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Protein arginine methyltransferase 7 has a novel homodimer-like structure formed by tandem repeats

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ABSTRACT

Protein arginine methyltransferase 7 (PRMT7) is a member of a family of enzymes that catalyze the transfer of methyl groups from *S*-adenosyl-L-methionine to nitrogen atoms on arginine residues. Here, we describe the crystal structure of *Caenorhabditis elegans* PRMT7 in complex with its reaction product *S*-adenosyl-L-homocysteine. The structural data indicated that PRMT7 harbors two tandem repeated PRMT core domains that form a novel homodimer-like structure. *S*-adenosyl-L-homocysteine bound to the N-terminal catalytic site only; the C-terminal catalytic site is occupied by a loop that inhibits cofactor binding. Mutagenesis demonstrated that only the N-terminal catalytic site of PRMT7 is responsible for cofactor binding.

1. Introduction

Protein arginine methylation is a common post-translational modification that has been implicated in numerous biological processes. Protein arginine methyltransferases (PRMTs) catalyze the transfer of methyl groups from *S*-adenosyl-L-methionine (AdoMet) to nitrogen atoms on arginine residues to produce methylarginine and *S*-adenosyl-L-homocysteine (AdoHcy). The mammalian PRMT family consists of nine members that are classified into three types (I, II, and III) according to their catalytic functions. Type I and II PRMTs produce asymmetric dimethylarginine and symmetric dimethylarginine, respectively, whereas type III PRMTs produce monomethylated arginine (Fig. S1a). All PRMTs contain a conserved core comprising a Rossmann fold domain and a β-barrel domain, as well as additional domains that confer various functions (Fig. S1b). PRMTs also contain a set of conserved sequence motifs and double E and threonine–histidine–tryptophan (THW) loops that are specific to the PRMT family (Fig. S2) [1].

It was reported recently that mouse and human PRMT7 exhibit type III activity [2,3]. The physiological roles of mammalian PRMT7 are associated with broad cellular processes [4,5], including methylation of Sm proteins, snRNP biogenesis [6], and the expression of genes involved in DNA repair [7]. Furthermore, PRMT7 appears to play a role in the maintenance of the pluripotent state of undifferentiated embryonic stem cells and germ cells [8], and a recent study reported that it antagonizes mixed-lineage leukemia 4-mediated cellular differentiation [9]. PRMT7 has also been associated with the promotion of breast cancer metastasis [10]. These findings suggest that PRMT7 is a potential target for anti-cancer therapeutics.

Structural studies have revealed that type I PRMTs contain a single PRMT core domain and dimerization of these proteins is required for their catalytic activity [11]. PRMT7 is unusual in that it contains two PRMT core domains in tandem (Fig. 1a). The sequence of the N-terminal domain of PRMT7 is well conserved between other PRMT family members; however, conservation of the C-terminal domain, including the residues within the conserved motifs, is relatively poor, suggesting that this domain is non-functional [12]. To obtain an insight into its structural organization, oligomerization, and function, we determined the crystal structure of *Caenorhabditis elegans* PRMT7 (*Ce*PRMT7) in complex with its substrate AdoHcy.

2. Materials and methods

2.1. Protein expression and purification

For protein expression, *Escherichia coli* (BL21 (DE3) pLysS) were transformed with the pGEX6P-1 plasmid encoding the full-length

Abbreviations: AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine; SAXS, small-angle X-ray scattering

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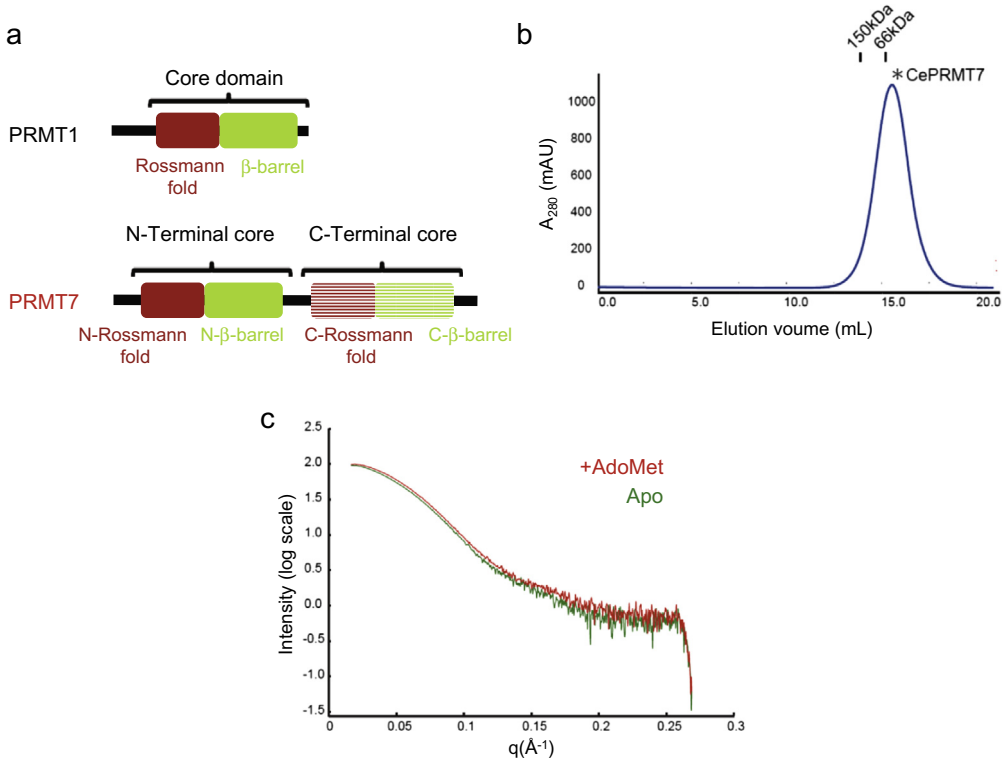

