PRMT7 Is a Member of the Protein Arginine Methyltransferase Family with a Distinct Substrate Specificity*

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We have identified a mammalian arginine N-methyltransferase, PRMT7, that can catalyze the formation of ω-N\(^2\)-monomethylarginine in peptides. This protein is encoded by a gene on human chromosome 16q22.1 (human locus AK001502). We expressed a full-length human cDNA construct in Escherichia coli as a glutathione S-transferase (GST) fusion protein. We found that GST-tagged PRMT7 catalyzes the S-adenosyl-[methyl-\(^3\)H]-l-methionine-dependent methylation of the synthetic peptide GGPGGRGGPGG-N\(_2\)H\(_4\) (R1). The radiolabeled peptide was purified by high-performance liquid chromatography and acid hydrolyzed to free amino acids. When the hydrolyzed products were separated by high-resolution cation-exchange chromatography, we were able to detect one identified species which co-migrated with an ω-N\(^2\)-S-monomethyl- \(\text{NG} \text{NG}'-\text{sym}-\text{monomethylarginine}\) standard. Surprisingly, GST-PRMT7 was not able to catalyze the in vitro methylation of a GST-fibrillarin (amino acids 1-148) fusion protein (GST-GAR), a methyl-accepting substrate for the previously characterized PRMT1, PRMT3, PRMT4, PRMT5, and PRMT6 enzymes. Nor was it able to methylate myelin basic protein or histone H2A, in vitro substrates of PRMT5. This specificity distinguishes PRMT7 from all of the other known arginine methyltransferases. An additional unique feature of PRMT7 is that it seems to have arisen from a gene duplication event and contains two putative AdoMet-binding motifs. To see if both motifs were necessary for activity, each putative domain was expressed as a GST-fusion and tested for activity with peptides R1 and R2 (acetyl-GGRGG-NH\(_2\)). These truncated proteins were enzymatically inactive, suggesting that both domains are required for functionality.

Protein arginine methylation is an apparently irreversible modification in which the guanidinium group of arginyl residues becomes methylated in a reaction with S-adenosylmethionine (AdoMet). This modification has been implicated in the regulation of signal transduction (1–4), transcription (5–7), RNA transport (8), and RNA splicing (9). Four different types of protein arginine methyltransferases have been described. Type I enzymes catalyze the formation of ω-N\(^2\)-monomethylarginine and ω-N\(^2\),N\(^2\)-asymmetric dimethylarginine residues. Type II enzymes form ω-N\(^2\)-monomethylarginine and ω-N\(^2\),N\(^2\)-sym-methyl dimethylarginine residues. Type III enzymes catalyze only the formation of ω-N\(^2\)-monomethylarginine residues. Finally, type IV enzymes form ω-N\(^2\)-monomethylarginine (for a review, see Ref. 10). The previously described mammalian enzymes PRMT1, PRMT3, PRMT4/CARM1, and PRMT6 and the yeast enzyme RMT1 have been shown to be responsible for type I methylation (5, 11–14). The mammalian enzyme PRMT5/JPBP1 catalyzes type II methylation (15), whereas the yeast RMT2 enzyme catalyzes type IV methylation (16). No activity has currently been defined for the mammalian PRMT2 gene product, although its amino acid sequence is similar to that of the other PRMT gene products (17).

In searching the human genome for novel protein arginine methyltransferases, we found a gene that appears to code for the seventh PRMT. A recombinantly expressed form of this PRMT is incapable of methylating GST-GAR, MBP, and histone H2A, typical substrates of the previously described protein arginine methyltransferases. However, this methyltransferase, now designated PRMT7, is able to methylate two arginine-containing peptides and exhibited type III enzymatic activity, catalyzing the formation of ω-N\(^2\)-monomethylarginine residues. A similar species has recently been described in Chinese hamster cells, but the biochemical reaction catalyzed by it has not yet been determined (18).

