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# Characterization of Protein Arginine Methyltransferases in Porcine Brain

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**Protein arginine methylation is a posttranslational modification involved in various cellular functions including cell signaling, protein subcellular localization and transcriptional regulation. We analyze the protein arginine methyltransferases (PRMTs) that catalyze the formation of methylarginines in porcine brain. We fractionated the brain extracts and determined the PRMT activities as well as the distribution of different PRMT proteins in subcellular fractions of porcine brain. The majority of the type I methyltransferase activities that catalyze the formation of asymmetric dimethylarginines was in the cytosolic S3 fraction. High specific activity of the methyltransferase was detected in the S4 fraction (high-salt stripping of the ultracentrifugation precipitant P3 fraction), indicating that part of the PRMT was peripherally associated with membrane and ribosomal fractions. The amount and distribution of PRMT1 are consistent with the catalytic activity. The elution patterns from gel filtration and anion exchange chromatography also indicate that the type I activity in S3 and S4 are mostly from PRMT1. Our results suggest that part of the type I arginine methyltransferases in brains, mainly PRMT1, are sequestered in an inactive form as they associated with membranes or large subcellular complexes. Our biochemical analyses confirmed the complex distribution of different PRMTs and implicate their regulation and catalytic activities in brain.**

**Keywords:** Arginine methylation, Methylarginine containing proteins, PRMT

## Introduction

Protein arginine methylation has been shown to play important roles in RNA processing, signal transduction, transcriptional regulation and recently DNA repair (Bedford and Richard, 2005; Boisvert *et al*., 2005; Pahlich *et al*., 2006). This posttranslational modification is catalyzed by protein arginine methyltransferase (PRMT) on various methylaccepting proteins. Before the identification of the PRMT genes, the type I methyltransferase activity that catalyze the formation of $N^G$-monomethylarginine (MMA) and di-ω-*N,N*-methylarginine (aDMA) on hnRNPA1 or histones has been purified in calf brain and rat liver and was designated as hnRNP/histone methyltransferase at that time (Gary and Clarke, 1998; Ghosh *et al*., 1988; Rawal *et al*., 1994). The type II methyltransferase activity catalyzes the formation of monomethylarginine and di-ω-*N,N'*-methylarginine (sDMA) in myelin basic protein (Ghosh *et al*., 1988; Rawal *et al*., 1994; Gary and Clarke, 1998).

The first protein arginine methyltransferase gene (PRMT1) was cloned independently as an interacting protein of an immediate-early gene product TIS 21 (Lin *et al*., 1996) and interferone α/β receptor (Abramovich *et al*., 1997). By now at least 9 PRMT genes are identified in the mammalian system and are conserved in the vertebrates (Bedford and Richard, 2005; Hung and Li, 2004; Krause *et al*., 2007). Among all these protein arginine methyltransferase, PRMT1 is the predominant type I arginine protein methyltransferase in mammals (Pawlak *et al*., 2000; Tang *et al*., 2000). PRMT3, cloned as a PRMT1-interacting protein, contains an N-terminal zinc finger (Tang *et al*., 1998) and is a ribosomal protein S2 methyltransferase (Bachand and Silver, 2004; Swiercz *et al*., 2004). PRMT4 (CARM1) was identified as a co-activator associated arginine methyltransferase and has been implied to play roles in methylation of histone and some transcription factors to regulate transcription (Chen *et al*., 1999; Xu *et al*., 2001; Chevillard-Briet *et al*., 2002). A PRMT1 paralogue PRMT8 (HRMTL3) is membrane associated by myristoylation and is specifically expressed in brain (Lee *et al*., 2005a). PRMT1, 3 and 4, 6 and 8 have been shown to have type I activity (Lin *et al*., 1996; Tang *et al*., 1998; Chen *et al*., 1999; Frankel *et al*., 2002; Lee *et al*., 2005a). There was no direct methyltransferase activity evidence of PRMT2. In

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comparison, PRMT5 and recently PRMT7 and PRMT9 are shown to have the type II methyltransferase activity (Branscombe *et al.*, 2001; Lee *et al.*, 2005b; Cook *et al.*, 2006). The members of the protein arginine methylaccepting substrates for specific PRMT enzymes are growing very fast (as reviewed in Bedford and Richard, 2005; Boisvert *et al.*, 2005; Pahlich *et al.*, 2006).

