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PRMT5 (Janus Kinase-binding Protein 1) Catalyzes the Formation of Symmetric Dimethylarginine Residues in Proteins*

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We have identified a new mammalian protein arginine *N*-methyltransferase, PRMT5, formerly designated Janus kinase-binding protein 1, that can catalyze the formation of ω-N^G-monomethylarginine and symmetric ω-N^G,$N^{G'}$-dimethylarginine in a variety of proteins. A hemagglutinin peptide-tagged PRMT5 complex purified from human HeLa cells catalyzes the *S*-adenosyl-L-[*methyl*-^{3}H]methionine-dependent *in vitro* methylation of myelin basic protein. When the radiolabeled myelin basic protein was acid-hydrolyzed to free amino acids, and the products were separated by high-resolution cation exchange chromatography, we were able to detect two tritiated species. One species co-migrated with a ω-N^G-monomethylarginine standard, and the other co-chromatographed with a symmetric ω-N^G,$N^{G'}$-dimethylarginine standard. Upon base treatment, this second species formed methylamine, a breakdown product characteristic of symmetric ω-N^G,$N^{G'}$-dimethylarginine. Further analysis of these two species by thin layer chromatography confirmed their identification as ω-N^G-monomethylarginine and symmetric ω-N^G,$N^{G'}$-dimethylarginine. The hemagglutinin-PRMT5 complex was also able to monomethylate and symmetrically dimethylate bovine histone H2A and a glutathione *S*-transferase-fibrillarin (amino acids 1–148) fusion protein (glutathione *S*-transferase-GAR). A mutation introduced into the *S*-adenosyl-L-methionine-binding motif I of a *myc*-tagged PRMT5 construct in COS-1 cells led to a near complete loss of observed enzymatic activity. PRMT5 is the first example of a catalytic chain for a type II protein arginine *N*-methyltransferase that can result in the formation of symmetric dimethylarginine residues as observed previously in myelin basic protein, Sm small nuclear ribonucleoproteins, and other polypeptides.

Protein arginine methylation has been implicated in the regulation of signal transduction, transcription, and RNA transport (1–5). It is an apparently irreversible modification in which the guanidinium group of arginyl residues becomes methylated in a reaction with *S*-adenosylmethionine (AdoMet)[1] as the methyl donor. Four different types of protein arginine methyltransferase enzymes have been discovered to date. Type I enzymes methylate arginine residues to form ω-N^G-monomethylarginine residues and asymmetric ω-N^G,N^G-dimethylarginine residues; type II enzymes catalyze the formation of ω-N^G-monomethylarginine residues and symmetric ω-N^G,$N^{G'}$-dimethylarginine residues; type III enzymes catalyze the formation of only ω-N^G-monomethylarginine residues; and type IV enzymes catalyze the formation of δ-N^G-monomethylarginine (for a review, see Ref. 6). Genes encoding enzymes responsible for type I methylation, such as the human *PRMT1* and *PRMT3* and yeast *RMT1*, and for type IV methylation, such as yeast *RMT2*, have already been identified (7–10). However, genes encoding species that are capable of the type II or type III methylation have yet to be reported.

The type II enzyme activity has been of much interest because it monomethylates and symmetrically dimethylates myelin basic protein (MBP) at arginine 107 in mammalian species (11–13). MBP is a highly conserved protein occurring as a single species in humans and cattle and as several different isoforms in rodents (for reviews, see Refs. 13 and 14). MBP is a major myelin protein constituting 30% of the total membrane-associated protein (15). The ratio of unmethylated arginine to ω-N^G-monomethylarginine to symmetric ω-N^G,$N^{G'}$-dimethylarginine at position 107 varies from species to species, and it has been postulated that older myelin is more highly methylated (16). Methylation of MBP has been implicated in maintaining the integrity of the myelin sheath. Subacute combined degeneration, a disorder characterized by the degeneration of the myelin sheath, can be induced in mice and monkeys when methylation of MBP is inhibited by exposure to an atmosphere containing 15% nitrous oxide (17, 18).

Until recently, symmetric dimethylarginine residues have been reported to be found only in myelin basic protein. However, two human Sm small nuclear ribonucleoproteins, D1 and D3, involved in mRNA splicing have recently been discovered to contain both ω-N^G-monomethylarginine and symmetric ω-N^G,$N^{G'}$-dimethylarginine residues in their C-terminal domains (19). We have recently found that purified D1 and D3 are methylated *in vitro* by PRMT5.[2] Although the role of methylation of these two proteins has yet to be determined, the methylation of D1 forms a major linear epitope for anti-Sm auto-

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[1] The abbreviations used are: AdoMet, *S*-adenosyl-L-methionine; SDMA, symmetric ω-N^G,$N^{G'}$-dimethylarginine; MMA, ω-N^G-monomethylarginine; lsqb]^{3}H]AdoMet, *S*-adenosyl-L-[*methyl*-^{3}H]methionine; JBP1, Janus kinase-binding protein 1; MBP, myelin basic protein; HA, hemagglutinin; GST, glutathione *S*-transferase.

[2] J.-H. Lee, J. R. Cook, S. Gunderson, and S. Pestka, unpublished data.