EXPERIMENTAL PROCEDURES

Construction of PRMT7, PRMT7ΔC, and PRMT7ΔN Expression Vectors—The plasmid pGEX-PRMT7 was constructed by PCR amplification of a 2.1-kb fragment from a template of an IMAGE human cDNA clone (clone ID 2900965; GenBank accession number AW675040) with primers PRMT7-N1 (5′-CTG TGG GCC-3′) and PRMT7-C1 (5′-CTG GCC GCG CGG-3′) and PRMT7-C1 (5′-CTG GCC GCG CGG-3′), which introduces a single N375K mutation to place a NotI site at the 3′ end of the fragment for the purpose of cloning. The PCR fragment was digested with SalI and NotI and ligated into pGEX/SN (19). The plasmid pGEX-PRMT7ΔC was constructed by PCR-amplification from pGEX/PRMT7 a 1.1-kb fragment with primers PRMT7-N1 and PRMT7-ΔC1 (5′-CGT TCC TCA CGG CCG CCG CTT CAA GAG CAG GAG CCG AGC-3′), which introduces a single N375K mutation to place a NotI site at the 3′ end of the fragment for the purpose of cloning. The PCR product was digested with SalI and NotI and ligated into pGEX/SN. The plasmid pGEX-PRMT7ΔN was constructed by PCR amplification of pGEX-PRMT7 with primers PRMT7-ΔN1 (5′-CTG TGG GCC GCG CGG-3′), and PRMT7-C1, resulting in a ω-N\(^2\)-monomethylarginine; [\(^3\)H]AdoMet, S-adenosyl-[methyl-\(^3\)H]-l-methionine; AdoHcy, S-adenosyl-l-homocysteine; HPLC, high-pressure liquid chromatography; MTA, 5′-deoxy-5′-methyl-thiodoasenosine; PRMT, protein arginine methyltransferase; THW loop, threonine-histidine/tryptophan loop.
950-bp fragment. PCR fragments were digested with SalI and NotI and ligated into pGEX(SN).

Purification of GST-GAR, GST-PRMT1, GST-PRMT7, GST-PRMT7/H9004C, and GST-PRMT7/H9004N—GST-GAR and GST-PRMT1 were constructed as described previously (11, 13). GST-GAR, GST-PRMT1, GST-PRMT7, GST-PRMT7/H9004C, and PRMT7/H9004N were overexpressed in Escherichia coli DH5α cells (Invitrogen) by induction with 0.4 mM isopropyl-β-D-thiogalactopyranoside. Cells were lysed in the presence of 100 μM phenylmethylsulfonyl fluoride with seven 20-s sonicator pulses (50% duty; setting 4) on ice with a Sonifier cell disrupter W-350 (Smith-Kline Corp.). The resulting lysate was centrifuged for 40 min at 23,000×g at 4°C. The protein was then purified from extracts by binding to glutathione-Sepharose 4B beads (Amersham Biosciences) according to the manufacturer’s instructions; the GST-fusion proteins were eluted with 30 mM glutathione, 50 mM Tris-HCl, pH 7.5, and 120 mM NaCl at protein concentrations of 0.1 to 0.2 mg/ml.