Protein arginine methyltransferases are localized in various subcellular compartments. For example, by immunofluorescence analysis in the RAT1 rat fibroblast cells, PRMT1 was shown to be predominantly localized in the nucleus while PRMT3 in the cytoplasm (Tang *et al.*, 1998). GFP-fusions of PRMT1 and PRMT2 appeared to be largely localized to the nucleus, but significant fluorescence was also observed in the cytoplasm. The signals from PRMT3 and PRMT5 GFP-fusions are excluded from the nucleus whereas PRMT6 and PRMT4 displayed strong nuclear localization (Lin *et al.*, 1996; Tang *et al.*, 1998; Chen *et al.*, 1999; Frankel *et al.*, 2002). A dynamic study *in vivo* showed that PRMT1 is predominantly cytoplasmic and is highly mobile both in the cytoplasm and the nucleus. Inhibition of methylation leads to a significant nuclear accumulation of PRMT1 (Herrmann *et al.*, 2005).

However, the localization revealed by immunostaining or GFP-fusions probably only suggested the presence of the majority of the PRMT proteins but not necessarily the active enzymes. The molecular masses of the PRMT monomers are of about 40 to 70 kDa. From the results of various laboratories as reviewed previously (Gary and Clarke, 1998) and in Table 1, the type I activity in the cytosolic or the supernatant fraction of high-speed centrifugation appeared to be of higher molecular mass than the PRMT monomers as determined by the gel filtration experiments. Moreover, various results have indicated that recombinant PRMT1 (Zhang and Cheng, 2003), PRMT3 (Zhang *et al.*, 2000), as well as PRMT5 (Rho *et al.*, 2001) can be dimers or even oligomers. In correlation with the classical biochemical characterization of these arginine methyltransferases, the PRMT gene products are likely to be the catalytic subunit in a methyltransferase complex. PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells (Pawlak *et al.*, 2000; Tang *et al.*, 2000). Numerous recent reports demonstrate the association of different PRMT proteins with other proteins or the presence of the PRMT proteins in various complexes. For example, PRMT1 has been reported to be in a fibrillarin complex (Yanagida *et al.*, 2004), and preferentially associated with hypomethylated hnRNP complex (SAF-1/hnRNPU) (Herrmann *et al.*, 2004). PRMT3 has been shown to be associated with ribosomes (Bachand and Silver, 2004; Swiercz *et al.*, 2004) and interact with a tumor suppressor DAL1/4.1B (Singh *et al.*, 2004). PRMT 5 is known to be in a complex containing pICln (Meister *et al.*, 2001) as a 20S methylosome (Friesen *et al.*, 2001).

Most of the studies described previously were conducted by transfection experiments with various PRMT constructs in different cellular systems or with recombinant enzymes. After the identification of the PRMT genes, the molecular weight of different PRMT containing complexes can be determined. Nevertheless, few biochemical reports are conducted for the native PRMT distributions in tissues since the identification of the genes. Lim *et al.* reported the multimerization of PRMT1 and PRMT5 in rat liver and pancreas (Lim *et al.*, 2005). In this report we utilized conventional biochemical approaches to isolate and analyze the protein arginine methyltransferases from porcine brain. We fractionated the brain extracts and found some interesting points about the distribution and activation of different PRMT enzymes. As the PRMT genes are conserved in the vertebrates (Hung and Li, 2004; Bedford and Richard, 2005; Krause *et al.*, 2007), our study of PRMT in porcine brain should provide general understanding of PRMT in mammals.

## Materials and Methods

**Fractionation of porcine brain extract.** Fresh porcine brains obtained from a slaughter house were stored in −80°C until use. Brain was weighed, washed two times with phosphate buffered saline, and then cut into small slices, resuspended into 3× (vol/wt) lysis buffer (0.32 M sucrose, 20 mM Tris, pH 7.3, 5 mM $MgCl_2$, 1 mM PMSF, 1 mg/ml aprotinin, 1 μg/ml pepstatin, 1 μg/ml leupeptin) and homogenized with a Teflon pestle with about 10 strokes. The fractionation scheme was illustrated in Fig. 1A. Briefly, the homogenate (S0) was centrifuged at 800 × g for 10 min and the pellet was P1. The supernatant S1 was further centrifuged at 9200 × g for 15 min. Then the supernatant and pellet fractions were designated as S2 and P2. Ultracentrifugation of the S2 fraction at 130,000 × g for 1 h resulted in the supernatant S3 (cytosolic) and pellet P3 (membrane) fraction. Part of the P3 was resuspended in

