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

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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, 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 in complex with its substrate AdoHcy.

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 (CePRMT7) 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
GST-fused CePRMT7 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 CePRMT7 was purified using Glutathione Sepharose 4B (GE Healthcare) and the GST-tag was removed using PreScission Protease (GE Healthcare). Additional purification of CePRMT7 was performed using HiTrapQ (GE Healthcare) and Superdex200 (GE Healthcare) columns. Purified CePRMT7 was concentrated to 15–22 mg/ml.

2.2. Crystallization

The crystal for the tetragonal form of CePRMT7 was obtained by incubating CePRMT7 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 CePRMT7 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), 15% (w/v) PEG MME 5000 and 1.25 mM KAu(CN)₄) 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 CePRMT7 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 CePRMT7 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 S củ atom of Cys347 and the N.att1 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 S củ atoms of Cys207 and Cys347 was 2.0 Å and the distance between the S củ 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...
measurements and statistics is shown in Table S1. The coordinate and structure factor data have been deposited to the Protein Data Bank (PDB) under the PDB IDs 3WSS and 3WST.

2.4. Small-angle X-ray scattering (SAXS)

SAXS data were collected using beamline BL-10C at the High Energy Accelerator Research Organization (KEK/PF). Phosphate-buffered saline containing 1 mM DTT with or without 0.8 mM AdoMet was used as the measurement solution. The radius of gyration was calculated using the Primus program [21]. Details of the experimental conditions and results are summarized in Table S2.

2.5. AdoMet binding assay

GST-fused CePRMT and its mutant (G72A) were used for the AdoMet binding assay. The binding assay and generation of the mutant were performed as described previously [13].

3. Results

3.1. Monomer structure of CePRMT7

First, we examined the solution properties of CePRMT7 using gel filtration column chromatography and SAXS analyses. The gel filtration profile indicated that CePRMT7 was eluted as a monomer (Fig. 1b). Consistent with this result, the SAXS analysis revealed that both apo-CePRMT7 and AdoMet-bound CePRMT7 exist as monomers in solution, demonstrating that the oligomeric state of the enzyme is independent of cofactor binding (Fig. 1c). Next, we determined the crystal structures of the tetragonal (P4_2_2) and trigonal (P3_1) forms of AdoHcy-bound CePRMT7. Fig. 2a and b shows the monomeric structure and topology of the protein, respectively. Analyses of the crystallization data indicated that the tetragonal form of CePRMT7 contains one molecule in an asymmetric unit, while the trigonal form contains eighteen molecules forming three spherical cages, each comprising six monomers in an asymmetric unit (Fig. S4a). Despite the differences in the crystallographic environments, the overall determined structures of the trigonal and tetragonal forms of CePRMT7 were almost identical (root mean square deviation, 0.7 Å). Because the quality of data for the tetragonal form of CePRMT7 was higher than that for the trigonal form, the tetragonal form was used for all further analyses, although it is worth noting that the trigonal form contains additional β-sheets at the N-terminal region (βN-1, βN0) (Fig. S4b and c).

3.2. Formation of a homodimer-like structure

Dimerization of the PRMT core domain is required for the enzymatic activity of type I PRMTs [11]. The dimerization interfaces of

