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Recent advances in protein methylation: Enzymatic methylation of nucleic acid binding proteins

Review Article

S. Kim[1], **G. H. Park**[1], and **W. K. Paik**[2]

[1]Department of Biochemistry, Medical School and Graduate School of Biotechnology, Korea University, Seoul, Korea
[2]Department of Biochemistry, School of Medicine, Ajou University, Suwon, Korea

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Summary. Heterogeneous nuclear RNP protein A1, one of the major proteins in hnRNP particle (precursor for mRNA), is known to be posttranslationally arginine-methylated *in vivo* on residues 193, 205, 217 and 224 within the RGG box, the motif postulated to be an RNA binding domain. Possible effect of N^G-arginine methyl-modification in the interaction of protein A1 to nucleic acid was investigated. The recombinant hnRNP protein A1 was *in vitro* methylated by the purified nuclear protein/histone-specific protein methylase I (S-adenosylmethionine:protein-arginine N-methyltransferase) stoichiometrically and the relative binding affinity of the methylated and the unmethylated protein A1 to nucleic acid was compared: Differences in their binding properties to ssDNA-cellulose, pI values and trypsin sensitivities in the presence and absence of MS2-RNA all indicate that the binding property of hnRNP protein A1 to single-stranded nucleic acid has been significantly reduced subsequent to the methylation. These results suggest that post-translational methyl group insertion to the arginine residue reduces protein-RNA interaction, perhaps due to interference of H-bonding between guanidino nitrogen arginine and phosphate RNA.

Keywords: Protein-arginine methylation – Nucleic acid binding protein – Protein methylase I – S-adenosyl-L-methionine – -RGG motiff

Abbreviations: hnRNP, heterogeneous ribonucleoprotein particle; AdoMet, S-adenosyl-L-methionine; AdoHcy, S-adenosyl-L-homocysteine; MBP, myelin basic protein; HMG, high mobility group; ss, single stranded.

I. Introduction

A large number of proteins biosynthesized at the polyribosomes are posttranslationally modified *in vivo* to yield functionally acitive and/or inac-