**Table 1.** Different preparation of type I protein methyltransferases

| Source | | MW methyltransferase Substrate | starting materials for purification | References |
|---|---|---|---|---|
| Rat liver | 450K | histone/hnRNPA1 | 100,000 g 60 min supernatant | Rawal *et al.*, 1994 |
| Calf brain | 275K | histone | 78,500 g 60 mim supernatant | Ghosh *et al.*, 1998 |
| HeLa cells | 450K | hnRNP complex; Recombinant hnRNPA1 | cytoplasmic fraction (3,000 g, 10min supernatant) | Liu and Dreyfuss, 1995 |
| Rat 1 cells | 317K | GST-GAR protein | cell soluble extract | Tang *et al.*, 1998 |
| Rat liver/pancreas | 440K | hnRNPA1 | 100,000 g 60 min supernatant | Lim *et al.*, 2005 |

lysis buffer with 0.5 M of KCl and subjected to ultracentrifuge again. The supernatant and the pellet fractions were S4 (high-salt-stripped soluble) and P4 (high-salt-stripped membrane pellet) respectively. The fractionation scheme is illustrated in Fig. 1a. The protein concentration in each fraction was determined using the BCA reagent (Pierce) with bovine serum albumin as the standard protein.

**Methyltransferase assay.** Different fractions of the porcine brain extracts prepared above were incubated with 1.5 μCi of [*methyl*-$^3$H]-AdoMet (60 Ci/mmol, Amersham) in a 15 μl reaction in methylation buffer (50 mM sodium phosphate, pH 7.5) with recombinant mouse fibrillarin protein as the methylaccepting substrate. After incubation at 37°C for 60 min, the reaction was terminated by the addition of one third of the volume of 4X SDS sample buffer, and the samples were subjected to SDS-PAGE (12.5% acrylamide) as described by Laemmli (Laemmli, 1970). The polyacrylamide gels were stained with coomassie brilliant blue, destained, and treated with EN$^3$HANCE (Perkin Elmer). Subsequently, the gels were dried and exposed to X-ray film (Kodak, MS) at −75°C for different time periods. Recombinant mouse fibrillarin protein (Pearson *et al*., 1999) was prepared as described.

**Western Blotting.** Different fractions of porcine brain extracts were separated by 12.5% SDS-PAGE and subsequently transferred to nitrocellulose membranes (Gelman Science). The membrane was then blocked in 5% skimmed dry milk in TTBS (10 mM of Tris-HCl, pH = 7.5; 100 mM of NaCl; 0.1% Tween 20) for 30 min, incubated with primary antibodies (1 : 200 dilution for hnRNP A2/B1 [Santa Cruz]; 1 : 100000 dilution for hnRNP M [Santa Cruz]; 1 : 2500 dilution for PRMT1 [Upstate], 1 : 500 dilution for PRMT3 [Upstate], 1 : 250 dilution for PRMT4 [Upstate], and 1 : 500 dilution for PRMT6 [IMGENEX]) for one hour at room temperature or 4°C overnight, washed three times in TTBS, and then incubated with secondary antibody (anti-mouse or rabbit or goat IgG horse radish peroxidase conjugate from Santa Cruz) for one hour. Chemiluminescent detection was performed using the Supersignal kit according to the instructions of manufacturer (Pierce).

**Analysis and Partial Purification by column chromatography.** For gel filtration chromatography, protein samples (250 μl) were applied onto a Superdex 200 column (GE Amersham Bioscience, 24 ml), and eluted in 20 mM $NaH_2PO_4$ buffer (pH = 7.3) at the flow rate of 0.4 ml/min using an FPLC system (AKTA purifier, GE Amersham Bioscience). Anion exchange chromatography was performed in the FPLC system with a monoQ column (GE Amersham Bioscience, 1 ml). The column was then washed with 5 ml of buffer A (20 mM of Tris, pH = 7.5), and eluted with buffer A with a NaCl gradient from 0 to 1 M in 5 bed volumes at the flow rate of 1 ml/min.

## Results

**Methyltransferase activity in subcellular fractions of porcine brain.** We fractionated the lysates of porcine brain by differential centrifugation (Fig. 1a). The activities of the type I arginine methyltransferase were assayed with recombinant mouse fibrillarin protein as the methylaccepting protein (Fig. 1b). The total and specific activities in each fraction were listed in Table 2. The majority of the proteins and methyltransferase activities remained in the P1 fraction, which represents the unhomogenated tissues and intact nuclei. Besides the P1 fraction, the supernatant fractions appeared to contain higher specific activity than the corresponding precipitation fractions. After ultracentrifugation, the majority of the activity was in the S3 (cytosolic) fraction. The P3 fraction precipitated from ultracentrifugation was mostly composed of membranes and macro-subcellular complexes such as ribosomes. Interestingly, the S4 fraction prepared by high salt stripping of P3 has the highest specific activity among all fractions. Furthermore, total activity in the S4 fraction appeared to be higher than that in the P3 membrane fraction, from which it was prepared. The results indicated that arginine methyltransferase was enriched in the S4 (high-salt stripped soluble) fraction. The PRMT activity in the "wash" solution of the P3 fraction (by the lysiss buffer without 0.5M KCl) was much lower than that in the S4 fraction, indicating that the PRMT activity in S4 was not likely to be due to residual S3 cytosolic PRMT in the P3 membrane fraction (data not shown).Therefore, the enzyme in the S4 fraction probably was activated due to its release from the P3 fraction by high-salt stripping, or high KCl concentration could activate the methyltransferase activity.

