Purification and characterization of S-adenosylmethionine–protein-arginine N-methyltransferase from rat liver

Nenoo RAWAL, Ramesh RAJPUROHIT, Woon Ki PAIK and Sangduk KIM*

Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140, U.S.A.

A protein methylase I (S-adenosylmethionine–protein-arginine N-methyltransferase; EC 2.1.1.23), with a high specificity for recombinant heterogeneous nuclear ribonucleoprotein particle (hnRNP) protein A1, was purified from rat liver. The purification method is simple and rapid; a single initial step of DEAE-cellulose DE-52 chromatography resulted in a 114-fold enrichment from the cytosol, and subsequent Sephadex G-200 chromatography and f.p.l.c. yielded a homogeneous preparation. Ouchterlony double-immunodiffusion analysis indicated that the rat liver enzyme is immunologically different from an analogous enzyme from the calf brain, nuclear protein/histone-specific protein methylase I [Ghosh, Paik and Kim (1988) *J. Biol. Chem.* **263**, 19024–19033; Rajpurohit, Lee, Park, Paik and Kim (1994) *J. Biol. Chem.* **269**, 1075–1082]. The purified enzyme has a

molecular mass of 450 kDa on Superose chromatography and 110 kDa on SDS/PAGE, indicating that it is composed of four identical-size subunits. The K_m values for protein A1 and S-adenosyl-L-methionine were 0.54×10^{-6} and 6.3×10^{-6} M respectively. S-Adenosyl-L-homocysteine and sinefungin were effective inhibitors of the enzyme with K_i values of 8.4×10^{-6} M and 0.65×10^{-6} M respectively. Bivalent metal ions such as Zn^{2+} , Mn^{2+} and Ni^{2+} were particularly toxic to the enzyme; at 1 mM Zn^{2+} , 99 % of the activity was inhibited. In addition, 50 % of the enzyme activity was lost by treatment with 0.12 mM *p*-chloromercuribenzoate, indicating a requirement for a thiol group for enzyme activity. Glycerol, a compound often used to prevent enzyme inactivation, inhibited over 80 % of the activity when present in the reaction mixture at a concentration of 20 %.

INTRODUCTION

Methylation of protein is one of several post-translational modification reactions catalysed by highly specific methyltransferases utilizing S-adenosyl-L-methionine (AdoMet) as the methyl donor (Kim and Paik, 1965; Paik and Kim, 1980). The reaction can be classified into three major categories (Paik and Kim, 1980, 1985; Clarke, 1985; Clarke et al., 1987; Kim et al., 1990): N-methylation involving methylation of arginine, lysine and histidine side chains; O-methylation of either the internal carboxy group of glutamate and isoaspartate residues or the C-terminal cysteine residue; and S-methylation of either cysteine or methionine residues.

Protein methylase I (S-adenosylmethionine–protein-arginine N-methyltransferase; EC 2.1.1.23) methylates the guanidino nitrogen of arginine residues in proteins yielding N^G -monomethylarginine (MeArg), $N^G N^G$ -dimethylarginine [Me_2 (sym)Arg] and $N^G N^G$ -dimethylarginine [Me_2 (asym)Arg] (Paik and Kim, 1968). The enzyme is present ubiquitously in mammalian tissues, plants and some protozoa (Paik and Kim, 1980; Kim et al., 1990). Studies have shown that it is not only specific for the kind of protein but also for the amino acid sequence around the methylatable arginine (Ghosh et al., 1990; Lischwe, 1990). In 1988, Ghosh et al. purified and characterized two subclasses of protein methylase I from calf brain, one specific for myelin basic protein (MBP) and the other for histone. The latter enzyme, however, has recently been shown to methylate recombinant heterogeneous nuclear ribonucleoprotein particle (hnRNP) protein A1 much more efficiently than histone (Rajpurohit et al., 1994).

We have recently demonstrated the presence of protein

methylase I in rat liver nuclei, where it methylates recombinant unmethylated hnRNP protein A1 (Rajpurohit et al., 1992). During the course of further subcellular fractionation, it was noted that, although the substrate protein A1 is present in the nuclei, the bulk of the enzyme activity is recovered from the cytosol. In the present paper, we report a simple method for purification from rat liver cytosol of a protein methylase I, which is highly specific for recombinant hnRNP protein A1. We also report on its properties.