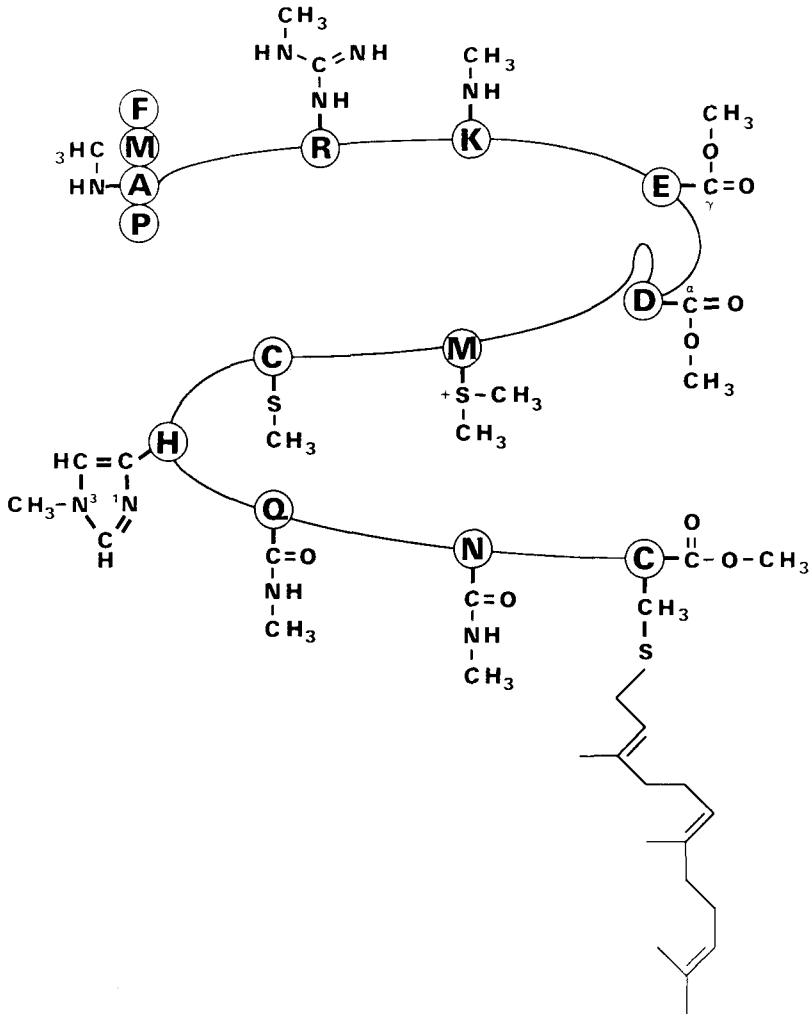

Fig. 1. Posttranslational methylation of a protein. Single letter sabbreviations are used for amino acids

tive proteins to modulate their functions (Wold, 1981), while preserving their nascent primary structure. One of such modification reactions is the methylation of protein (Fig. 1); this occurs on the several side chains of amino acids, catalyzed *in vivo* by a group of highly protein- and amino acid-specific methyltransferases (Paik and Kim, 1980; Paik and Kim, 1985; Kim et al., 1990). It is quite likely that there are more methylated amino acids in nature yet to be identified. The most extensively studied protein methyl-modification reactions include; N-methylation of arginine, lysine, and histidine side chains; O-methylation of either internal carboxyl groups of glutamate or isoaspartate residues and COOH-terminal lipidated cysteine residues; and S-methylation of either cysteine or methionine residues. We review in this article the most recent up-to-date information on the protein-arginine methylation, emphasizing an enzymology on the subclasses of the enzyme, and possible biological

significance of arginine-methylation *in vivo* and *in vitro* of nucleic acid binding proteins: These subjects gained much progress in recent years in regards to the biological role of protein methylation in general. Readers should be refered to many review articles on the other protein methylation reactions (Siegel et al., 1990; Johnson and Aswad, 1990; Clarke, 1985; Duerre et al., 1991).

II. Protein-arginine methyltransferase (protein methylase I)

Protein methylase I (S-adenosylmethionine:protein-arginine N-methyltransferase; EC.2.1.1.23) methylates the guanidino nitrogen of arginine residues utilyzing S-adenosyl-L-methionine (AdoMet) as the methyl donor (Paik and Kim, 1980; Kim et al., 1990). As shown in Fig. 2, the reaction yields three methylated derivatives; N^G-monomethylarginine, N^G,N^G-dimethylarginine and N^G,N'^G-dimethylarginine with liberation of S-adenosyl-L-homocysteine (AdoHcy), the demethylated AdoMet. AdoHcy is a potent product inhibitor for all known AdoMet-dependent transmethylation reactions with K_i values close to the K_m for AdoMet in most cases. There are many natural and synthetic structural analogs of AdoHcy which are widely used as inhibitor for methylation reactions (Oliva et al., 1980; Lawrence and Robert-Gero, 1990).