**The type I arginine methyltransferase activity and high salt concentration.** To evaluate the effect of KCl on the type I PRMT activity, we adjusted the KCl concentration in the S3 cytosolic and P3 membrane fractions to 0.5 M before assaying the methyltransferase activity. As shown in Fig. 2, upon the addition of KCl, the methyltransferase activity in the P3 fraction was significantly elavated while the activities in S3 fraction were not affected. The activity of recombinant GST-PRMT1 enzyme was not affected by KCl (data not shown). Other monovalent salts (NaCl, NaF) have similar effects as KCl to elevate the type I methyltransferase activity in P3 (data not shown). The results indicated that high concentration of the monovalent ions *per se* probably cannot generally activate the type I methyltransferase activity and the activation is specific to the methyltransferase in P3. Therefore, the increased methyltransferase activity in S4 was more likely to be due to the dissociation of the enzyme from certain inhibiting factors in the P3 membrane fraction.

**Detection of different type I PRMT proteins in the porcine brain fractions.** At least 5 different PRMTs (PRMT1, 3, 4, 6, and 8) in mammals have been demonstrated as catalytically active type I enzyme. Except PRMT4, other type I enzyme including PRMT1, 3, 6, 8 can catalyze the methylation of a recombinant GAR protein containing the RGG methylation sites in human fibrillarin (Frankel *et al*., 2002; Lee *et al*., 2005a; Tang *et al*., 1998). Since mouse fibrillarin with similar