In Vitro Labeling of MBP, Histone H2A, GST-GAR, Peptide R1, and Peptide R2 and Chemical Analysis of the Methylated Species—10 μg of myelin basic protein (MBP) (purified from bovine brain, lyophilized powder; Sigma), 10 μg of histone H2A (purified from calf thymus, Roche Applied Science), 10 μg GST-GAR, 52 μg peptide R1 (GPGGRGPPG-NH₂, California Peptide Research, Inc.) or 26.5 μg of peptide R2 (acetyl-GGRGG-NH₂, California Peptide Research, Inc.) was incubated with 2 μg of GST-PRMT7. 52 μg of peptide R1 or 26.5 μg of peptide R2 were
in vacuo. Reactions were initiated by the addition of buffer (pH 7.5) was added to all reactions to a final concentration of 100 mM. Reactions were initiated by the addition of 3 μl of S-adenosyl-L-[methyl-3H]methionine ([3H]AdoMet, Amersham Pharmacia Biotech; 72.0–79.0 Ci/mmol; 13–14 μM) in a final volume of 60 μl. Reactions were allowed to proceed at 37 °C for 1 h. As a control, 2 μg of GST-GAR was added to 2 μl of GST-PRMT1 and sodium phosphate buffer (pH 7.5) for a final concentration of 100 mM and incubated at 37 °C for 30 min with 3 μl of [3H]AdoMet in a final volume of 30 μl. Reactions were stopped by freezing on dry ice. The in vitro reactions for MBP, histone H2A, and GST-GAR were mixed with 20 μg of bovine serum albumin as a carrier protein and an equal volume of 25% (w/v) trichloroacetic acid in a 6 × 50-mm glass vial and incubated at room temperature for 30 min. The precipitated protein was then centrifuged at 4000 × g for 30 min at 25 °C, the supernatant was drawn off and discarded, and the pellets were allowed to air dry. Acid hydrolysis was then carried out on these reactions in a water Pico-Tag Vapor-Phase apparatus in vacuo for 20 h at 110 °C using 200 μl of 6 N HCl. The in vitro reactions for peptides R1 and R2 were added to a 6 × 50-mm glass vial and dried by speed vacuum. 50 μl of 6 N HCl was added to each vial, and the samples were hydrolyzed in vacuo for 20 h at 110 °C.

Hydrolyzed samples were resuspended in 50 μl of water and mixed with 1.0 μmol of each of the standard ω-Nϵ-monomethylarginine (ω-MMA, acetate salt, Sigma) and asymmetric ω-Nϵ,Nϵ-dimethylarginine (ADMA, hydrochloride, Sigma) for amino acid analysis by column chromatography. 500 μl of citrate dilution buffer (0.2 mM Na3PO4, pH 2.2) was added to the hydrolyzed samples before loading onto a cation-exchange column (Beckman AA-15 sulfonated polystyrene beads; 0.9-mm inner diameter × 7-cm column height) equilibrated and eluted with sodium citrate buffer (0.35 mM Na3PO4, pH 5.27) at 1 ml/min at 55 °C.

Analysis of Column Fractions—Amino acid standards were determined by a ninhydrin assay as described previously (13). Radioactivity in column fractions was quantitated using a Beckman LS6500 counter as an average of three 3-min counting cycles. The efficiency of counting was determined to be 36% using a calibrated standard.

High-pressure Liquid Chromatography (HPLC) Purification of Methylated Peptide R1 and Chemical Analysis of the Purified Methylated Product—520 μg of peptide R1 was added to 20 μg of GST-PRMT7 and incubated with 10 μl of [3H]AdoMet at 37 °C for 5 h in a final volume of 600 μl and a final concentration of 100 mM sodium phosphate. All reactions were stopped by freezing on dry ice. Samples were then thawed and dried under vacuum in a Speed Vac apparatus and resuspended in 60 μl of water. An equal volume of 10% trichloroacetic acid was added to acidify the reaction and precipitate out the proteins that were present. The sample was spun at 10,000 × g for 5 min, and the supernatant was injected onto a C18 reverse-phase column (5-micron spherical particles, column dimensions 4.6 × 250 mm; Alltech Econosphere) equilibrated at room temperature in solvent A (0.1% trifluoroacetic acid in water). At a flow rate of 1 ml/min, the sample was eluted with increasing proportions of solvent B (0.1% trifluoroacetic acid, 0.9% water in acetonitrile) using the following gradient: 0–5 min, 0% B; 5–55 min, 0–100% B; 55–65 min, 100% B; and 65–70 min, 100% B–0 B. The column effluent was monitored at 214 nm and 1-ml fractions were collected. Fractions corresponding to the peptide peak were pooled and concentrated under vacuum to ~200 μl. Samples were then injected onto the C18 column and eluted under the same conditions as before. Fractions corresponding to the peptide peak were dried under vacuum in a 6 × 50-mm glass vial. 50 μl of 6 N HCl was added to each 6 × 50-mm glass vial, and acid hydrolysis was then carried out on the reactions in a water Pico-Tag Vapor-Phase apparatus in vacuo for 20 h at 110 °C. The hydrolyzed samples were resuspended in 50 μl of water and analyzed on a cation exchange column as described above.