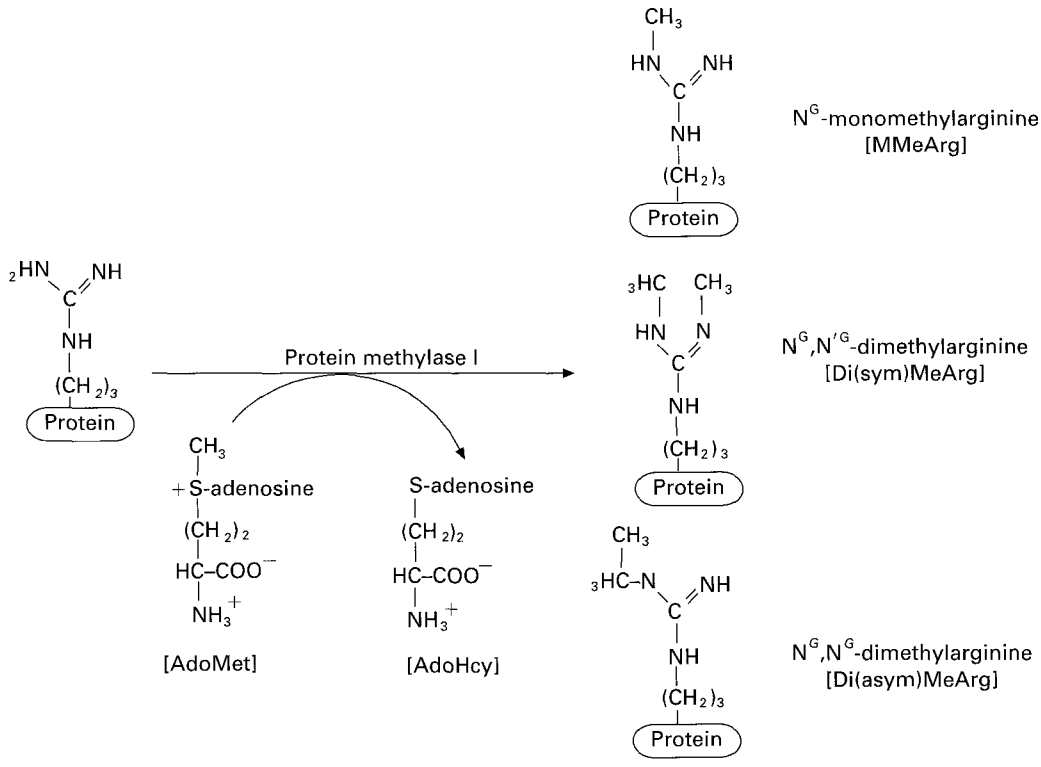

Fig. 2. Reaction of protein methylase I (protein-arginine N-methyltransferase)

Table 1. Natural occurrence of N^G-methylarginine

Protein	Authors and year of finding
Histone	Paik & Kim (1967)
Urine and Serum (as free amino acids)	Kakimoto & Akazawa (1970)
Myelin Basic Protein	Baldwin & Carnegie (1971)
	Brostoff & Eylar (1971)
Myosin	Reporter & Cobin (1971)
Ribosomal protein A1 & A2	Chang et al. (1976)
hnRNP protein A1 & A2	Boffa et al. (1977)
	Beyer et al. (1977)
Tooth matrix protein	Kalasz et al. (1978)
HMG 1 & 2	Boffa et al. (1979)
Heat shock protein	Wang et al. (1982)
Nucleolin (C23 nucleolar phosphoprotein)	Lischwe et al. (1985)
Fibrillarin (Scleroderma antigen; 34 kDa nucleolar protein	Lischwe et al. (1985)
ssDNA binding protein(UP1)	Williams et al. (1985)
Basic fibroblast growth factor	Sommer et al. (1989)
	Burgess et al. (1991)

As shown in Table 1, these unusual methylated amino acid residues are found in wide variety of proteins. It is noted that the majority of these proteins are structural proteins such as myelin basic protein (MBP) (Baldwin and Carnegie, 1971; Brostoff and Eylar, 1971), hnRNP protein (Bofffa et al., 1977; Beyer et al., 1977), nucleolin (Lischwe et al., 1985), fibrillarin (Lischwe et al., 1985), HMG chromosomal protein (Boffa et al., 1979), heat shock protein (Wang et al., 1982), and tooth matrix protein (Kalasz et al., 1978). This diversity and wide occurrence of the methylated arginine residues together with the ubiquitous distribution of the protein-arginine methylating enzyme led to the discovery of a multiplicity of enzymes which are specific to each methyl-acceptor protein.