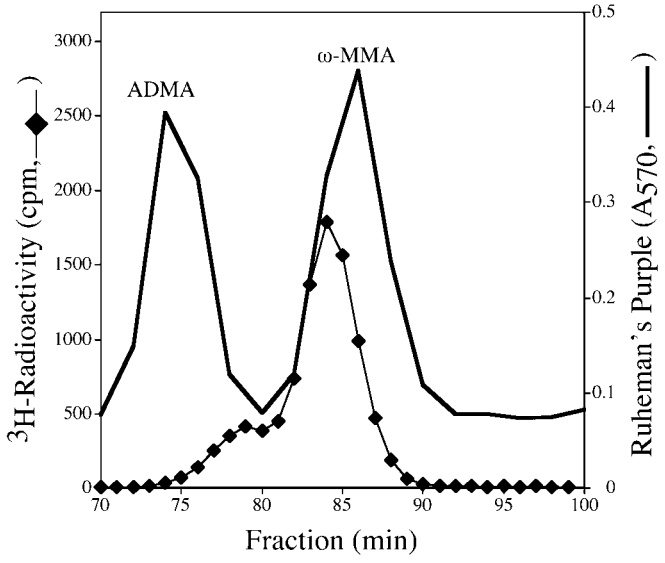

FIG. 1. **Amino acid analysis of myelin basic protein modified by the PRMT5 methyltransferase.** Myelin basic protein was methylated by HA-PRMT5 *in vitro* with [^{3}H]AdoMet (see "Experimental Procedures"). Labeled proteins were precipitated with trichloroacetic acid and acid-hydrolyzed as described under "Experimental Procedures." Samples were resuspended in water and mixed with 1.0 μmol of each of the standards (ω-N^G-monomethylarginine and asymmetric ω-N^G,N^G-dimethylarginine). 500 μl of citrate sample dilution buffer (0.2 M citrate in Na^+ containing 2% thiodiglycol and 0.1% phenol, pH 2.2) was added to the hydrolyzed sample before loading onto an amino acid cation exchange column (Beckman AA-15 sulfonated polystyrene beads; 0.9-cm inner diameter × 11-cm column height) equilibrated and eluted with sodium citrate buffer (0.35 M citrate in Na^+, pH 5.27) at 1 ml/min at 55 °C. ^{3}H radioactivity was determined by counting a 200-μl aliquot of every fraction diluted with 400 μl of water in 5 ml of fluor (Safety Solve; Research Products International) three times for 3 min. The unlabeled amino acid standards were analyzed using a ninhydrin assay with 100-μl aliquots of every other fraction (5).

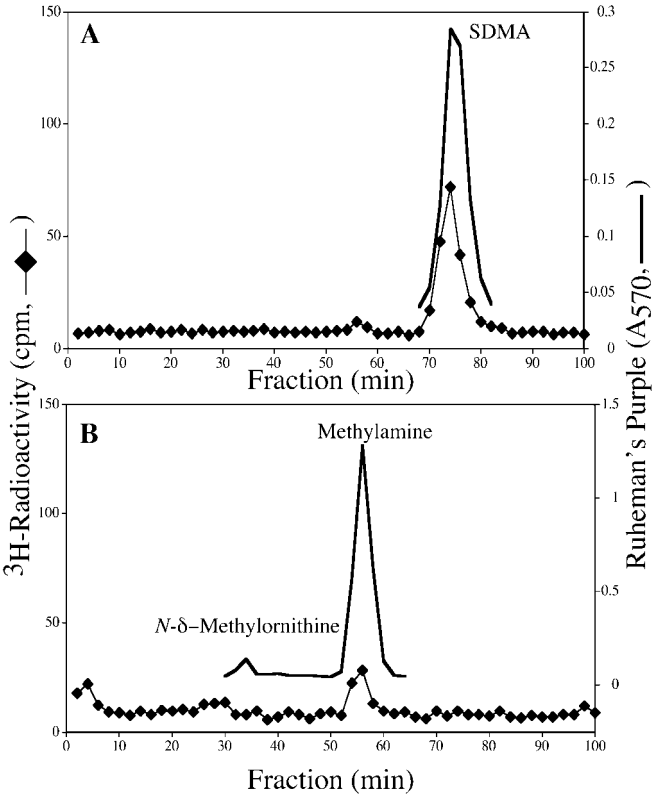

FIG. 2. **Base treatment of the ^{3}H-methylated arginine derivative from MBP produces [^{3}H]methylamine and not δ-N^G-[^{3}H]monomethylornithine.** Fractions 76–79 from the chromatograph shown in Fig. 1 were pooled, mixed with 50 μl of 10 mM symmetric ω-N^G,$N^{G'}$-dimethylarginine, and desalted by gel filtration as described under "Experimental Procedures." The desalted pool was then concentrated to ~1.0 ml by vacuum centrifugation in a Speedvac apparatus. *A*, a 200-μl aliquot of the desalted pool was spiked with 20 μl of 10 mM symmetric ω-N^G,$N^{G'}$-dimethylarginine and brought to a pH of 2.0 with 6 N HCl. The sample was then re-chromatographed on the amino acid cation exchange column. *B*, a 200-μl aliquot of the desalted pool was mixed with 50 μl of 10 M NaOH and incubated at 55 °C for 24 h. The sample was then diluted with 4.25 ml of water and mixed with 50 μl of ~0.02 M δ-*N*-methylornithine, which was synthesized as described previously (23), and 5 μl of 0.2 M methylamine. The sample was brought to a pH of 2.0 and re-chromatographed as described above.

antibodies, which are found in people with systemic lupus erythematosus (19).

In a yeast two-hybrid screen to search for proteins that bind the second polypeptide chain of Janus kinase 2, a protein arginine methyltransferase was identified as Janus kinase-binding protein 1 (JBP1) and shown to catalyze the methylation of MBP and histones H4 and H2A *in vitro* (20), as does its yeast homologue Hsl7p (21). However, the type of methylation of these proteins was not established, although JBP1 showed amino acid sequence similarity to the family of previously identified type I protein arginine methyltransferase catalytic subunits (20). Recent evidence has been presented that the methyl-accepting substrates are in fact protein arginine residues; however, whether the enzyme catalyzes a type I or type II reaction was not determined (22). Here we provide evidence that JBP1 can catalyze the formation of ω-N^G-monomethylarginine and symmetric ω-N^G,$N^{G'}$-dimethylarginine on a variety of substrates *in vitro*. Therefore, JBP1, now designated PRMT5, represents the first gene encoding a type II protein arginine *N*-methyltransferase. This PRMT5 polypeptide is a candidate catalytic unit that may be responsible for the modification of myelin basic protein *in vivo*, the Sm ribonucleoproteins, and additional proteins that require the presence of symmetric dimethylarginine residues.