MATERIALS AND METHODS

Materials

S-Adenosyl-L-[methyl- ^{14}C]methionine (specific radioactivity, 47 mCi/mmol) and S-adenosyl-L-[methyl- 3H]methionine (specific radioactivity, 60 Ci/mmol) were purchased from ICN Radiochemicals. DEAE-cellulose DE-52 was obtained from Fisher Chemicals, and Sephadex G-200, S-adenosyl-L-homocysteine (AdoHcy), sinefungin, *p*-chloromercuribenzoate, phenylmethanesulphonyl fluoride (PMSF), 2-mercaptoethanol, *p*-phthalaldehyde, histone II-AS (calf thymus) and γ -globulin (bovine) were from Sigma Chemical Co. All other chemicals used were of the highest purity available. MBP was purified from bovine brain as described previously (Chanderkar et al., 1986; Deibler et al., 1972). Recombinant protein A1 was purified from overexpressed *Escherichia coli*, containing plasmid pEx11 carrying the cDNA coding sequence for rat protein A1, by single-stranded DNA–cellulose column chromatography as described (Cobianchi et al., 1988). Two subclasses of calf brain protein methylase I (MBP-specific and nucleic acid/histone-

Abbreviations used: AdoMet, S-adenosyl-L-methionine; AdoHcy, S-adenosyl-L-homocysteine; hnRNP, heterogeneous nuclear ribonucleoprotein particle; MBP, myelin basic protein; PMSF, phenylmethanesulphonyl fluoride; PEG, poly(ethylene glycol); ND, non-denaturing; MeArg, N^G -monomethylarginine; Me_2 (sym)Arg, $N^G N^G$ -dimethylarginine; Me_2 (asym)Arg, $N^G N^G$ -dimethylarginine.

* To whom correspondence should be addressed.

specific) were purified essentially as described (Ghosh et al., 1988).

Assay for protein methylase I

Protein methylase I activity was determined as described previously (Paik and Kim, 1968; Ghosh et al., 1988) with slight modifications. An incubation mixture contained 0.1 M Tris/HCl (pH 7.6), 40 μ M Ado[methyl- 14 C]Met (103 d.p.m./pmol) or Ado[methyl- 3 H]Met (450 d.p.m./pmol), substrate protein A1 (50 μ g) and enzyme preparation in a total volume of 0.125 ml. The reaction was initiated by the addition of Ado[methyl- 14 C]Met, following a 5 min preincubation at 37 °C. After 15 min at 37 °C, the reaction was terminated by the addition of 5 ml of 15 % trichloroacetic acid followed by the addition of 0.2 ml of γ -globulin (4 mg) as a carrier. The precipitate was washed three times with 15 % trichloroacetic acid and then once with ethanol. The precipitate was then suspended in 5 ml of scintillation mixture (Ecolite; ICN) and counted for radioactivity in an LKB 1209 RACKBETA liquid-scintillation counter. To estimate the activity in crude enzyme preparations such as cytosol (100 000 g supernatant), the first trichloroacetic acid precipitate was treated to remove nucleic acids, phospholipids and carboxymethyl esters formed by protein methylase II as described (Kim, 1984; Ghosh et al., 1988). The specific activity of the enzyme is defined as pmol of [methyl- 14 C] group transferred/min per mg of enzyme protein, and unit activity is defined as pmol of [methyl- 14 C] group transferred/min.

Protein concentration was estimated by the Coomassie Blue method of Bradford (1976) modified by Pierce Chemical Co. (cat. no. 23200) using BSA as the standard.

Purification of protein methylase I and rat liver

All procedures were carried out at 4 °C unless otherwise stated. Fresh rat liver (10 g) was cut into small pieces and homogenized in 4 vol. of 5 mM sodium phosphate (pH 7.4) containing 5 mM EDTA and 0.32 M sucrose. The homogenate was centrifuged first at 39 000 g for 30 min and then at 100 000 g for 60 min.

DEAE-cellulose DE-52 chromatography

The supernatant obtained at 100 000 g was mixed with an equal volume of 10 mM Mops (pH 7.4) containing 2 mM EDTA, 0.5 mM PMSF and 1 mM 2-mercaptoethanol (buffer A), and applied to a DE-52 cellulose column (3 cm $\times$ 8 cm) which was pre-equilibrated with buffer A. The column was washed with buffer A containing 0.1 M NaCl until the absorbance at 280 nm was less than 0.05 (about 500 ml required), and then proteins were eluted with a linear salt gradient established by 100 ml each of 0.1 M NaCl/buffer A and 0.25 M NaCl/buffer A. Fractions of 1.7 ml were collected at a flow rate of 20 ml/h. A sample from each fraction was assayed for enzyme activity using protein A1 or histone as the methyl-acceptor substrate (Figure 1). Enzymically active fractions were then pooled and concentrated by using poly(ethylene glycol) (PEG) 20000.