A. Mammalian organs

Earlier studies on the tissue distribution of protein methylase I in rodent indicated that the enzyme is widely present in most organs, having decreasing order of the activity in testis, brain, thymus, spleen, kidney, and liver (Paik and Kim 1980). At present, two subclasses of protein methylase I have been identified and purified to apparent homogeneity from calf brain and rat liver: MBP-specific and nuclear protein/histone-specific protein methylase I, respectivley (Ghosh et al., 1988; Rajpurohit et al., 1994; Rawal et al., 1994). In the brain cytosol, both subclasses of the methylases are present. The native molecular mass of the MBP-specific protein methylase I was approximately ~500-kDa consisting of ~100- and ~72-kDa hetero-subunits, whereas that of the nuclear protein/histone-specific enzyme was ~275-kDa with ~110- and ~75-kDa hetero-subunits. On the other hand, rat liver nuclear protein/

Table 2. Comparative properties of MBP- and nuclear protein/histone-protein methylase I

Characteristic	MBP-PM I calf brain	Nuclear protein/histone-PM I	
		calf brain	rat liver
Mr (native)	500 kDa	275 kDa	450 kDa
Subunit (SDS-PAGE)	100 kDa, 72 kDa	110 kDa, 75 kDa	110 kDa
pI	5.09	5.68	
K_m value (M)			
protein A1	–	0.19×10^{-6}	0.54×10^{-6}
histone	1.0×10^{-4}	21.0×10^{-6}	–
MBP	0.23×10^{-6}	inhibitor	–
AdoMet	4.4×10^{-6}	8.0×10^{-6}	6.3×10^{-6}
K_i value (M)			
AdoHcy	1.8×10^{-6}	2.3×10^{-6}	8.4×10^{-6}
sinefungin	7.0×10^{-6}	6.6×10^{-6}	0.65×10^{-6}
MBP	–	3.42×10^{-6}	not inhibitor
50% inactivation			
PCMB	0.46 mM	0.15 mM	0.12 mM
Guanidine-HCl	3.1 mM	0.3 mM	100 mM
At 50°C for 5 min	99% remained	60% remained	17% remained

Data are from Ghosh et al. (1988), Rajpurohit et al. (1994), and Rawal et al. (1994).

histone-specific methylase was shown to be molecular mass of ~450-kDa consisting of four ~100-kDa homo-subunits.

At this point, a brief background of the identification of nuclear protein/histone-specific protein methylase I should be in order. For most of the earlier investigations on protein methylase I, histones (unfractionated) were the commonly used substrate for the methylase assays, even though it was not the most ideal substrate in terms of its purity and the methyl-accepting efficiency. However, since an unmethylated recombinant hnRNP protein A1 became available (Cobianchi et al., 1988), subsequent reinvestigation on its substrate activity using both protein A1 or histones indicated that the former was by far superior substrate for previously thought the "histone-specific" enzyme, evidenced by the fact that the K_m value for the recombinant protein A1 was two orders of magnitude lower than that of histone (0.19 μM vs. 21 μM). The maximal extent of methylation between these two substrates differed greatly; whereas 1.08 mol of methyl groups were incorporated into the protein A1, only 0.04 mol were incorporated into the histone (Rajpurohit et al., 1994). The greater capacity of the protein A1 to be methylated together with its higher affinity for the protein suggested it more likely to be an *in vivo* substrate for this enzyme than histone. Consequently, in 1994, hitherto "histone-specific" methylase I was renamed as "nuclear protein/histone-specific" protein methylase I (Rajpurohit et al., 1994). Table 2 shows the overall molecular and catalytic properties of three protein methylase I's. It shows that not only the native molecular weight but also subunit sizes of the three methylases are

quite different. An immunological difference between MBP-specific and nuclear protein/histone-specific enzyme has been verified by Western immunoblot analyses using respective antibodies raised against the purified enzymes (Rajpurohit et al., 1994). Sensitivities toward p-chloromercuribenzoate and guanidine-HCl as well as heat inactivation profiles also differ markedly among the enzymes. Interestingly, MBP, a high affinity substrate for MBP-specific protein methylase I, acted as an inhibitor for the nuclear protein/histone-specific enzyme with K_i value of 3.42×10^{-6}M (Park et al., 1986). Related to this, our recent study showed that several basic amino acids such as arginine, lysine and histidine, as well as polyamines were found to inhibit the rat liver protein methylase I at relatively high concentrations (Yoo et al., 1998).