Fig. 1. Overview of PRMT1 and PRMT7, and the solution properties of *Ce*PRMT7. (a) Schematic representation of the domain structures of PRMT1 and PRMT7. The Rossmann fold domain and the β-barrel domain are shown in red and green, respectively. PRMT1 contains a single core domain whereas PRMT7 contains two core domains in tandem. (b) Gel filtration chromatography profile of *Ce*PRMT7. For comparison, the elution volumes of bovine serum albumin (66 kDa) and alcohol dehydrogenase (150 kDa) are shown above the *Ce*PRMT7 elution profile. (c) SAXS analysis of *Ce*PRMT7. The scattering curves of the apo and AdoMet-bound forms of *Ce*PRMT7 are shown in green and red, respectively.

GST-fused *Ce*PRMT7 gene [13]. The cells were grown in LB medium until they reached a density of 0.4–0.5 at 660 nm. After the addition of 0.13 mM IPTG, the cells were incubated at 18 °C overnight. GST-fused *Ce*PRMT7 was purified using Glutathione Sepharose 4B (GE Healthcare) and the GST-tag was removed using PreScission Protease (GE Healthcare). Additional purification of *Ce*PRMT7 was performed using HiTrapQ (GE Healthcare) and Superdex200 (GE Healthcare) columns. Purified *Ce*PRMT7 was concentrated to 15–22 mg/ml.

2.2. Crystallization

The crystal for the tetragonal form of *Ce*PRMT7 was obtained by incubating *Ce*PRMT7 with 0.6 mM histone H4 peptide and 1 mM AdoHcy. Sitting drops were set up at 20 °C by mixing the protein solution with reservoir solution containing 50 mM Bis–Tris (pH 6.7–7.0) and 9.5–13% (w/v) PEG MME 5000. The trigonal form crystal was obtained by sitting drop vapor diffusion at 15 °C by mixing *Ce*PRMT7 with 1.4 mM AdoHcy and the mother liquid containing 100 mM Tris (pH 8.5) and 2 M ammonium phosphate. Heavy atom derivatives were prepared with a solution containing 44 mM Bis–Tris (pH 6.9), 15% (w/v) PEG MME 5000, and 1.25 mM $KAu(CN)_4$. After soaking for 26 h, the derivatives were transferred to the harvesting solution (50 mM Bis–Tris (pH 6.9) and 16% (w/v) PEG MME 5000) and then to cryoprotectant solution (50 mM Bis–Tris (pH 6.9), 22% (w/v) PEG MME 5000). The native crystals were transferred to a harvesting solution (25 mM Bis–Tris (pH 6.7–7.0), 15% (w/v) PEG MME 5000) and the solution was changed to cryoprotectant containing 1 mM AdoHcy by vapor-diffusion overnight (reservoir solution: 50 mM Bis–Tris (pH 6.7–7.0), 30% (w/v) PEG MME 5000). The data were collected on a BL-5A beamline at The Photon Factory (PF; Tsukuba, Japan) and on a BL-44XU beamline at SPring-8 (Harima, Japan). The collected data were processed using the HKL2000 package [14].

2.3. Structure determination

The structure of the tetragonal form of *Ce*PRMT7 was solved by single-wavelength anomalous diffraction using the SHARP/autoSHARP program [15]. The trigonal structure was determined by molecular replacement using the Phaser program [16] and the structure of the tetragonal form of *Ce*PRMT7 as a search model. Initial model building was performed using the ARP/wARP program [17]. Manual model building and structure refinement was performed using the Coot [18] and Refmac5 [19] packages, respectively. We added the H4 peptide to determine the structure complexed with the peptide, but the electron density corresponding to the peptide was not observed. The geometry of the structure was verified using the PROCHECK program [20]. After initial phase determination, huge electron density was observed around Cys207, Cys345, Cys347, and His350, which were positioned in close proximity (Fig. S3a). During the refinement process, a disulfide bond between Cys207 and Cys345 and a covalent bond between the Sγ atom of Cys347 and the Nδ1 atom of His350 were incorporated into the model to minimize the residual density. Dual conformers for Cys345 and Cys347 were also incorporated (Fig. S3b). The distance between the Sγ atoms of Cys207 and Cys347 was 2.0 Å and the distance between the Sγ atoms of Cys345 and Cys347 was 1.8 Å; these distances were small enough to enable the formation of disulfide bonds. Therefore, the electron density map was derived from various combinations of covalent bonds between these residues. However, we were unable to generate a more precise model due to the limited resolution. Uninterpretable electron density was observed around SAH molecule. A summary of the crystallographic