EXPERIMENTAL PROCEDURES

Purification of GST-GAR—A GST-human fibrillarin (residues 1–148) fusion protein (designated GST-GAR) (8) was overexpressed in *Escherichia coli* DH5α cells (Life Technologies, Inc.) by induction with a final concentration of 0.4 mM isopropyl-β-D-thiogalactopyranoside. The protein was then purified from extracts by binding to glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech) according to the manufacturer's instructions, but in the presence of 100 μM phenylmethylsulfonyl fluoride and eluted with 30 mM glutathione, 50 mM Tris-HCl, pH 7.5, 120 mM NaCl, and 2% glycerol. The protein was then desalted on a Sephadex G-25 gel filtration column (13.5-cm column height × 2.5-cm inner diameter; 60-ml bed volume) in 50 mM sodium phosphate, pH 7.5.

Construction of myc-PRMT5 and myc-PRMT5ΔGAGRG—Wild type *myc*-PRMT5 was cloned as described previously (20). The mutant *myc*-PRMT5ΔGAGRG (amino acids 365–369) was constructed by site-directed mutagenesis with phage M13 (23). The oligonucleotide used to introduce the deletion was 5′-CTGATGGTGCTGCCCCTGGTGAACGCTTCCC-3′.

In Vitro Labeling of MBP, Histone H2A, and GST-GAR—HA-JBP1 (PRMT5) was immunoprecipitated with anti-HA antibody from the HeLa-HA-SPMT cell line as described previously (20, 21). 10–100 μg of MBP (Sigma product M1891; purified from bovine brain; lyophilized powder), 10–100 μg of histone H2A (Boehringer Mannheim product 1,034,740; purified from calf thymus), or 2 μg of GST-GAR was added to the HA-PRMT5 (immunoprecipitated from 1×10^7 HeLa cells/reaction) bound to protein A/G beads, which were then incubated at 37 °C for 5 h with 3 μl of *S*-adenosyl-L-[*methyl*-^{3}H]methionine ([^{3}H]AdoMet; Amersham Pharmacia Biotech; 72.0–79.0 Ci/mmol; 13–14 μM) for GST-GAR and 5 μl of [^{3}H]AdoMet for MBP and histone H2A in a final volume of 30 μl for GST-GAR and 50 μl for histone H2A and MBP (21). Reactions were stopped by freezing on dry ice.

For *myc*-tagged proteins, *myc*-PRMT5 and *myc*-PRMT5ΔGAGRG were immunoprecipitated with anti-*myc* antibody from 7.5×10^6 COS-1 cells as described previously (20). 10 μg of MBP was added to either *myc*-PRMT5 or *myc*-PRMT5ΔGAGRG, which was then incubated at

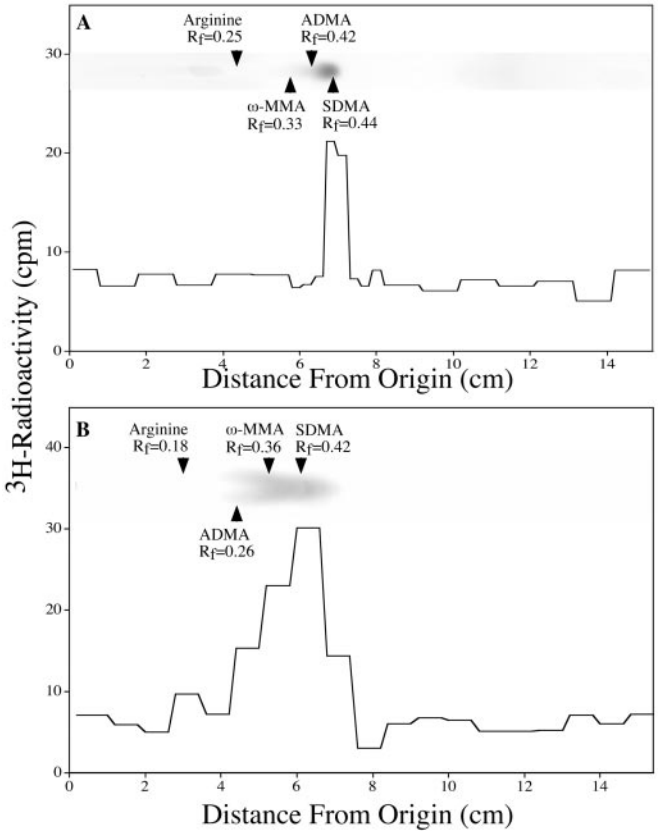

FIG. 3. **HA-PRMT5 can dimethylate arginine residues symmetrically as shown by the co-migration of its radiolabeled amino acid products with symmetric $N^G,N^{G'}$-dimethylarginine on thin layer chromatography.** 10 μl of the desalted pool from Fig. 2 was analyzed by thin layer chromatography at room temperature on a 20-cm sheet coated with either a layer of silica gel (*A*) or a layer of cellulose (*B*). *A* had a mobile phase consisting of methanol:14.8 N ammonium hydroxide (3:1, v/v), and *B* had a mobile phase consisting of *n*-butanol:acetic acid:water (4:1:1, v/v/v). The sheets were heated at 37 °C to evaporate the solvents and then sprayed with ninhydrin (10 mg/ml in acetone). The sheets were incubated again at 37 °C until the Ruheman's Purple color appeared. The ^{3}H radioactivity was determined by dividing the lanes into slices and scraping either the silica or cellulose into scintillation vials. Slices were then diluted with 500 μl of water in 5 ml of fluor and counted three times for 3 min.