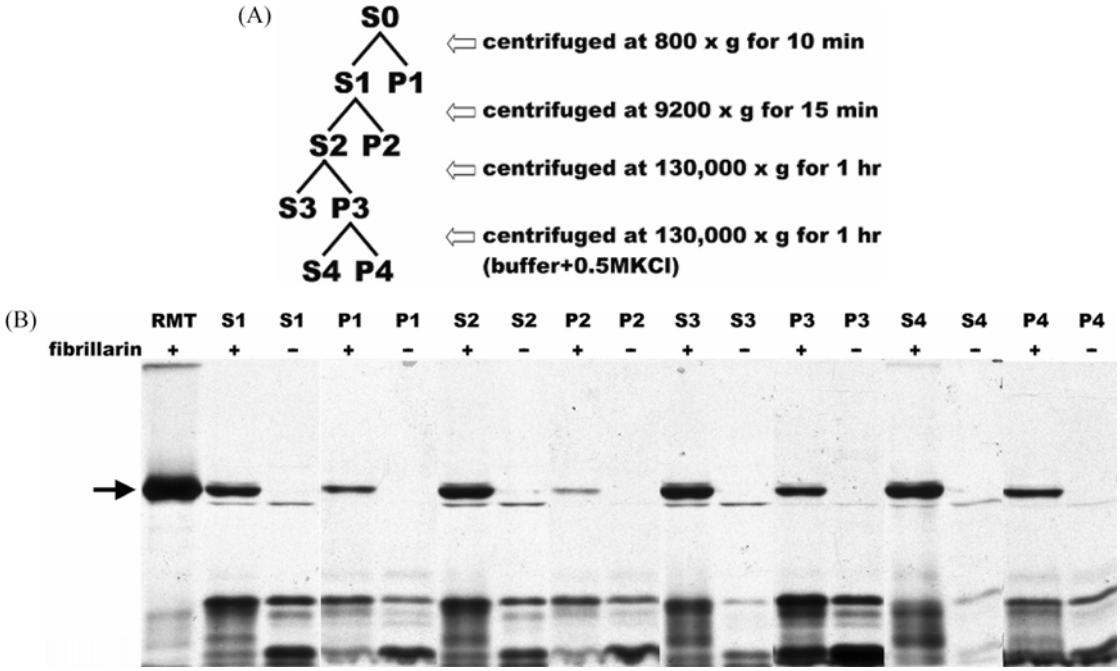


**Fig. 1.** Fractionation of porcine brain and the type I protein arginine methyltransferase activity in the porcine brain fractions. (A) Fractionation scheme of porcine brain. Porcine brain was disrupted by homogenization in lysis buffer. The brain homogenate was then fractionated by centrifugation. The P3 fraction was resuspended in lysis buffer with 0.5 M KCl and subjected to ultracentrifuge again to obtain S4 and P4. The fractionation procedures are illustrated, with *S* indicating supernatant and *P* indicating pellet. (B) Analyses of the type I activity in the porcine brain fractions. *In vitro* protein methylation reactions were performed in the presence of various fractions (20 μg of protein), 1.5 μCi of the methyl group donor [$^3$H] AdoMet, and with or without a arginine methylaccepting substrate fibrillarin to a final reaction volume of 15 μl. The reaction products were visualized by SDS-PAGE and fluorography. Recombinant fibrillarin methylated by recombinant yeast arginine methyltransferase resolved in the RMT lane was used as a positive control and to normalize the results of different reactions. The position of the recombinant mouse fibrillarin was indicated by an arrow.

RGG motif was used as the methylaccepting substrate in our protein arginine methyltransferase assay, the type I activity we detected might be from either PRMT1, 3, 6 or 8. We then used antibodies against different PRMT proteins to analyze their distribution in the porcine brain fractions. As PRMTs in mammals are highly conserved (Bedford and Richard, 2005; Hung and Li, 2004; Krause *et al.*, 2007), the commercially available PRMT antibodies that can detect the PRMTs in human and mouse should be able to detect the porcine PRMTs. PRMT1 proteins could be detected in all fractions, and generally were more abundant in the supernatant than in the corresponding precipitation fractions (Fig. 3a). The distribution of PRMT1 is in good consistence with the type I activity detected in the subcellular fractions shown in Fig. 1b. The PRMT6 protein was most abundant in the supernatant fractions and P4 (Fig. 3b). The commercially available anti-PRMT3 antibody detected multiple signals. The signals corresponded to the molecular mass of PRMT3 about 68 kD were slightly detected in S1 and S3, and more clearly in S4 as well as in P4 (data not shown). In addition, the signals of PRMT4/CARM1 were mostly detected in the supernatant fractions S1, S2 and S3, but slightly in S4 (Fig. 3c).

To confirm the effectiveness of the fractionation scheme to separate proteins of different subcellular compartments, we further performed western blot analyses with antibodies against other protein methyltransferase and hnRNP proteins. A cytoplasmic protein carboxyl methyltransferase PCMII was present in the S3 fraction but not in the S4 fraction as expected (data not shown). The majority of a hnRNP protein

**Table 2.** Methyltransferase activity in different porcine brain fractions

| | Total protein (mg) | Total activity[a] | Sp. Activity (U/mg)[a] |
|---|---|---|---|
| $S_1$ | 124.55 | 16180 | 129.9 |
| $P_1$ | 710.32 | 48660 | 68.5 |
| $S_2$ | 103.57 | 14930 | 144.2 |
| $P_2$ | 21.41 | 362 | 16.9 |
| $S_3$ | 98.34 | 13940 | 141.7 |
| $P_3$ | 17.03 | 1327 | 77.9 |
| $S_4$ | 4.32 | 695 | 160.6 |
| $P_4$ | 15.76 | 1266 | 80.3 |

[a]All of the methyltransferase assay reactions were analyzed by SDS-PAGE and fluorography with a standard normalized reaction. One unit of the type I methyltransferase activity was designated as the activity that can catalyze the methylation of fibrillarin to 1/3 of the density by recombinant RMT (1 μg).

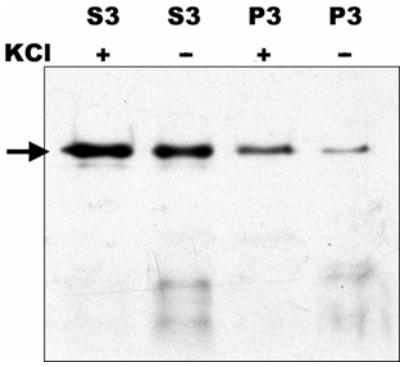


**Fig. 2.** The effects of high KCl concentration on the type I methyltransferase activity in the S3 and P3 fractions. The S3 and P3 fractions were incubated in the presence of 0.5M KCl for 1hr followed by in vitro protein methylation reaction. The "+" symbol represents the addition of 0.5 M KCl before the reaction. The position of the recombinant mouse fibrillarin was indicated by an arrow.

hnRNPA2/B1, which has been shown to accompany mRNA into cytoplasm until translation, was detected in the S4 fraction (Fig. 3d). Another hnRNP protein hnRNPM known to reside mainly in the nucleus was present in the P1 fraction and was marginally detected in S4 (Fig. 3e). The results indicate that the fractionation procedures is able to separate proteins of different subcellular compartments properly.