Sephadex G-200 gel filtration

The concentrated enzyme (about 2 ml) from the above DE-52 chromatography was applied to a Sephadex G-200 column (1.6 cm $\times$ 113 cm) pre-equilibrated with buffer A containing 2 %

glycerol, and elution was continued with the same buffer at a flow rate of 2 ml/h. Fractions of volume 2 ml were collected and assayed for enzyme activity. Enzymically active fractions were pooled, concentrated with PEG 20000 to about 1 ml and rechromatographed on the same Sephadex column as described above. Unless otherwise stated, most of the following studies were carried out with this enzyme preparation.

F.p.l.c.

The enzyme preparation obtained from Sephadex chromatography was further purified by f.p.l.c. (Pharmacia) using a Superose 6 column (1 cm $\times$ 30 cm) which was previously equilibrated with a buffer containing 50 mM sodium phosphate (pH 7.6) and 0.15 M NaCl at a flow rate of 6 ml/h. The elution was monitored at A_{214} with a Pharmacia UVII detector (see Figure 3). Fractions of volume 0.25 ml were collected and assayed for enzyme activity. About 70 % of the enzyme activity was lost during this procedure.

PAGE

Non-denaturing (ND) PAGE was carried out on a 7.5 % polyacrylamide gel at 4 °C and 30 mA/plate as described previously (Ghosh et al., 1988). SDS/PAGE was performed by the method of Laemmli (1970) with 10 % acrylamide for the running gel and 3 % for the stacking gel at a constant current of 35 mA for 5 h at room temperature.

Production of antibodies

The nucleic acid/histone-specific protein methylase I was purified from calf brain as described (Ghosh et al., 1988) and subjected to ND PAGE. The enzyme protein band corresponding to 275 kDa on the gel was excised and used for antibody production. Rabbits (about 2 kg body weight) were injected intradermally at multiple sites on the back with about 30–40 μ g of the purified enzyme emulsified with an equal volume of complete Freund's adjuvant. Booster injections were given every 2 weeks and the animals were bled after 8 weeks. Control preimmune serum was obtained before immunization. Immunoglobulin was purified as described (Ey et al., 1978). Ouchterlony double-immunodiffusion analysis was carried out by the method of Vaittukaitus et al. (1971).

Amino acid analysis by h.p.l.c.

The enzymically prepared [methyl- 14 C]protein A1 was subjected to 6 M HCl hydrolysis at 100 °C for 24 h *in vacuo*. After removal of the acid by lyophilization, the methylated amino acids were analysed by h.p.l.c. as described (Rawal et al., 1992). Briefly, separation of the methylated amino acids was achieved on a strong cation amino acid analysis column (0.4 cm $\times$ 25 cm; Waters Associates) by isocratic elution with 0.3 M sodium citrate buffer, pH 3.8. The eluted amino acids, derivatized with *o*-phthaldialdehyde, were detected with a fluorescence detector. Simultaneously, fractions (1 min) were collected and incorporation of radioactivity was determined.

RESULTS

Purification of protein methylase I from rat liver

Purification of protein methylase I from rat liver was relatively simple compared with that from calf brain (Ghosh et al., 1988). Our initial studies on the subcellular distribution of protein A1-methylating activity in rat liver indicated that more than 90 % of

Figure 1 DE-52 chromatography of rat protein methylase I

Rat liver 100 000 *g* supernatant (225 mg of protein) was applied to a column (3 cm × 8 cm) of DE-52 and the enzyme was eluted with an NaCl gradient (—) as described in the Materials and methods section. A portion (30 μ l) from each fraction was assayed for enzyme activity using 50 μ g of protein A1 (●) or 0.5 mg of histone (○) as the methyl-acceptor substrate in a total incubation mixture of 0.125 ml., A_{280} .