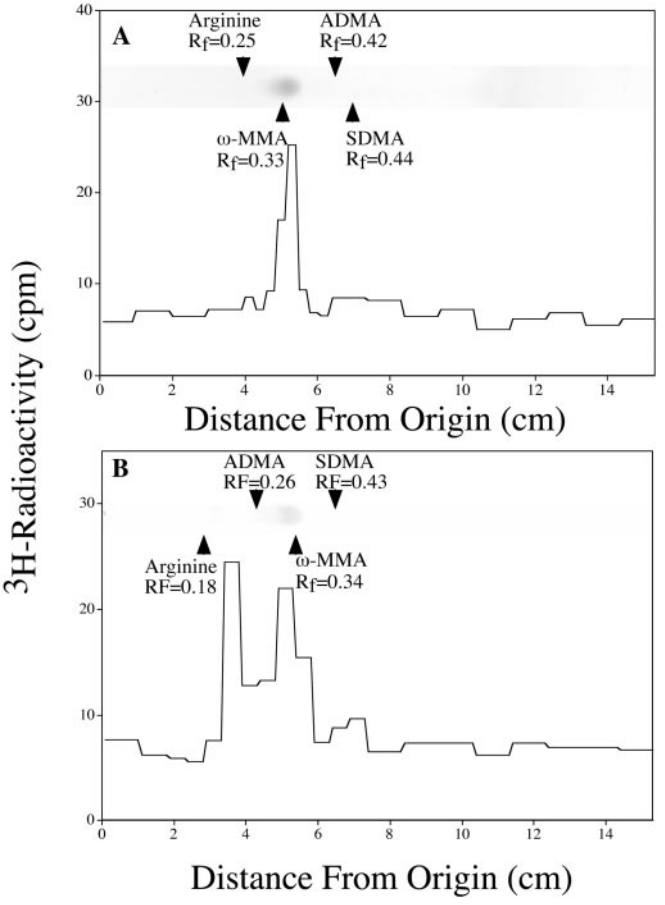

FIG. 4. **HA-PRMT5 can monomethylate arginine at the ω nitrogen as shown by co-migration with ω-N^G-monomethylarginine on thin layer chromatography.** A desalted pool of fractions 83–86 from Fig. 1 was desalted by gel filtration chromatography as described for fractions 76–79 in the Fig. 2 legend, and the sample was then concentrated to 1.5 ml in a Speedvac apparatus and analyzed by thin layer chromatography at room temperature on a 20-cm sheet coated with either a layer of silica gel (*A*) or a layer of cellulose (*B*) as described in the Fig. 3 legend.

37 °C for 5 h with 5 μl of [^{3}H]AdoMet (Amersham Pharmacia Biotech; 82.0 Ci/mmol; 12 μM) in a final volume of 30 μl.

Chemical Analysis of Methylated Species—A 5-μl aliquot of the above-mentioned *in vitro* reaction for MBP and histone H2A and a 30-μl aliquot of the above-mentioned *in vitro* reaction for GST-GAR were mixed with 11.1 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 20 min. The precipitated protein was then centrifuged at 4000 × *g* for 40 min at 25 °C, the supernatant was drawn off and discarded, and the pellets were washed once with an equal volume of acetone at −20 °C. After centrifugation for 20 min as described above, the acetone was discarded, and the pellets were allowed to dry. Acid hydrolysis was carried out on the dried pellet in a Waters Pico-Tag vapor-phase apparatus *in vacuo* for 20 h at 110 °C using 200 μl of 6 N HCl, and the hydrolyzed samples were resuspended in 50 μl of water for analysis on an amino acid cation exchange column. The column was regenerated with 0.2 N NaOH. Standard amino acids included ω-N^G-monomethylarginine (Sigma product M7033; acetate salt), asymmetric ω-N^G,N^G-dimethylarginine (Sigma product D4268; hydrochloride), and symmetric $N^G,N^{G'}$-dimethylarginine (Sigma product D0390; di-(*p*-hydroxyazobenzene-*p'*-sulfonate) salt).

Desalting of the Methylated Arginine Derivatives—Pooled fractions from the cation exchange column were loaded onto a Sephadex G-15 column (1.5 cm in diameter and 77 cm in length) at 25 °C equilibrated with 0.1 M acetic acid. Fractions (3.5 ml) were collected, and their conductance was determined with a YSI conductance meter model 35. Radioactivity was measured by counting a 200-μl aliquot of every fraction diluted with 400 μl of water in 5 ml of Safety Solve scintillation fluor (Research Products International). A 100-μl aliquot was tested for the nonradioactive standards using the ninhydrin assay as described previously (5).

RESULTS AND DISCUSSION

To determine the specific products of the methylation reaction catalyzed by PRMT5 (JBP1), HA-tagged PRMT5 was isolated from HeLa cells and incubated with MBP and [^{3}H]AdoMet. Protein reaction products were precipitated with trichloroacetic acid, acid-hydrolyzed to their amino acid components, and fractionated on a high-resolution amino acid analysis cation exchange column along with standards of unlabeled asymmetric ω-N^G,N^G-dimethylarginine and ω-N^G-monomethylarginine, species that are well separated under our conditions (Fig. 1). We found that most of the radioactivity eluted just ahead of the ω-N^G-monomethylarginine standard at 86 min in a position expected for its tritiated derivative (9, 24, 25). However, a peak that consistently migrated at about 79 min between the positions of asymmetric ω-N^G,N^G-dimethylarginine and ω-N^G-monomethylarginine standards was also observed. In Fig. 2*A*, we show that the radioactivity in this second peak at 76–79 min elutes just prior to the unlabeled symmetric ω-$N^G,N^{G'}$-dimethylarginine standard, suggesting that PRMT5 may catalyze the formation of the symmetric dimethylarginine derivative. However, δ-*N*-monomethylarginine, the product of