**Elution patterns of the type I methyltransferase activity in S3 and S4 by liquid chromatography of different separation modes.** We are interested in whether the arginine methyltransferases in the S4 (high-salt-stripped soluble) fraction were silimar to that in the cytosolic S3 fraction. We thus partially purified and characterized the methyltransferases in S3 and S4 fractions by liquid chromatography. The type I methyltransferase activity in the eluted fractions were then assayed with recombinant mouse fibrillarin as the methyl-accepting protein. As shown in Fig. 4a, the activity in both S3 and S4 were eluted almost at the same position from a gel filtration (Superdex 200) column at fraction 28-31, with calculated molecular mass of about 250 kD.

To determine which PRMT contributed to the activities in these fractions, we further detected different PRMT proteins in the S200 fractions by western blot analyses (Fig. 4b). When either S3 or S4 were analyzed, PRMT1 was detected in the same fractions containing active type I methyltransferase. PRMT4 in both S3 and S4 fractions was mostly eluted at fraction 30 and 31 from the S200 column, corresponding to the molecular mass of about 220 kDa (Fig. 4c). The PRMT6 protein in both S3 and S4 fractions was eluted at fractions 25-37, with double peaks detected at fraction 26 and 34, suggesting its presence in complexes ranging from 120 to 450 kDa (data not shown). The molecular mass determined by gel filtration chromatography for PRMT1, 4 and 6 were apparently

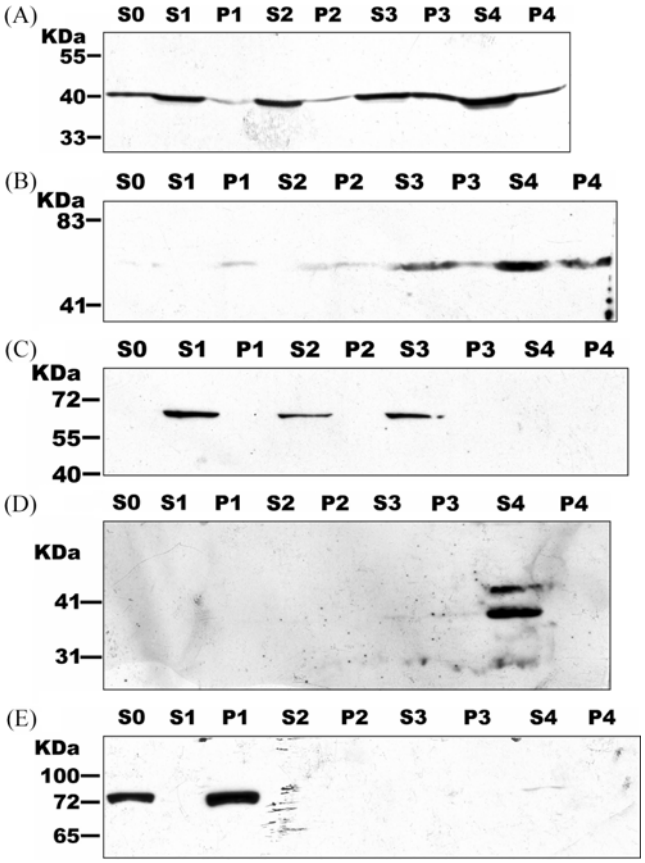


**Fig. 3.** Presence of different type I PRMT proteins in the porcine brain fractions. Subcellular fractions of porcine brain (30 μg) were resolved by SDS-PAGE, transferred to nitrocellulose membrane, and then analyzed by western blot analyses as desecribed in the materials and methods. The primary antibodies used were anti-PRMT1 (A), anti-PRMT6 (B), anti-PRMT4 (C), anti-hnRNPA2/B1 (D), and anti-hnRNPM (E).

larger than their monomeric PRMT proteins. Thus these PRMT are likely to be homo-or heteromultimers of the monomeric PRMT proteins in the porcine brain.

Furthermore, analysed by a monoQ anion exchange column, the activities in the S3 fractions were eluted at about fractions 14-16 while the activities in S4 were eluted at about fractions 15-18 (Fig. 4d). The presence of PRMT1 protein in the mono Q column fractions detected by anti-PRMT1 was again consistent with the active fractions (data not shown). We further used an arginine sepharose 4B column for separation of of the arginine methyltransferases in view that the enzyme might bind specifically to the arginine moity on the resins. However, this column basically functioned as an anion exchanger similar to monoQ and no specific binding affinity was observed (data not shown). Our results demonstrated that the PRMT1 complex in S3 and S4 were of similar molecular mass but slighly different pI.

