and purified in parallel with the Smr domain protein; this control sample was not catalytically active (Fig. 2a). The metal cofactors magnesium or manganese was required for the nicking endonuclease activity (Fig. 2b), while barium, cadmium, and zinc failed to support this reaction (data not shown). The temperature optimum for this reaction (Fig. 2c) is in good agreement with the observed temperature-dependent unfolding of the Smr domain, as determined by thermofluor analysis[17] (Fig. 2d). These data indicate that the N-terminally extended B3BP–Smr domain is functionally active.

The B3BP–Smr domain folds as an α–β two-layer sandwich

The NMR structure of the B3BP–Smr domain (1688–1770) reveals a classical α2β4 sandwich structure with a βαβαββ succession of four β-strands and two α-helices. As shown in Fig. 1c, all four β-strands form one slightly twisted β-sheet, where strands β_1 (1690–1692), β_2 (1724–1728), and β_3 (1756–1761) run parallel, with the antiparallel β_4 (1764–1768) inserting between β_2 and β_3. The α-helices pack against one side of the β-sheet, with the longer slightly bent helix α_1 (1698–1719) running along strands β_1 and β_2, while helix α_2 (1742–1753) stacks against strands β_3 and β_4. The helices diverge towards their C-termini at an angle of ca 30°.

A prominent structural feature is the extended β_2–α_2 loop L_3 (1729–1741) protruding at the bottom of the α2β4 sandwich opposite both termini of the Smr domain (Fig. 1c, green). This loop is in close