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Fig. 2. Structure of CePRMT7. (a) The monomeric structure of CePRMT7 in the tetragonal form. The N-terminal Rossmann fold domain, the N-terminal β-barrel domain, and the N-terminal dimerization arm are shown in red, green, and yellow respectively. The C-terminal Rossmann fold domain, C-terminal β-barrel domain, and C-terminal dimerization arm are shown in magenta, dark green, and coral, respectively. The connecting loop between the N-terminal and C-terminal core domains is shown in black. AdoHcy in the N-terminal Rossmann fold domain is also indicated in stick model. (b) A topological illustration of CePRMT7. The secondary structures were assigned according to the structure in the tetragonal form. Helices and strands are denoted by cylinders and arrows, respectively. (c) The dimerization interface of CePRMT7. The residues forming the interface are shown in Fig. S2. The residues in the N-terminal Rossmann fold domain, N-terminal β-barrel domain, and N-terminal dimerization arm that form the interface are shown in red, green, and yellow respectively; the residues in the equivalent C-terminal domains are shown in magenta, dark green, and coral, respectively. The residues in the connecting loop is shown in black.
type I PRMTs consists of a Rossmann fold domain and a dimerization arm in the β-barrel domain. Dimeric PRMTs usually include a cavity at the center of their core. Notably, although CePRMT7 exists as a monomer in solution, the structural analysis revealed that it forms a homodimer-like structure comprising its N-terminal and C-terminal core domains (Fig. 2a). Analysis of the crystal structure revealed that the N-terminal and C-terminal domains of CePRMT7 are topologically similar (Fig. 2b) and are packed tightly with buried surface areas of 2372 Å² (Fig. 2c). The crystallization data also indicated that the N-terminal and C-terminal Rossmann folds of CePRMT7 (amino acids 21–171 and 349–481, respectively) are structurally similar to the Rossmann fold of rat PRMT1 (amino acids 38–175), with root mean square deviations of 1.5 Å and 2.5 Å, respectively (Fig. 3a). Similarly, the N-terminal and C-terminal β-barrel domains of CePRMT7, excluding the dimerization arm, are topologically similar to that of rat PRMT1, with root mean square deviations of 2.4 Å and 2.9 Å, respectively (Fig. 3b). The dimerization arm in the N-terminal core domain of CePRMT7 is a loop-rich structure with one short α-helix, while the C-terminal core domain has a helical dimerization arm containing two short β-strands (Fig. 3b). The orientations of the N-terminal and C-terminal dimerization arms of CePRMT7 are different (Fig. S5); as a result, the cavity in the homodimer is almost completely closed by the N[11]–N[17], N[11]–N[12], C[17]–C[17], and C[11]–C[12] regions, creating a new dimerization interface (Figs. 2c and S2).

3.3. Structure of the active site of CePRMT7 and its ability to bind AdoMet

A clear electron density corresponding to the AdoHcy cofactor was observed in the N-terminal Rossmann fold of the CePRMT7 crystal structure (Fig. 4a). CePRMT7 recognized AdoHcy in a manner similar to that of other PRMTs, indicating that PRMT family members share similar mechanisms of cofactor binding and catalysis. By contrast, no electron density was observed in the cofactor binding pocket of the C-terminal Rossmann fold. Unlike the N-terminal binding pocket, the C-terminal binding pocket was occupied by the double E loop and motif I of the C-terminal Rossmann fold domain, where the loops were stabilized by multiple hydrogen bonds, thereby inhibiting cofactor binding (Figs. 4b and c, and S2). Because of its differential structural features and the fact that the C-terminal active site lacks the catalytic glutamate residue in the double E loop that is conserved in all PRMT family members (Fig. S2), we hypothesized that the C-terminal domain of CePRMT7 is non-functional. To confirm this hypothesis, we performed a competitive assay using 3H-labeled and non-isotopically labeled AdoMet. CePRMT7 was able to bind to 3H-AdoMet binding ability and this binding was competitively abrogated by the addition of an excess of non-isotopically labeled AdoMet. Mutation of CePRMT7 at Gly72 (G72A) in motif I reduced its AdoMet binding ability markedly (Fig. 4d). These results demonstrate that the N-terminal PRMT core of CePRMT7 is responsible for its cofactor binding ability.