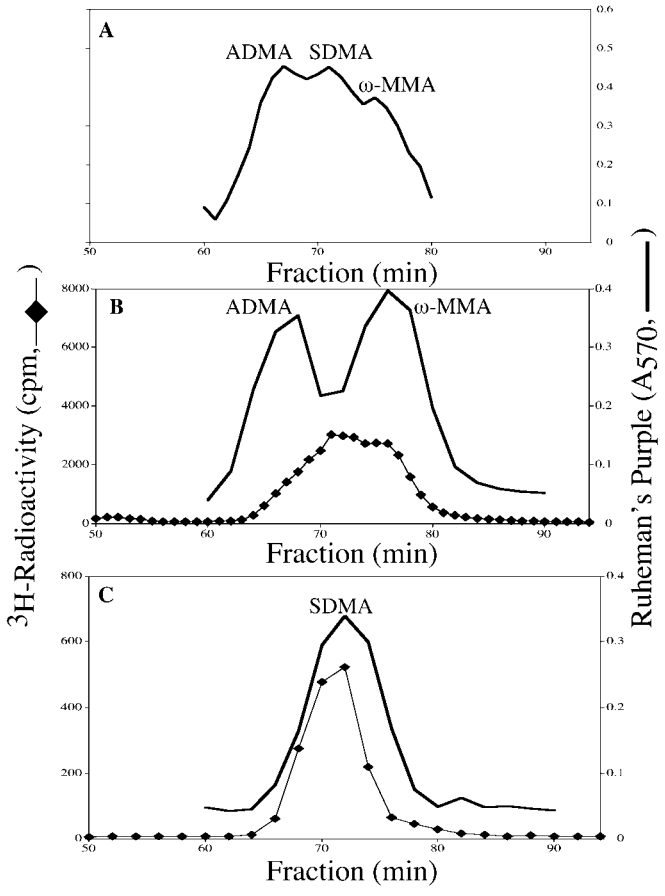

FIG. 5. **HA-PRMT5 can monomethylate and symmetrically dimethylate arginine residues on GST-GAR.** *A*, 1.0 μmol of each of the standards (ω-N^G-monomethylarginine, ω-N^G,N^G-dimethylarginine, and ω-N^G,$N^{G'}$-dimethylarginine) was added to 500 μl of citrate sample dilution buffer and fractionated on the amino acid cation exchange column at a flow rate of 1 ml/min. 100-μl aliquots of every other fraction were analyzed by the ninhydrin assay. *B*, purified GST-GAR was methylated *in vitro* as described under "Experimental Procedures." GST-GAR was then precipitated with trichloroacetic acid, acid-hydrolyzed, and run on the cation exchange column as described in the Fig. 1 legend. *C*, fractions 67–69 from *B* were diluted with 14 ml of water and brought to a pH of 2.0 with 6 N HCl. 1.0 μmol of the standard ω-N^G,$N^{G'}$-dimethylarginine was added to the pooled fractions, and the mixture was rerun on the amino acid cation exchange column as described above.

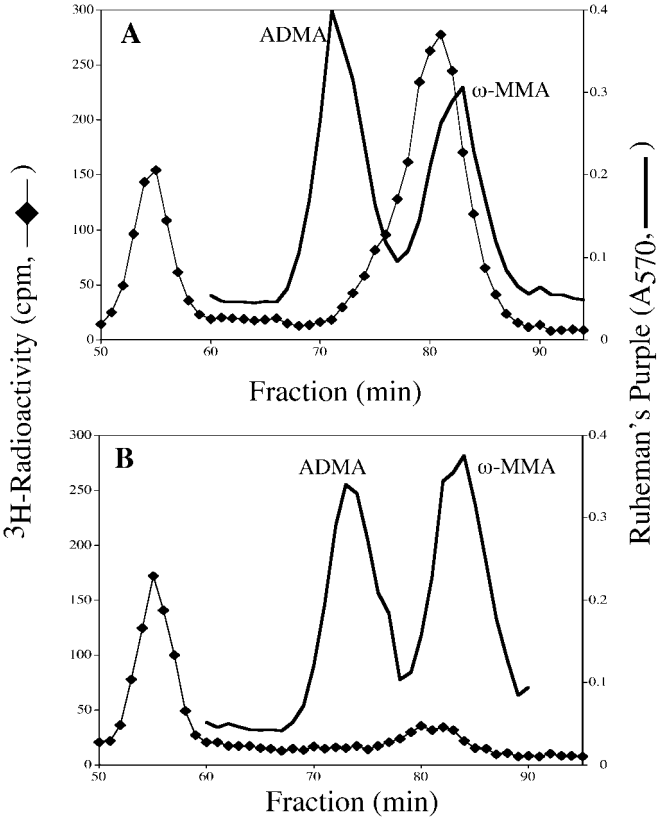

FIG. 6. **Mutation of PRMT5 in the AdoMet-binding motif I leads to a loss of methyltransferase activity.** 10 μg of MBP was added to immunoprecipitated *myc*-PRMT5 (*A*) or *myc*-PRMT5ΔGAGRG (*B*) that had been expressed in COS-1 cells. These mixtures were then incubated at 37 °C for 5 h with 5 μl of [^{3}H]AdoMet as described under "Experimental Procedures." After precipitation with trichloroacetic acid and acid hydrolysis, methylated amino acid standards were added, and the hydrolysate was fractionated using cation exchange chromatography as described in the Fig. 1 legend. The *solid line* represents the ninhydrin color of the asymmetric ω-N^G,N^G-dimethylarginine and ω-MMA standards; the *diamonds* represent the radioactivity present in 200 μl of each fraction.

the type IV methyltransferase, has also been determined to elute in a similar position on this cation exchange column (25). To distinguish between these two methylarginine derivatives, we examined their breakdown products upon base treatment. It has previously been shown that when δ-*N*-monomethylarginine is treated with base, it forms δ-*N*-methylornithine (26), whereas ω-N^G,$N^{G'}$-dimethylarginine and ω-N^G-monomethylarginine yield methylamine, and ω-N^G,N^G-dimethylarginine yields dimethylamine (27). We thus treated the material eluting with the symmetric standard with 2 M NaOH at 55 °C for 24 h and then re-chromatographed it on the cation exchange column along with unlabeled δ-*N*-methylornithine and methylamine standards (Fig. 2*B*). No radioactivity was found to co-elute with the δ-*N*-methylornithine standard. However, radioactivity did co-elute with the methylamine standard, suggesting that the peak in fractions 76–79 (Figs. 1 and 2*A*) may be attributed to symmetric ω-N^G,$N^{G'}$-dimethylarginine.