the total activity was present in the cytosol and approximately 5% in the nuclear pellet. When rat liver cytosol was subjected to anion-exchange (DE-52) column chromatography (Figure 1), over 90% of the total protein applied was eluted in the run-off fraction showing a deep-reddish colour (not shown). The subsequent salt-gradient elution resulted in 114-fold increase in enzyme specific activity with a yield of 738%, with protein A1 as the substrate (Table 1 and Figure 1). It is clear from Figure 1 that histone is very inferior to protein A1 as a substrate in all the enzymically active fractions. The unusually high yield of enzyme activity is probably due to the removal of cytosolic protein inhibitor of protein methyltransferases which is known to be present in high amount particularly in rat liver cytosol (Kim and Paik, 1971; Chiva and Mato, 1984; Hong et al., 1986; Park et al., 1993). The purified inhibitor was shown to be a peptide of molecular mass approx. 2600 Da and inhibited all the AdoMet-dependent methyltransferases so far examined, including protein A1-arginine methyltransferase (Park et al., 1993). This was further confirmed in the present study by an experiment in which extensive dialysis of the 100 000 *g* supernatant against buffer A resulted in a 2.5-fold increase in enzyme activity over the undialysed preparation (results not shown). The subsequent two steps of molecular-sieve chromatography on Sephadex G-200 further increased the enzyme activity to 195-fold purification.

Table 1 Purification of rat liver protein methylase I

Enzyme activity was measured using recombinant protein A1 as substrate. Specific activity is defined as pmol of methyl group transferred/min per mg of protein under the assay conditions described in the Materials and methods section.

Purification steps	Total protein (mg)	Enzyme activity		Purification (fold)	Yield (%)
		Specific activity	Total activity		
Supernatant at 100 000 <i>g</i>	225	0.42	95	1	100
DE-52 column chromatography	14.6	48	701	114	738
First Sephadex G-200 chromatography	4.5	67	302	160	318
Second Sephadex G-200 chromatography	1.6	82	131	195	138

Figure 2 Ouchterlony double immunodiffusion analysis of rat protein methylase I

The immunoplate was incubated overnight in a moist chamber at room temperature and then at 4 °C for 48 h. The central well contained anti-immunoglobulin against purified calf brain nuclear protein/histone protein methylase I, and out wells contained: well 1, purified calf brain nuclear protein/histone protein methylase I (25 μ g); well 2, purified rat liver protein methylase I (36 μ g); well 3, purified calf brain MBP protein methylase I (42 μ g).

Immunological differences between rat liver and calf brain enzyme

Immunological identity of rat liver protein methylase I judged by antibodies raised against calf brain nuclear protein methylase I (formerly called histone-protein methylase I) was also investigated by Ouchterlony double-immunodiffusion analysis. As shown in Figure 2, the purified rat liver enzyme did not cross-react with antibodies against the brain enzyme, indicating that the rat enzyme is immunologically distinguishable from the brain enzyme, possibly exhibiting species and/or tissue specificity.

Molecular size of protein methylase I

In order to estimate the molecular size of the rat liver enzyme, the Sephadex G-200 preparation was subjected to Superose 6 f.p.l.c. (Figure 3). Although this chromatographic procedure inactivated about 70% of the enzyme, perhaps as a result of the buffer composition (which is devoid of glycerol and 2-mercaptoethanol), a homogeneous enzyme preparation was obtained. An attempt to preserve the enzyme activity by inclusion of 2-mercaptoethanol in the f.p.l.c. buffer was unsuccessful, because the thiol compound itself exhibited high A_{214} resulting in high background on the chromatogram which obscured the enzyme peak. The molecular size of the native enzyme was shown to be approximately

Figure 3 Purification of rat liver protein methylase I by f.p.l.c. on a Superose 6 column

The enzyme preparation obtained from Sephadex G-200 chromatography was further purified on a Superose 6 column. Fractions (250 μ l) were collected at a flow rate of 6 ml/h. A portion (155 μ l) from each fraction was assayed for enzyme activity using 40 μ M Ado[methyl- 3 H]Met (350 d.p.m./pmol) at 37 $^{\circ}$ C for 2 h. Details of other conditions are the same as described in the text. —, Relative absorbance; ▨, radioactivity.

Figure 4 Estimate of molecular mass of rat protein methylase I by Superose 6 chromatography

The experimental conditions were the same as those described in Figure 3. The distribution coefficient (K_{av}) was determined by the equation $(V_e - V_0)/(V_t - V_0)$, where V_e is the elution volume of the protein, V_0 the void volume and V_t the total gel bed volume. Estimation of molecular mass was by the linear correlation between K_{av} and log (molecular mass) of standard globular proteins. The elution volume of rat protein methylase I was calculated from Figure 3.