3.4. Arginine binding pocket

In general, the THW loop, double E loop and αY helix of PRMTs form an arginine binding pocket adjacent to the cofactor binding site (Fig. 5a) [22]. Analysis of the crystal structure of CePRMT7 revealed that, in this protein, the THW loop is positioned in close proximity to the double E loop, enabling the formation of a hydrogen bond between Thr144 and the main chain atom of Met302 in the THW loop. Moreover, the THW loop is positioned in close proximity to the αY helix due to an interaction between Asp35 in the αY helix and Asp299 in the THW loop, which liberates the end of the αY helix. These conformational changes cause Phe33 in the αY helix to be located close to Glu149 (Fig. 5b and c); as a result, the entrance to the arginine binding pocket of CePRMT7 is much narrower than those of rat PRMT1 and human PRMT5 (Fig. 5c). The THW loop in CePRMT7 is fixed by a network of direct and water-mediated hydrogen bonds that include Asp299 (corresponding to “Thr” in the THW loop),

![Fig. 3. Comparison of the Rossmann fold and β-barrel domains in CePRMT7 and rat PRMT1.](image-url)
involved in the formation of the newly created dimerization functionality [11], a single PRMT7 molecule can form a homodimer-like arrangement. The crystal structure of PRMT7, which harbors unique core domains in tandem. Analysis of the crystal structure indicated that, unlike PRMT1 through its cavity[11], it is possible that substrate access PRMT7 from the top surface due to the closure of the corresponding cavity. Acidic residues are concentrated at the N-terminal Rossmann fold on the top surface of PRMT7; this acidic environment might be important for substrate binding (Fig. S6).

The huge and unclear electron density of CePRMT7 was derived from complicated covalent bonds formed by Cys207, Cys345, Cys347, and His350. These covalent bonds likely contribute to stabilization of the intra-domain interactions, but their exact function remains unknown.

CePRMT5 has four conserved residues in its active site that are likely to be important for type II activity: Phe379, Lys385, Ser503, and Ser669 (corresponding to Phe327, Lys333, Ser439, and Ser578 in human PRMT5) [23,24]. The corresponding residues in type I PRMTs are Met, Arg, Tyr, and His, respectively. Notably, PRMT7 contains type I PRMT-like residues (Met36, Arg42, Thr144, and His300), all of which are conserved among PRMT7s (Fig. S2). It should also be noted that these residues are located at structurally similar positions. In PRMT1, the bulky Tyr148 residue seems to push out the THW and double E loops, making the entrance to the binding site wider. Conversely, a similar structural change in PRMT7 would not occur due to the small side chain of Thr144 (Fig. 5a and b). The Phe33 residue in CePRMT7, which makes the entrance to the arginine pocket narrower, is converted to Tyr in PRMT7 proteins from other species (Fig. S2). These bulky Tyr residues would likely play a similar role to Phe33 in CePRMT7, suggesting that the relatively narrow entrance to the arginine binding pocket is a common structural feature of PRMT7 proteins. Monomethylated arginine is unlikely to enter the binding pocket due to the steric hindrance. Processive PRMT could facilitate dimethylation by forming a homodimer [11,25], because the product of

Tyr537, and Gln532 (Fig. 5b). Notably, these residues are conserved among PRMT7 proteins from various species (Fig. S2) and are involved in the formation of the newly created dimerization interface.

4. Discussion

To our knowledge, this study is the first description of the crystal structure of PRMT7, which harbors unique core domains in tandem. Analysis of the crystal structure indicated that, unlike type I PRMTs, which require dimerization for enzymatic functionality [11], a single PRMT7 molecule can form a homodimer-like arrangement. The structural analysis indicated that the cavity of PRMT7 is occupied, suggesting the restriction of substrate access. However, although substrates access the binding site of PRMT1 through its cavity [11], it is possible that substrate access PRMT7 from the top surface due to the closure of the corresponding cavity. Acidic residues are concentrated at the N-terminal Rossmann fold on the top surface of PRMT7; this acidic environment might be important for substrate binding (Fig. S6).

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the first methylation reaction can enter the active site of the second molecule of the dimer without releasing the substrate from PRMT. Assuming that PRMT7 catalyzes the reaction in a manner similar to processive PRMT, the unique non-functional C-terminal domain and the narrow arginine binding pocket entrance of PRMT7 may explain its function as a type III enzyme (Fig. S7).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.03.053.

References