To further confirm that the *in vitro* methylated MBP acid hydrolysis products are in fact symmetric ω-N^G,$N^{G'}$-dimethylarginine and ω-N^G-monomethylarginine, the methylated arginine derivatives, were analyzed by thin layer chromatography. Fig. 3 shows that the radioactive material eluting at 76–79 min in Figs. 1 and 2*A* co-elutes exactly with a symmetric ω-N^G,$N^{G'}$-dimethylarginine standard in two different thin layer chromatography systems. The radioactive species that co-elutes with the ω-N^G-monomethylarginine standard at 83–86 min on the cation exchange column in Fig. 1 is also shown to co-elute with the ω-N^G-monomethylarginine standard in these two chromatography methods (Fig. 4). These results indicate that HA-PRMT5 is capable of catalyzing the formation of ω-N^G,$N^{G'}$-dimethylarginine and ω-N^G-monomethylarginine in polypeptides.

We next asked whether the formation of symmetric dimethylarginine residues occurred only with MBP, or whether the specificity for symmetric dimethylation resided in the enzyme itself. GST-GAR, an artificial polypeptide that has been shown to be an excellent *in vitro* substrate for type I enzymes that catalyze asymmetric dimethylation (8), was also found to be radiolabeled *in vitro* with HA-PRMT5. As shown in Fig. 5, amino acid analysis of this reaction product clearly demonstrates that arginine residues in GST-GAR are monomethylated (Fig. 5*B*) and symmetrically dimethylated (Fig. 5*C*), but significantly, these residues are not asymmetrically dimethylated by HA-PRMT5. Additionally, we found that histone H2A, another methyl-accepting substrate of HA-PRMT5 (21), also forms the symmetric ω-N^G,$N^{G'}$-dimethylarginine and ω-N^G-monomethylarginine derivatives (data not shown). Our results, therefore, suggest that PRMT5 is capable of specific type II