Recently, the primary amino acid sequence of nuclear protein/histone-specific methylase I of rat liver was found to be identical with that of the rat liver 10-formyltetrahydrofolate dehydrogenase [EC. 1.5.1.6; Cook et al., 1991] (unpublished results). Since the dehydrogenase is known to be a multifunctional enzyme molecule having not only the 10-formyltetrahydrofolate dehydrogenase but also aldehyde dehydrogenase activities (Krupenko et al., 1997), the above findings require further investigation to define catalytic domain of the dehydrogenase whose sequence is responsible for the methylase activity. In view of these unexpected findings, it is tempting to speculate that there must be some relationship between catalysis of transmethylation and dehydrogenation in the pathway of one-carbon folate metabolism.

B. Cultured cells

Studies on protein arginine-methylation in several cell lines showed that there exist multiple endogenous substrates. For example, metabolic labeling of the PC12 cells with Ado[methyl-^{3}H]Met indicated the presence of over 50 methyl acceptors with molecular weights ranging 18 to 120-kDa, when the cells were hypomethylated by the treatment of adenosine dialdehyde (Najbauer et al., 1993). The methylation of these endogenous proteins was inhibited by synthetic peptide containing methyl acceptor sequence of nucleic acid binding protein (but devoid of N^G-methyl-groups in arginine residues). Thus, it was concluded that the majority of the endogenous proteins which contain N^G-methylarginine are likely to interact with RNA. Park et al. (Park et al., 1997) studied hnRNP protein A1-arginine-methylation in HCT-48 colon cancer cells synchronized in culture, and found about 35-kDa endogenous substrate protein whose methylation was severly inhibited by the addition of exogenous hnRNP protein A1, indicating that the 35-kDa endogenous methyl-acceptor protein compete with hnRNP protein A1 during the methylation reaction. In addition, there present a hitherto uncharcterized highly methylated 20-kDa protein species. The extents of methylation of these two protein species were highest during the S-phase of the cell cycle, indicating possible correlation between cellular proliferation and arginine-methylation of these endogenous

proteins. Using HeLa cells in culture, histone f3 (arginine-rich histone) was also shown to be arginine methylated at maximum level during S phase (Borun et al., 1972). Although protein-arginine methyltransferase is not abundant enzyme in HeLa cells, the enzyme was partially purified and enzymatically active species was shown to be ~450-kDa with major protein bands of ~100- and ~45-kDa on SDS-PAGE (Liu and Dryfuss, 1995). The substrate specificity of the HeLa cell enzyme was shown to be similar to that of the mammalian nuclear protein/histone-specific protein methylase I.

Employing two hybrid analysis, a protein which interacted with TIS21 immediate-early gene and leukemia-associated BTG gene product was shown to be protein arginine N-methyltransferase in RAT1 cell (PRMT1; Lin et al., 1996). Amino acid sequence deduced from DNA sequence of this cloned methyltransferase indicated its molecular mass to be 40.5-kDa composed of 353 amino acid residues which showed high sequence homology with ODP1 gene product from *Saccharomyces cerevisiae* encoding a 348 amino acid residues of 39.8-kDa molecular mass (Feldman et al., 1994; Gary et al., 1996). Interestingly, TIS21 and BTG fusion protein of glutathion S-transferase qualitatively and quantitatively modulated protein-arginine methyltransferase in RAT1 cell. More recently, to identify proteins which could interact with the intracytoplasmic domain of the interferon-α, β receptors, two hybrid screening has been also employed (Abramovich et al., 1997): Among several positive clones, IR1B4 was identified as the protein arginine-methyltransferase from RAT1 cell. These authors speculated that methylated proteins by the methyltransferase which can attach to the intracytoplasmic domains of receptors may be a signaling mechanism complementing protein phosphorylation. These interesting observations obtained from two hybrid analyses require further study to elucidate the relationship between protein-arginine methyltransferase and the interacting receptor proteins.