UV Crosslinking with [3H]AdoMet—Either 7.2 μg of GST-PRMT7, 3.2 μg of GST-PRMT7ΔC, or 2 μg of GST-PRMT7ΔN were incubated with 0.5 μl [3H]AdoMet (72 Ci/mmol) and 5 mM dithiothreitol in 50 mM sodium phosphate, pH 7.4, in a total volume of 50 μl. In control reac-
The PRMT family of enzymes exhibits amino acid conservation within the characteristic seven β-strand methyltransferase motifs I, post I, II, and III (20), and in other portions of the polypeptide chain (14, 21). A search of the GenBank NR database of the human PRMT7 sequence revealed clear orthologs in a variety of organisms including mice (85% identity over 690 residues), the frog Xenopus laevis (64% identity over 686 residues), the fly Drosophila melanogaster (36% identity over 706 residues), the worm Caenorhabditis elegans (30% identity over 700 residues), and the higher plant Arabidopsis thaliana (31% identity over 667 residues). However, we find no homologs in the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe or in prokaryotes.

A plasmid encoding a fusion construct of GST and the full-length coding sequence of PRMT7 was expressed in E. coli cells and purified by affinity chromatography, as described under “Experimental Procedures.” A single major polypeptide of 108 kDa was observed, consistent with that expected for GST linked to the 692-residue PRMT7 polypeptide (Fig. 2A). We first asked if the GST-PRMT7 fusion protein could bind to the methyl donor AdoMet. GST-PRMT7 was exposed to UV irradiation at 254 nm in the presence of [3H]AdoMet. The reaction mixture was then fractionated by SDS gel electrophoresis and analyzed by autoradiography. As shown in Fig. 2B, a radiolabeled band was observed migrating at 108 kDa, corresponding to the GST-PRMT7 fusion polypeptide. In control experiments, addition of the non-isotopically labeled AdoMet and AdoHcy substrate and product fully inhibited the crosslinking reaction, whereas the addition of the UV-absorbing non-substrate ATP had no effect. An intermediate effect was seen with the substrate analog MTA (Fig. 2B).

Histones, MBP, and GST-GAR have been used as methyl-accepting substrates to identify, partially purify, and characterize protein-arginine N-methyltransferases (12, 15, 25). To identify the possible methyltransferase activity of PRMT7, the GST-fusion protein was incubated with GST-GAR, MBP, or histone H2A in the presence of [3H]AdoMet. Protein reaction products were precipitated with trichloroacetic acid, acid-hydrolyzed to their amino acid components, and fractionated by high-resolution amino acid cation-exchange chromatography along with standards of unlabeled symmetric ADMA and ω-MMA (Fig. 3). No protein arginine methyltransferase activity was observed when GST-GAR, MBP, or histone H2A was used as a substrate for GST-PRMT7. However, activity was seen in a control experiment when GST-GAR was used as a substrate for GST-PRMT7 under these conditions (Fig. 3A). Because PRMT7 cannot use these substrates as methyl-acceptor enzymes, they may have a substrate specificity that is distinct from the other known arginine methyltransferases.