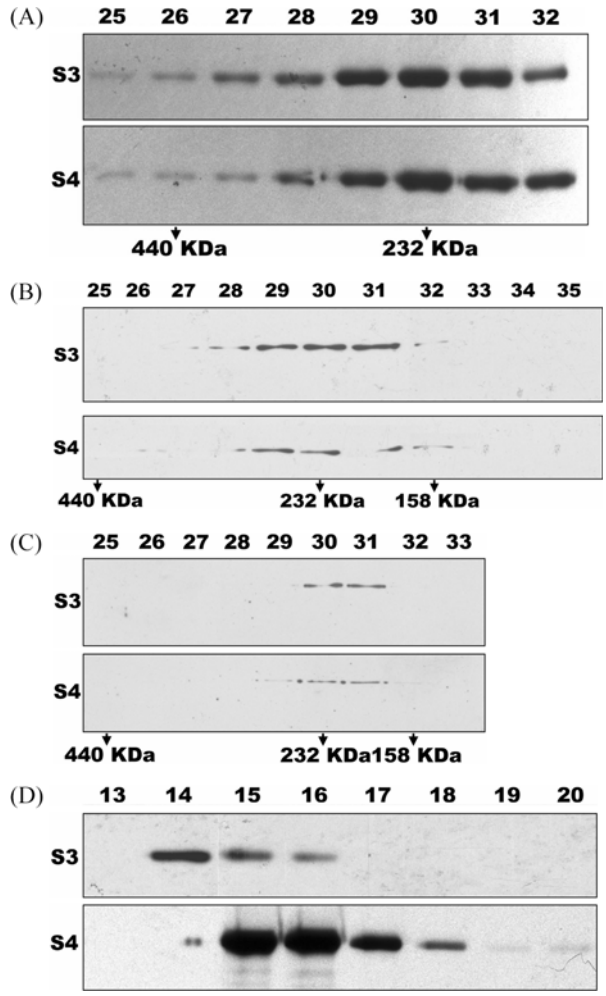


**Fig. 4.** Analyses of the type I methyltransferase activity in S3 and S4 by size exclusion and anion exchange liquid chromatography. S3 or S4 porcine brain fractions were loaded onto a Superdex 200HR gel filtration column (24 ml). The column was equilibrated and eluted in 20 mM $NaH_2PO_4$, pH 7.3 buffer at a flow rate of 0.4 ml/min and the fractions were collected per minute. The elution pattern of the chromatography monitored by the absorbance at 280 nm is shown in supplementary Fig. 1. The type I protein methyltransferase activity in the column fractions were demonstrated in (A). PRMT1 and PRMT4 proteins in the S200 column fractions were determined by western blot analyses using anti-PRMT1 or PRMT4 as shown in (B) and (C). Alternatively, S3 and S4 were analyzed by monoQ anion exchange column. The column was equilibrated in 0.08 M Tris PH 7.5 and eluted in 0.08 M Tris, PH 7.5 with increasing concentration of NaCl (from 0 to 1 M NaCl in 15 min), at a flow rate 1 ml/min and the fractions were collected per minute. The fractions were collected every 0.5 min. The type I protein methyltransferase activity in the column fractions were demonstrated in (D). The UV trace of the chromatography is shown in supplementary Fig. 2.

## Discussion

In this study, we determined the protein arginine methyltransferase activities as well as the distribution of different PRMT proteins in subcellular fractions of porcine brain. After ultracentrifugation, the majority of the type I methyltransferase activities assayed by recombinant mouse fibrillarin was in the cytosolic S3 fraction. Interestingly, high specific activity of the methyltransferase was detected in the S4 fraction. The fraction comes from high-salt stripping of the ultracentrifugation precipitants (P3 fraction) which represents peripherally associated proteins of membrane and ribosomal fractions. After comparing different type I PRMT proteins in the subcellular fractions, the amount and distribution of PRMT1 is consistent with the type I catalytic activity and is most likely to be responsible for the detected activity.