III. Arginine-methylation of nucleic acid binding proteins

A. Heterogeneous RNP particle and nucleic acid binding proteins

Heterogeneous ribonucleoprotein particle (hnRNP) 40–50S particle is located in the nucleoplasm and known to serve as the platform to process mRNA after series of reactions involving splicing, packaging and transport to cytoplasm (Beyer et al., 1977). Several structurally related proteins (A1, A2, B1, B2, C1 and C2) are associated with the particle, A1 being the major core protein. Protein A1 is a basic protein with a molecular mass of 34-kDa consisting of 319 amino acid residues and contains 3.1 mol of N^G,N^G-dimethylarginines per mol of the protein (Kumar et al., 1986). The notable property of protein A1 is to bind single-stranded (ss) RNA or DNA (Cobianchi et al., 1988; Kumar et al., 1990), and stimulates α DNA polymerase activity *in vitro* (Herick et al., 1976).

The rat hnRNP protein A1 was cloned and overexpressed in *Escherichia coli* (Cobianchi et al., 1988). Analysis of the primary structure of protein A1 indicated that it contained two major domains, i.e., the residues 1–195 region

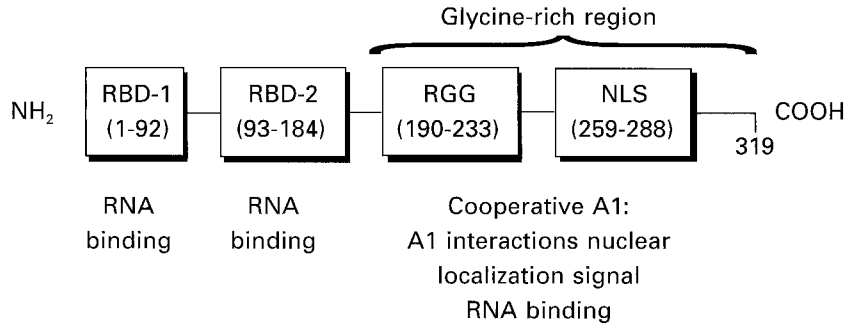

Fig. 3. Schematic depiction of functional domains in hnRNP protein A1. Structural alignments of domains are based on the following references: Merrill et al., 1986; Kiledjian and Dreyfus, 1992; Weighardt et al., 1995; Kim et al., 1997

and the glycine-rich 196–319 COOH-terminal domain (Merrill et al., 1986). The NH_2-terminal 1–195 region contains a region of internal sequence homology to an extent of 32% when residues 3–93 are aligned with 94–184. The two internal repeats have been postulated to represent two independent nucleic acid binding domains (confer Fig. 3). The similar domains have been found in more than 100 other eukaryotic RNA binding proteins (Adams et al., 1986; Birney et al., 1993; Kenan et al., 1991), and this domain of about 90 amino acid residues is referred to as the RNP motif RNA-binding domain (RBD). Several nucleic acid binding proteins [nucleolin and the poly(A) binding protein] were found to have from one to four of these domains (Kenan et al., 1991).

One-third of the COOH-terminal protein A1 (residue 185–319) contains 45% of the glycine residues which is not homologus with the NH_2-terminal two thirds. The COOH-terminal region contains several Arg-Gly-Gly (RGG box) sequences that have been proposed to constitute a conserved RNA-binding motif (Dreyfus et al., 1992). This region of protein A1 contains six arginines, five of which occur in Arg-Gly-(Gly) repeats boxes. Numerous other RNA-binding proteins such as splicing factors, hnRNPs, RNA helicases, nucleolin, and fibrillalin also contain RGG repeats (interspersed with aromatic amino acids) that have a similar spacing within the RNP-binding motif as that found in A1. Nucleolin is associated with the pre-rRNA particle present in nucleolus, and contains four residues of N^G-dimethylarginines in the RGG motif (Dreyfuss et al., 1992).