The R1 peptide, GGFGGRGGFG-amide, derived from the fibrillarin consensus methyl-acceptor site for PRMT enzymes, encodes a 692-amino acid hypothetical protein FLJ10640. We compare the sequence of this protein, now designated PRMT7, with the six previously described human PRMTs in Fig. 1. PRMT7, although sharing conserved seven β-strand methyltransferase motifs I and post I with other PRMTs, appears more distantly related at motifs II and III. Significantly, PRMT7 shares additional sequences common to only the PRMT family in the N-terminal region (residues 22–65) in the region between motifs I and post I and at two active-site regions, the “double E” loop and adjacent sequences between motifs II and III, and the THW loop and adjacent sequences at residues 312–330 (21, 24). Interestingly, although the two glutamate residues are present at the conserved positions in the double E loop, there is only marginal similarity in the residues in between that are generally conserved in most of the other PRMT enzymes. Furthermore, PRMT7 is unique among these enzymes in that the threonine residue of the THW loop is replaced by an aspartic acid residue in PRMT7.

A protein-protein BLAST search of the GenBank NR database with the human PRMT7 sequence revealed clear orthologs in a variety of organisms including mice (85% identity over 690 residues), the frog Xenopus laevis (64% identity over 686 residues), the fly Drosophila melanogaster (36% identity over 706 residues), the worm Caenorhabditis elegans (30% identity over 700 residues), and the higher plant Arabidopsis thaliana (31% identity over 667 residues). However, we find no homologs in the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe or in prokaryotes.

**RESULTS AND DISCUSSION**

The PRMT family of enzymes exhibits amino acid conservation within the characteristic seven β-strand methyltransferase motifs I, post I, II, and III (20), and in other portions of the polypeptide chain (14, 21). A search of the GenBank NR amino acid database using the gapped-BLAST method (22) for sequences that match known PRMT enzymes identified a full-length cDNA as part of the National Institutes of Health Mammalian Gene Collection (23). This cDNA (locus AK001502) is encoded by a gene on human chromosome 16q22.1 and itself...
has been used to characterize protein-arginine N-methyltransferase activity present in cultured cells and in mammalian tissues along with the activity of PRMT1 (11, 26). Even though PRMT7 was unable to methylate the parent protein GST-GAR, we decided to ask whether PRMT7 might be able to methylate the consensus site in a peptide context. Reactions with peptide R1 alone, peptide R1 plus GST-PRMT7, or GST-PRMT7 alone were incubated with [3H]AdoMet. The R1 peptide was then separated from other reaction components by C18 HPLC. 3H-radioactivity was found to co-migrate with peptide R1 on the C18 column in all three reaction mixtures (data not shown). However, when the fractions corresponding to the peptide peak were pooled in each case, acid-hydrolyzed, and then fractionated by high-resolution amino acid analysis, we found radioactivity to elute with the /H9275-NG-monomethylarginine derivative only in the reaction containing GST-PRMT7 and peptide R1, and not in the control reactions containing PRMT7 alone or peptide alone (Fig. 4).

Because GST-PRMT7 is able to methylate a small peptide, we decided to see whether PRMT7 could methylate free arginine. We were intrigued by the presence of free /H9275-N^G-monomethylarginine and asymmetric /H9275,N^G-dimethylarginine in mammals and their role as endogenous inhibitors of the nitric oxide signaling pathway (27, 28). Free arginine (1 mM) was incubated with GST-PRMT7 and [3H]AdoMet. Analysis of the reaction by high-resolution cation exchange chromatography did not reveal the presence of any /H-methylated arginine derivatives, suggesting that free arginine is not, in fact, a substrate of PRMT7 (data not shown).

PRMT7 appears to have resulted from a gene duplication event resulting in two PRMT domains (Fig. 5). The N-terminal

![Fig. 5. PRMT7 contains two putative methyltransferase domains. A, a diagram of PRMT7 indicating the position of the two methyltransferase domains. The positions of the signature methyltransferase motifs I, post I, II, and III are displayed in black. B, the amino acid sequence alignment of PRMT1 (splice variant 2) with PRMT7ΔC and PRMT7ΔN with conserved amino acid sequences in black boxes to highlight the majority consensus. Signature seven β-strand methyltransferase motifs I, post I, II, and III are indicated by boxes. The PRMT-conserved glutamate residues of the “double E” loop are indicated by asterisks, and the PRMT-conserved THW loop is also boxed.](#)