450 kDa, estimated by both Superose 6 chromatography (Figure 4) and ND PAGE (results not shown). On SDS/PAGE, however, the purified enzyme exhibited a single band at approximately 110 kDa (Figure 5), indicating that the native form of the enzyme consists of four identical-size subunits.

Effect of glycerol on protein methylase I activity

During the course of enzyme purification, we encountered high variability in enzyme activity. This led us to uncover the inhibitory effect of glycerol on enzyme activity if present in the incubation

Figure 5 PAGE of purified rat protein methylase I

The enzyme purified by f.p.l.c. on a Superose 6 column as described in Figure 3 was subjected to PAGE. SDS/PAGE was performed in 10% acrylamide using 3 μ g of the purified enzyme as described in the text. Prestained low-molecular-mass markers (Bio-Rad, cat. no. 161-0305) were used; phosphorylase *b* (106 kDa), BSA (80 kDa), ovalbumin (49.5 kDa), bovine carbonic anhydrase (32.5 kDa) and soya-bean trypsin inhibitor (27.5 kDa) are shown.

Table 2 Protein substrate specificity of rat liver protein methylase I

Enzyme reaction was carried out in the presence of recombinant protein A1 (50 μ g) or the indicated proteins (500 μ g each) as the methyl-acceptor substrate in a standard reaction mixture as described in the Materials and methods section. An activity of 100% indicates 12 pmol of [methyl- 14 C] group transferred/15 min at 37 $^{\circ}$ C using protein A1 as the substrate.

Substrates	Activity (%)
Protein A1	100
MBP	23.3
Histone	11.0
Cytochrome <i>c</i>	0
BSA	0
γ -globulin	0

mixture; nearly 80 % of the activity was inhibited in the presence of 20 % glycerol (results not shown). This finding was quite perplexing, as glycerol was routinely included in the buffer to stabilize the enzyme during purification.

Substrate specificity of protein methylase I

Table 2 shows the relative substrate capacity of various protein substrates; rat liver methylase showed high specificity toward recombinant protein A1 with a K_m value of 0.54×10^{-6} M (summarized in Table 5). MBP and histone exhibited only 23 % and 11 % of the protein A1-methylating activity respectively, and cytochrome *c*, γ -globulin and BSA did not serve as substrate at all. Interestingly, with the calf brain enzyme, MBP was an inhibitor with a K_i value of 3.45×10^{-5} M [Table II in Ghosh et al. (1988)]. The K_m values for protein A1 and AdoMet were 0.54×10^{-6} M and 6.3×10^{-6} M respectively. AdoHcy and sinefungin, well-known inhibitors of most AdoMet-dependent methyltransferases, were both competitive inhibitors, with K_i values of 8.4×10^{-6} M and 0.65×10^{-6} M respectively (Figure 6).

Effect of various chemicals on protein methylase I

Table 3 illustrates the effect of bivalent metal ions on rat protein methylase I activity using protein A1 as the substrate. Of the

Figure 6 Effect of various concentrations of AdoHcy and sinefungin on rat liver protein methylase I

Enzyme activity was determined using recombinant protein A1 as the methyl-acceptor substrate as described in the text. (a) Enzyme activity with no inhibitor (○) and in the presence of AdoHcy at 3 μ M (●), 6 μ M (△) and 9 μ M (▲). (b) Enzyme activity with no inhibitor (●) and in the presence of sinefungin at a concentration of 3 μ M (▲), 6 μ M (○) and 9 μ M (△). Insets are secondary plots of the respective slopes.

Table 3 Effect of bivalent metal ions on rat liver protein methylase I activity

Enzyme reaction was carried out in a standard reaction mixture (125 μ l) in the presence of the indicated amount of the test compound. Details of assay conditions are described in the Materials and methods section. An activity of 100% represents 18.6 pmol of [*methyl*- 14 C] group transferred/30 min at 37 $^{\circ}$ C.