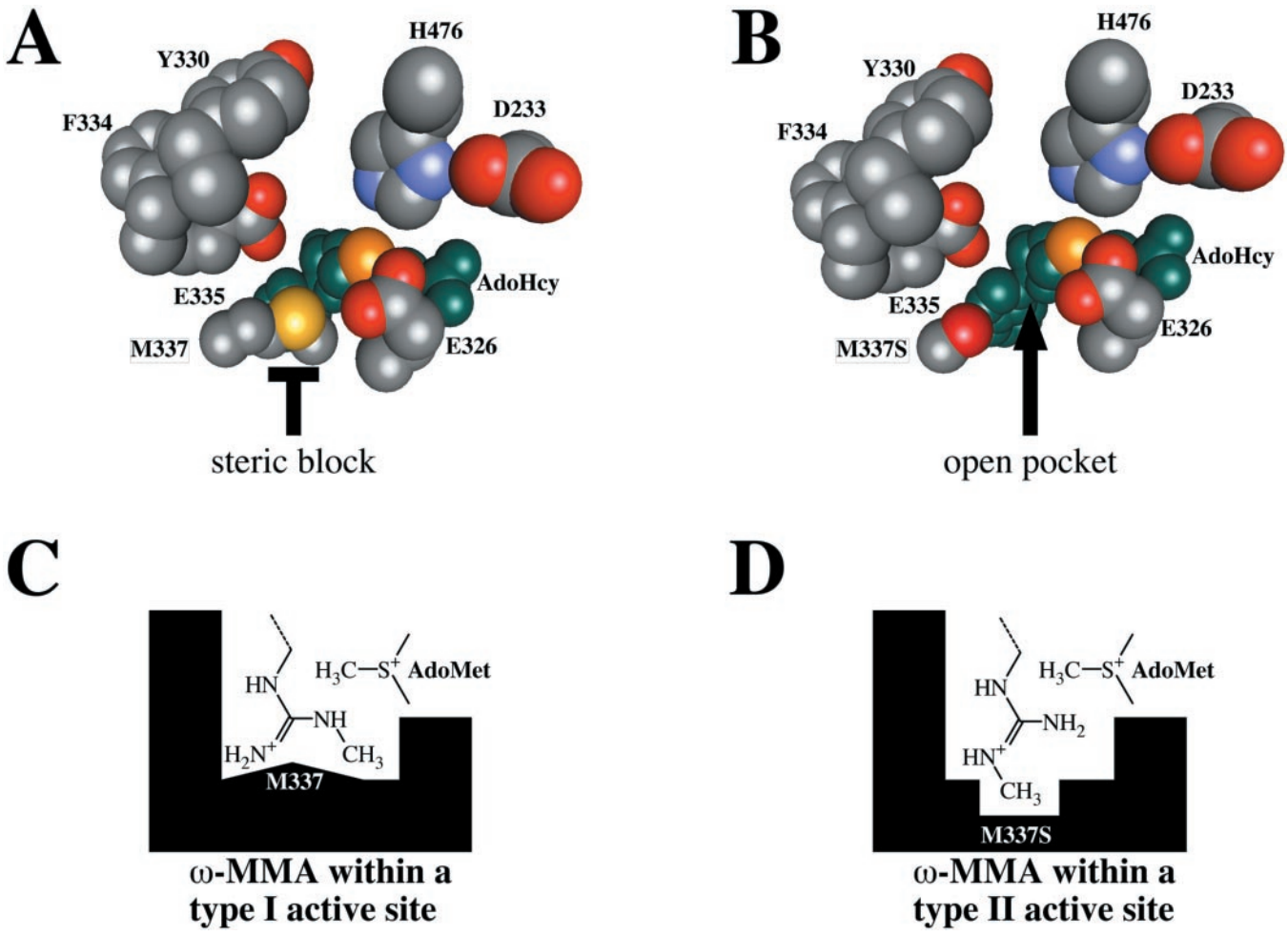

FIG. 7. **Proposed active sites for type I and type II protein arginine *N*-methyltransferases.** The active site residues of the rat PRMT3 crystal structure with *S*-adenosyl-L-homocysteine (32) are modeled using WebLab™ ViewerLite 3.2 in *A*. The colors used to indicate the atoms are as follows: carbon atoms are *gray*, nitrogen atoms are *light blue*, oxygen atoms are *red*, the sulfur atom of methionine is *yellow*, the sulfur atom of *S*-adenosyl-L-homocysteine is *orange*, and all other atoms of *S*-adenosyl-L-homocysteine are *green*. It has been proposed that Met-337 (*M337*) forces the binding of ω-MMA in a configuration where the second methylation reaction must occur on the same nitrogen atom to give the type I asymmetric ω-N^G,N^G-dimethylarginine product (*B*) (30). The formation of SDMA would be prevented by the steric block of the Met-337 residue, which may not allow ω-MMA to bind with the methylated amino nitrogen in this configuration. A change in residue 337 from methionine to serine, as in the PRMT5 enzyme model in *C,* opens a pocket that may allow such binding and the formation of the SDMA derivative (*D*).

(symmetric dimethylation) activity in a substrate-independent fashion.

To verify that the type II activity we see is due to PRMT5 and not an impurity in the enzyme preparation, we constructed *myc*-tagged mutants of PRMT5 that were transfected into COS-1 cells, and we analyzed the immunoprecipitates for arginine methyltransferase activity. Because the sequence G*X*GRG of motif I is highly conserved in PRMT5 and its homologues and is important for AdoMet binding (20), the sequence GAGRG (amino acids 365–369) was deleted to form *myc*-PRMT5ΔGAGRG. As shown in Fig. 6, there is a dramatic reduction in the formation of ω-[^{3}H]MMA and [^{3}H]SDMA in the mutated enzyme preparation. The small amount of radioactivity seen in the PRMT5ΔGAGRG mutant preparation may represent co-precipitated endogenous enzymatic activity because a similar amount of radioactivity is seen in a vector-only control of immunoprecipitated COS-1 cell extracts (data not shown). We also constructed a mutant in which the GAAGR sequence of motif I was mutated to GAAAG. When this mutant was analyzed for enzymatic activity, the formation of ω-[^{3}H]MMA and [^{3}H]SDMA was approximately half that of the wild type enzyme (data not shown). In both the deletion and substitution mutants, Western analysis with anti-*myc* antibodies showed the presence of equivalent amounts of PRMT5 expression.

A MBP-specific arginine methyltransferase activity has been previously purified to "near" homogeneity from calf brain as a large complex (500 kDa) containing two major polypeptides of 75 and 100 kDa (13). Attempts to dissociate the complex into smaller enzymatically active molecules have failed (13). The relationship between these polypeptides and the expected 72-kDa PRMT5 (JBP1) polypeptide is unknown at present, although the 75-kDa protein found in the MBP-specific methyltransferase could represent the catalytic PRMT5 polypeptide, and the 100-kDa polypeptide could be an additional subunit that is involved in regulation or substrate specificity. Here we show that PRMT5 purified as a native hemagglutinin-tagged complex from HeLa cells or as a *myc*-tagged complex from COS-1 cells, which should contain other tightly bound subunits as well, has type II arginine methyltransferase activity. However, it is likely that the 75-kDa protein contains this activity in the absence of additional subunits because it has been demonstrated that the polypeptide itself has methyltransferase activity (22).[3]

We have shown that the PRMT5 complex can methylate MBP, GST-GAR, and histone H2A *in vitro*, although its physiological methyl-accepting substrate(s) has yet to be deter-

[3] L. Izotova and S. Pestka, unpublished data.

mined. Three potential substrates, MBP and the Sm nuclear ribonucleoproteins D1 and D3, have been shown to be symmetrically dimethylated *in vivo* (13, 19). All of these three substrates have a consensus sequence that consists of an arginine residue sandwiched between two glycine residues. This consensus sequence is similar to that found for type I substrates (6). As we show here with GST-GAR, type I substrates can be symmetrically dimethylated *in vitro*. We have also been able to asymmetrically dimethylate MBP, a type II substrate, with type I enzymes *in vitro*.[4] These results suggest that it is not the "consensus sequence" or secondary structure of the substrate that is crucial in determining the type of methylation the arginine residue will undergo, but rather the type of specific methyltransferase complex that acts on the methyl acceptor.

The role of PRMT5 *in vivo* has yet to be determined. PRMT5 was isolated because of its ability to interact with Janus kinase 2 (20). Homologues of PRMT5 have been identified in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Hsl7, the *S. cerevisiae* homologue, has been shown to be a negative regulator of the protein kinases Swe1 and Ste20 (28, 29) and exhibits protein methyltransferase activity (21). Disruption of the gene leads to cell cycle abnormalities and elongated buds (28). The mammalian *PRMT5* gene has been found to partially rescue this phenotype when introduced into *S. cerevisiae* on a plasmid (21). Skb1, the *S. pombe* homologue of Hsl7, interacts with the Shk1 kinase (the *S. pombe* homologue of Ste20), and disruption of its gene also leads to cell cycle abnormalities (30, 31). This suggests that PRMT5 may be involved in numerous functions, including cell cycle progression and cellular signaling. We note the connections of enzymes that catalyze post-translational modifications by phosphorylation and methylation in these systems, and it is possible that both types of reactions interact to achieve metabolic control.