![Fig. 6. Deletion of PRMT7 C-terminal or N-terminal domain leads to a loss of methyltransferase activity. Peptide R1 (A) or peptide R2 (B) were incubated with [3H]AdoMet and either GST-PRMT7 (●), GST-PRMT7ΔC (amino acids 1–378, ▲), or GST-PRMT7ΔN (amino acids 379–992, ▼) as described under “Experimental Procedures.” After reactions were dried and acid-hydrolyzed, methylated standards were added, and the hydrolysate was fractionated using cation exchange chromatography, as described under “Experimental Procedures.” 3H-radioactivity was determined by counting a 600-μl aliquot of every other fraction diluted with 400 μl of water in 5 ml of fluor three times for 3 min. The unlabeled amino acid standards were analyzed using a ninhydrin assay with 100-μl aliquots of every other fraction.](#)
Peptidyl Arginine Methyltransferase Activity of PRMT7

half of PRMT7 shown in Fig. 1 (amino acids 1–378) contains a PRMT domain that is most similar to the catalytic core of PRMT5, sharing 33% sequence identity, and is least similar to PRMT2 and PRMT6 (21% identity). The C-terminal half of PRMT7 (amino acids 379–692) shares 26% sequence identity with PRMT1 and 22% sequence identity with the N-terminal half of the protein (Fig. 5). The C-terminal half of PRMT7 shows less conservation in the AdoMet-binding motif I than the N-terminal half and other PRMT enzymes, although it has an identifiable THW loop. To determine whether the PRMT7 domains can function independently, N-terminal and C-terminal fusion proteins (GST-PRMT7ΔC and GST-PRMT7ΔN) were purified from E. coli and analyzed for peptide arginine methyltransferase activity. When either peptide R1 (Fig. 6A) or peptide R2 (Fig. 6B) was used as a substrate, neither fusion protein showed any methyltransferase activity, whereas the full-length construct was able to catalyze the formation of ω-N^2-monomethylarginine in both peptides. UV crosslinking experiments using [3H]AdoMet were also performed with GST-PRMT7 and ethylarginine in both peptides. UV crosslinking experiments were performed to determine whether PRMT7 may be a peptide-specific enzyme. PRMT7, or to the presence of additional subunits or regulatory domains, it is possible that this difference is species-dependent. Because the human and hamster proteins differ at 102 residues, it is hard to rule out the possibility that the activity seen with the hamster enzyme preparation may be due to a contaminating PRMT activity in the immunoprecipitated PRMT7, or to the presence of additional subunits or regulatory posttranslational modifications (12, 15).

In this work, we present direct evidence that GST-PRMT7 is an arginine methyltransferase that can catalyze the formation of ω-N^2-monomethylarginine in peptides, although its physiological substrate(s) have yet to be determined. The facts that PRMT7 has an apparent duplication of methyltransferase domains and is incapable of methylating the common PRMT substrates GST-GAR, MBP, or histone H2A distinguishes it from all other known arginine methyltransferases. It is not clear whether PRMT7 may be a peptide-specific enzyme in vivo or may be specific for yet unidentified substrates. It also remains to be determined whether PRMT7 would be capable of the addition of a second methyl group to an arginine residue in an endogenous substrate to generate the asymmetric ADMA residue of type I enzymes or the ω-N^2,ω-N^4-symmetric dimethylarginine residues of type II enzymes. It was previously suggested that a crucial difference between enzymes that catalyze type I versus type II methylation reactions is the size of the side chain at the position corresponding to residue 163 in PRMT1 (15, 21). Here, the type I PRMT1, PRMT3, PRMT4, and PRMT6 enzymes contain a methionine residue, and the type II PRMT5 enzyme contains a serine residue; the latter may allow the second methylation reaction to occur on the opposite guanidino nitrogen atom. In the case of PRMT7, the residue here is an alanine, suggesting the possibility of type II catalysis.

REFERENCES