Addition	Concentration (mM)	Activity (%)
None	—	100
EDTA	10	89.1
Ca $^{2+}$	1	104.2
	5	64.2
Mg $^{2+}$	1	94.6
	5	67.1
Fe $^{2+}$	1	79.4
	5	34.1
Ni $^{2+}$	1	86.7
	5	2.9
Mn $^{2+}$	1	15.4
	5	2.3
Zn $^{2+}$	0.1	90.9
	1	1.1

various metals tested, Zn $^{2+}$ was the most potent, inhibiting almost all of the enzyme activity at 0.1 mM. Mn $^{2+}$ and Ni $^{2+}$ also inhibited most of the activity but at a concentration of 5 mM. Enzyme activity was not significantly affected by EDTA, indicating that the enzyme may not require any metal ion for activity. It was extremely sensitive to *p*-chloromercuribenzoate; 50% of the activity was lost at 0.12 mM, and nearly all of the activity at 0.5 mM, indicating a requirement for a thiol group for activity. In the presence of guanidinium chloride, a denaturing reagent, 50% of the activity remained at 100 mM.

Table 4 Analysis of products of rat liver protein methylase I activity

The methylation reaction was carried out in a volume of 0.5 ml (four times the standard reaction mixture) in the presence of 40 μ M Ado[*methyl*- 14 C]Met and 43.5 μ g of the Sephadex G-200-purified rat liver protein methylase I at 37 $^{\circ}$ C for 60 min, using 0.2 mg of recombinant protein A1 or 2 mg of calf thymus histone II-AS as the methyl-acceptor substrate. After the reaction, the [*methyl*- 14 C]protein was recovered by precipitation with trichloroacetic acid, hydrolysed with 6 M HCl, and [*methyl*- 14 C]amino acid was analysed by h.p.l.c. as described in the text. Specific activity is defined as pmol of [*methyl*- 14 C] group transferred/min per mg of enzyme protein. Values in parentheses indicate the percentage of [*methyl*- 14 C] group recovered as *N* $^{\epsilon}$ -methylarginines from the respective substrate.

Substrate	Specific activity	[<i>methyl</i> - 14 C] group incorporated (pmol)		
		MeArg	Me $_2$ (asym)Arg	Me $_2$ (sym)Arg
Protein A1	73.2	113 (59)	39.2 (41)	0
Histone II-AS	11.8	15 (49)	7.82 (51)	0

Various other properties of the enzyme

Purified rat liver protein methylase I has an optimum pH around 7.6 with protein A1 as the methyl-acceptor substrate (results not shown). The stability of the enzyme at high temperatures was studied by preincubating the enzyme at the indicated temperature for 5 min, followed by the standard enzyme assay as described previously (Ghosh et al., 1988): Approximately 15% of the activity was lost at 40 $^{\circ}$ C and nearly all of the activity at 60 $^{\circ}$ C (results not shown). In the case of calf brain nuclear protein/histone methylase I, 20% of the activity was lost at 45 $^{\circ}$ C, and 80% at 60 $^{\circ}$ C, indicating a similar heat-inactivation profile.

Identification of the methylation product

To identify the methylation product, [*methyl*- 14 C]protein A1 was hydrolysed in 6 M HCl, and analysed for 14 C-labelled amino

acids by h.p.l.c. Two peaks of radioactivity coinciding with the standard MeArg and Me₂(asym)Arg were identified in a ratio of 59:41 (Table 4). A dimethyl isomer, Me₂(sym)Arg, was not detected among the methylated products.

DISCUSSION

The present paper described the purification from rat liver cytosol of protein methylase I which is quite similar to the recently described nuclear protein/histone protein methylase I from calf brain (Ghosh et al., 1988; Rajpurohit et al., 1994). Both enzymes show a high specificity toward recombinant hnRNP protein A1; the K_m value for the former is 0.54×10^{-6} M and that for the latter, 0.19×10^{-6} M. The K_m values of these enzymes for AdoMet are also similar (6.3×10^{-6} M and 12.5×10^{-6} M for the rat liver and calf brain enzymes respectively) (Table 5). Furthermore, both enzymes yielded only MeArg and Me₂(asym)Arg as the methylation products, and no Me₂(sym)Arg. On the other hand, they are intrinsically quite different in their molecular properties: whereas rat liver methylase I was shown to have a molecular mass of 450 kDa possibly consisting of four identical-size subunits (110 kDa) (Figure 5), that of the calf brain enzyme was 275 kDa with two non-identical subunits (110 and 75 kDa; Table 5) (Ghosh et al., 1988). Most importantly, however, the rat liver enzyme did not cross-react with polyclonal antibodies raised against the calf brain enzyme, indicating that they are immunologically distinguishable. It should also be noted that these enzymes exhibited quite different sensitivity to an inhibitor; the K_i value for sinefungin for the rat enzyme was 0.65×10^{-6} M, whereas that for the calf brain enzyme was 6.6×10^{-6} M (Ghosh et al., 1988). On the basis of these properties together with the fact that protein A1 is the only high-affinity substrate for both enzymes, it can be surmised that these enzymes are indeed species/tissue-specific isozymes.