It is to note that besides the high specific activity in S4 high-salt-stripped soluble faction, total type I activity in S4 was higher than that in the P3 membrane fraction. Incubation with 0.5 M KCl did not elevate the type I activity of the arginine methyltransferase in S3 as well as recombinant PRMT1. It is thus less likely that high KCl concentration generally activates the PRMT actitivy. Furthermore, the PRMT activity in the P3 membrane fraction can be activated by KCl and other monovalent ions. When the type I methyltransferase has been stripped from the P3 membrane fraction by 0.5 M KCl in S4, removing KCl by dialysis or gel filtration column chromatography did not significantly affect the methyltransferase activity. Adding back KCl did not increase the type I PRMT activity in the S4 fraction any further (data not shown). Consequently, the increased S4 methyltransferase activity was more likely due to the dissociation of specific PRMT enzymes from the P3 pellet by monovalent salts.

The elution patterns of the activities and the PRMT proteins from a gel filtration column showed that PRMT1 should account for the majority of the activity in either S3 cytosolic or S4 high salt-stripped fraction. From further gel filtration chromatographic analyses, the type I PRMT activity and the PRMT proteins in the S3 fraction are of similar molecular mass as those in S4. The consistency of the elution pattern of type I activity with that of the PRMT1 protein in both S3 and S4 furtehr indicated that the major type I activity observed in both subcellular fractions were PRMT1. The result is consistant with previous reports that PRMT1 is the predominant PRMT (Pawlak *et al*., 2000; Tang *et al*., 2000).

Furthermore, from anion exchange chromatography, the activity in S3 were eluted slightly faster than that from S4. Therefore, it is likely that arginine methyltransferase in S4 released from P3 membrane was not a monomer but as a similar yet non-identical protein complex as that in S3. Our

results of the PRMT in the S4 high salt-stripped fraction provide biochemical evidences that part of the type I arginine methyltransferase, mainly PRMT1, are associated with components of membranes or large subcellular complexes. The PRMT1 proteins in the associated form, mostly fractionated in P3, are very likely to be sequestered in an inactive form and are released to S4 under high salt treatment as an active form in this study. It thus leads to the possibility that *in vivo* the sequestered PRMT1 in brain can be released at specific conditions and initiate certain signaling.

Before identification of the PRMT genes, the protein arginine methyltransferase analyzed by biochemical methods indicated that the type I protein arginine methyltransferase activities were of 275 kDa from calf brain (Ghosh *et al*., 1988) and 450 kDa in rat liver (Rawal *et al*., 1994) or HeLa cells. Native PRMT1 complex from RAT1 cell extract was estimated to be of 317 kDa (Tang *et al*., 1998). Recently it was reported that the enzyme activities of PRMT1 in the cytosol of rat liver and pancreas were about 440 kDa (Lim *et al*., 2005). The type I activity as well as the PRMT1 protein we detected in porcine brain was about 250 kDa, close to the results from calf brain. PRMT1 is likely to be the catalytic subunit in the methyltransferase complex. In addition, we observed that PRMT4 or PRMT6 eluted from the size exclusion column with the calculated molecular mass of 220 kDa and 450 to 120 kDa respectively. Similar results were obtained for S3 and S4, indicating that they are also present as similar complex in both subcellular fractions.

Recently PRMT8 (HRMT1L3), a PRMT1 paralogue in vertebrates (Hung and Li, 2004), has been demonstrated to be a membrane-bound and brain-specific member of the PRMT family. It has the type I activity and can homodimerize or heterodimerize with PRMT1 (Lee *et al*., 2005a). In the twenty amino acid sequences of the antigenic peptide of the PRMT1 antibody used in this study, five amino acid residues are different between PRMT1 and PRMT8. The signals detected by anti-PRMT1 thus are less likely to be partially from PRMT8. We could not exclude the possibility that some of the methyltransferase activity detected in S4 was contributed by PRMT8. However, 0.5 M KCl treatment is less likely to dissociate myristoylated PRMT8 from membrane. It is rather probable that part of the PRMT1 in the S4 fraction might be originally associated with PRMT8 in the plasma membrane fraction in P3 and then released by the high salt treatment. Moreover, PRMT1 has been shown to be physically associated with other membrane protein such as interferone α/β receptor (Abramovich *et al*., 1997). Besides, hnRNP particles containing the predominant substrates of the enzyme interact with PRMT1 via scaffold attachment factor A (SAF-A, hnRNPU) and the interaction is enhanced after the inhibition of methylation (Herrmann *et al*., 2004). Specifically, we detected some hnRNP proteins such as hnRNPA1 and A2/B1 highly concentrated in the S4 fraction. Therefore, part of the PRMT enzyme in S4 is likely to be from PRMT 1 originally associated with large hnRNP particles precipitated in P3. In conclusion, our biochemical analyses further confirmed the complex distribution of the PRMT proteins and implicate the regulation of different PRMT proteins and catalytic activities in brain.

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