Our demonstration that PRMT5 catalyzes type II activity distinguishes it from all of the known mammalian protein *N*-arginine methyltransferase homologues that catalyze type I activity. The crystal structures of two type I enzymes, the rat PRMT3 catalytic core (amino acids 201–528) in complex with *S*-adenosyl-L-homocysteine (32) and RMT1 (amino acids 22–348) (33), have recently been solved. Both structures exhibit virtually identical core structural features with the predicted site for the arginine methyl-accepting substrate falling within an acidic pocket. Interestingly, all of the PRMTs except Hsl7 and PRMT5 contain an active site methionine residue (amino acid 337 for PRMT3 and amino acid 143 for RMT1) that has been proposed to exclude binding of ω-MMA in a conformation that would allow its symmetric methylation (32). However in both PRMT5 (amino acid 446) and Hsl7 (amino acid 474), the residue corresponding to Met-337 is serine. The smaller bulk of the side chain of this residue may now allow for SDMA formation with PRMT5 and possibly Hsl7 (Fig. 7).

[4] T. L. Branscombe, A. Frankel, and S. Clarke, unpublished data.

REFERENCES

1. McBride, A. E., Weiss, V. H., Kim, H. K., Hogle, J. M., and Silver, P. A. (2000) *J. Biol. Chem.* **275,** 3128–3136
2. Chen, D., Ma, H., Hong, H., Koh, S. S., Huang, S., Schurter, B. T., Aswad, D. W., and Stallcup, M. R. (1999) *Science* **284,** 2174–2177
3. Altschuler, L., Wook, J. O., Gurari, D., Chebath, J., and Revel, M. (1999) *J. Interferon Cytokine Res.* **2,** 189–195
4. Tang, J., Kao, P. N., and Herschman, H. R. (2000) *J. Biol. Chem.* **275,** 19866–19876
5. Bedford, M. T., Frankel, A., Yaffe, M. B., Clarke, S., Leder, P., and Richard, S. (2000) *J. Biol. Chem.* **275,** 16030–16036
6. Gary, J. D., and Clarke, S. (1998) *Prog. Nucleic Acids Res. Mol. Biol.* **61,** 65–130
7. Lin, W. J., Gary, J. D., Yang, M. C., Clarke, S., and Herschman, H. R. (1996) *J. Biol. Chem.* **271,** 15034–15044
8. Tang, J., Gary, J. D., Clarke, S., and Herschman, H. R. (1998) *J. Biol. Chem.* **273,** 16935–16945
9. Gary, J. D., Lin, W. J., Yang, M. C., Herschman, H. R., and Clarke, S. (1996) *J. Biol. Chem.* **271,** 12585–12594
10. Niewmierzycka, A., and Clarke, S. (1999) *J. Biol. Chem.* **274,** 814–824
11. Brostoff, S., and Eylar, E. H. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68,** 765–769
12. Baldwin, G. S., and Carnegie, P. R. (1971) *Science* **171,** 579–581
13. Kim, S., Chanderkar, L. P., and Ghosh, S. K. (1990) in *Protein Methylation* (Paik, W. K., and Kim, S., eds), pp. 77–95, CRC Press, Boca Raton, FL
14. Stoner, G. L. (1984) *J. Neurochem.* **43,** 433–447
15. Eylar, E. H. (1972) *Ann. N. Y. Acad. Sci.* **195,** 481–491
16. Aspillaga, M. O., and McDermott, J. R. (1977) *J. Neurochem.* **28,** 1147–1149
17. Dinn, J. J., Weir, D. G., McCann, S., Reed, B., Wilson, P., and Scott, J. M. (1980) *Ir. J. Med. Sci.* **149,** 53–58
18. Scott, J. M., Wilson, P., Din, J. J., and Weir, D. G. (1981) *Lancet* **2,** 334–337
19. Brahms, H., Raymuckers, J., Union, A., Keyser, F., Meheus, L., and Luhrmann, R. (2000) *J. Biol. Chem.* **275,** 17122–17129
20. Pollack, B. P., Kotenko, S. V., He, W., Izotova, L. S., Barnoski, B. L., and Pestka, S. (1999) *J. Biol. Chem.* **274,** 31531–31542
21. Lee, J.-H., Cook, J. R., Pollack, B. P., Kinzy, T. G., Norris, D., and Pestka, S. (2000) *Biochem. Biophys. Res. Commun.* **274,** 105–111
22. Rho, J., Choi, S., Seong, Y. R., Cho, W. K., Kim, S. H., and Im, D. S. (2001) *J. Biol. Chem.* **276,** 11393–11401; Erratum (2001) *J. Biol. Chem.* **276,** 16592
23. Norrander, J., Kempe, T., and Messing, J. (1983) *Gene* (*Amst.*) **26,** 101–106
24. Gottschling, H., and Freese, E. (1962) *Nature* **196,** 829–831
25. Xie, H., and Clarke, S. (1993) *J. Biol. Chem.* **268,** 13364–13371
26. Zobel-Thropp, P., Gary, J. D., and Clarke, S. (1998) *J. Biol. Chem.* **273,** 29283–29286
27. Paik, W. K., and Kim, S. (1970) *J. Biol. Chem.* **245,** 88–92
28. Ma, X., Lu, Q., and Grunstein, M. (1996) *Genes Dev.* **10,** 1327–1340
29. Fujita, A., Tonouchi, A., Hiroko, T., Inose, F., Nagashia, T., Satoh, R., and Tanaka, S. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 8522–8527
30. Gilbreth, M., Yang, P., Wang, D., Frost, J., Polverino, A., Cobb, M. H., and Marcus, S. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 13802–13807
31. Gilbreth, M., Yang, P., Bartholomeusz, G., Pimental, R. A., Kansra, S., Gadiraju, R., and Marcus, S. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 14781–14786
32. Zhang, X., Zhou, L., and Cheng, X. (2000) *EMBO J.* **19,** 3509–3519
33. Weiss, V. H., McBride, A. E., Soriano, M. A., Filman, D. J., Silver, P. A., and Hogle, J. M. (2000) *Nat. Struct. Biol.* **7,** 1165–1171