Protein A1 is one of the major proteins of nuclear ribonucleoprotein particle (Beyer et al., 1977) which is known to

be a precursor for mRNA (Dreyfuss, 1986; Choi et al., 1986). The protein is highly basic in nature and contains clusters of Gly, Phe and Arg/Me₂(asym)Arg in the C-terminal domain (Lischwe, 1990), which has been implicated as the site for co-operative binding to nucleic acid (Kumar et al., 1990). Protein A1 binds to RNA and single-stranded DNA (Herrick and Alberts, 1976; Williams et al., 1985), and stimulates DNA polymerase α *in vitro* (Riva et al., 1980; Sapp et al., 1985). The protein has also been classified as a helix-destabilizing protein (Planck and Wilson, 1980) and a nucleic-acid-unwinding protein (UP1; proteolysis product of protein A) (Herrick and Alberts, 1976; Williams et al., 1985).

The primary amino acid sequence of protein A1 isolated from rat liver was shown to contain 3.1 mol% of Me₂(asym)Arg (Kumar et al., 1986). Although several other proteins in the nucleus, such as nucleolin (Lischwe et al., 1985a), fibrillarin (Lischwe et al., 1985b), chromosomal high-mobility groups 1 and 2 (Boffa et al., 1979), also contain N^G-methylated arginines, over 90% of the methylating enzyme activity has been found to reside in the cytosol (R. Rajpurohit, W. K. Paik and S. Kim, unpublished work). Thus it is extremely intriguing to speculate on the role that enzymic methylation plays in the processing/maturation and nuclear transport of protein A1 after its biosynthesis on the polysomes.

Enzymic methylation of specific amino acid residues (such as arginine and lysine) in a protein molecule has been shown to bring about drastic structural changes in the protein as a result of the modification. One dramatic example is the enzymic methylation of Lys-72 of yeast cytochrome *c*: a drastic decrease in the pI value (Park et al., 1988) and enhancement of the importation of cytochrome *c* into mitochondria (Park et al., 1987) results. It seems to be somewhat premature to speculate on the biochemical role of arginine methylation of nucleic acid-binding proteins, but preliminary studies have indicated that the pI value of enzymically methylated protein A1 is significantly lower than that of the unmethylated species. Furthermore, it would be interesting to investigate the relative binding properties of unmethylated and methylated A1 to either RNA or single-stranded DNA. As both purified protein methylase I and recombinant unmethylated protein A1 are now available in this laboratory, it is planned to carry out several comparative studies.

This work was supported in part by grants from the National Cancer Institute (5-P30-CA12227), the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases (AM09602) and Temple University (BRSG PRO5417).

Table 5 Comparative properties of rat liver and calf brain nuclear protein/histone protein methylase I

ND indicates not determined.

Characteristics	Rat liver enzyme	Calf brain enzyme*
Molecular mass of native enzyme (by molecular sieve and non-denaturing PAGE) (kDa)	450	275
Subunit (by SDS/PAGE) (kDa)	110	110, 75
K_m values (M)		
Protein A1	0.54×10^{-6}	$0.19 \times 10^{-6}\dagger$
Histone	ND	$21.0 \times 10^{-6}\dagger$
AdoMet	6.3×10^{-6}	$12.5 \times 10^{-6}\dagger$
K_i values (M)		
MBP	Not an inhibitor	3.42×10^{-5}
AdoHcy	8.4×10^{-6}	2.3×10^{-6}
Sinefungin	0.65×10^{-6}	6.6×10^{-6}
IC_{50} (mM)		
<p>-Chloromercuribenzoate</p>	0.12	0.15
Guanidinium chloride	100	300
Percentage inactivation at 50 °C for 5 min	83	40

* Data from Ghosh et al. (1988), unless otherwise specified.

† Data from Rajpurohit et al. (1994).

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