B. In vitro *effect of methylation on hnRNP protein A1*

Among many proteins known to contain N^G-methylarginine residues, protein A1 is one of the most highly *in vivo* methylated proteins containing 3.1–4.0 mol of dimethylarginines per mol of the protein (Kumar et al., 1986; Kim et al., 1997). This makes it an ideal model molecule to study structure-

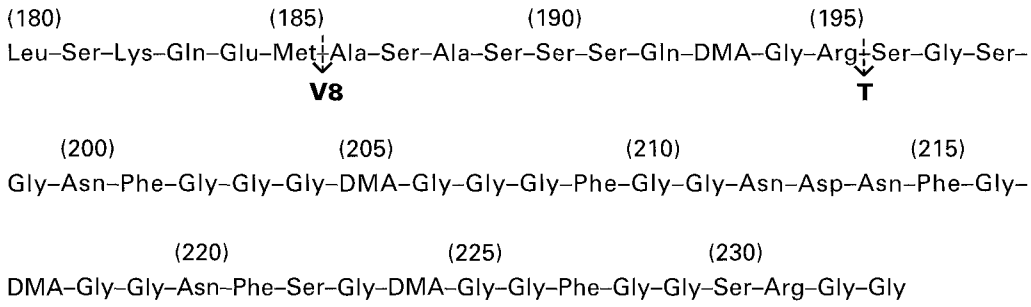

Fig. 4. Amino acid sequence of the RGG box region of hnRNP protein A1 containing four N^G,N^G-dimethylarginines, and protease sensitive sites. *V8* indicates cleavage site for V8 endoprotease and *T* for trypsin

functional relationship of this posttranslational modification reaction. However, in the past, the major difficulty for the study has been an unavailability of unmethylated methyl-acceptor proteins, because proteins isolated from normal cells are already methylated *in vivo*. To overcome this hurdle, we used recombinant hnRNP protein A1 which is not methylated and available in milligram quantities. The unmethylated recombinant protein A1 was methylated up to 1.45 mol methyl-groups per mol of protein (maximum extent of *in vitro* methylation) by purified nuclear-protein/histone methylase I (Rajpurohit et al., 1994). The methylation site has been identified by specific cleavage of [methyl-^{3}H]protein A1 by *Staphylococcus aureus* V8 endoprotease and trypsin. Trypsin selectively cleaves at Arg-195 whereas V8 cuts at Glu-184 (Fig. 4), thus yielding two similar-size fragments; the larger NH_2-terminal (22- or 24-kDa) and the smaller COOH-terninal (10- or 12-kDa) fragments. Analysis of the digests on SDS-PAGE and autoradiography indicated that the Arg 193 of the NH_2-terminal fragment and the Arg 205, 217 and 224 of the COOH-terminal fragments were methylated (confer Fig. 4). However, ultraviolet absorption maxima of the methylated and unmethylated protein A1 were identical at 278.5 nm.

Several biochemical properties were compared between the unmethylated and the methylated protein A1. The methylated A1 was completely digested with 5 min, whereas the unmethylated A1 still remained undigested even after 10 min of digestion (Fig. 5). The difference in sensitivity of the two species of the protein toward trypsin was much more pronounce in the presence of ssRNA. In the presence of coliphage MS2-RNA, the $T_{1/2}$ was 2.41 min for the methylated and 4.30 min for the unmethylated protein (Table 3). The pI values were also found to be different between the methylated and the unmethylated protein A1 [9.41 for the former and 9.48 for the latter (Rajpurohit et al., 1994)]. In agreement with the above observation, an experiment carried out by Cornnell et al. (1993) demonstrated that the motifs bound to arginine-affinity gel were eluted with arginine, however, much less efficiently with N^G-methylarginine.

Based on the above parameters such as pI value, the binding properties to ssDNA-cellulose column, together with the fluorescence quenching in the

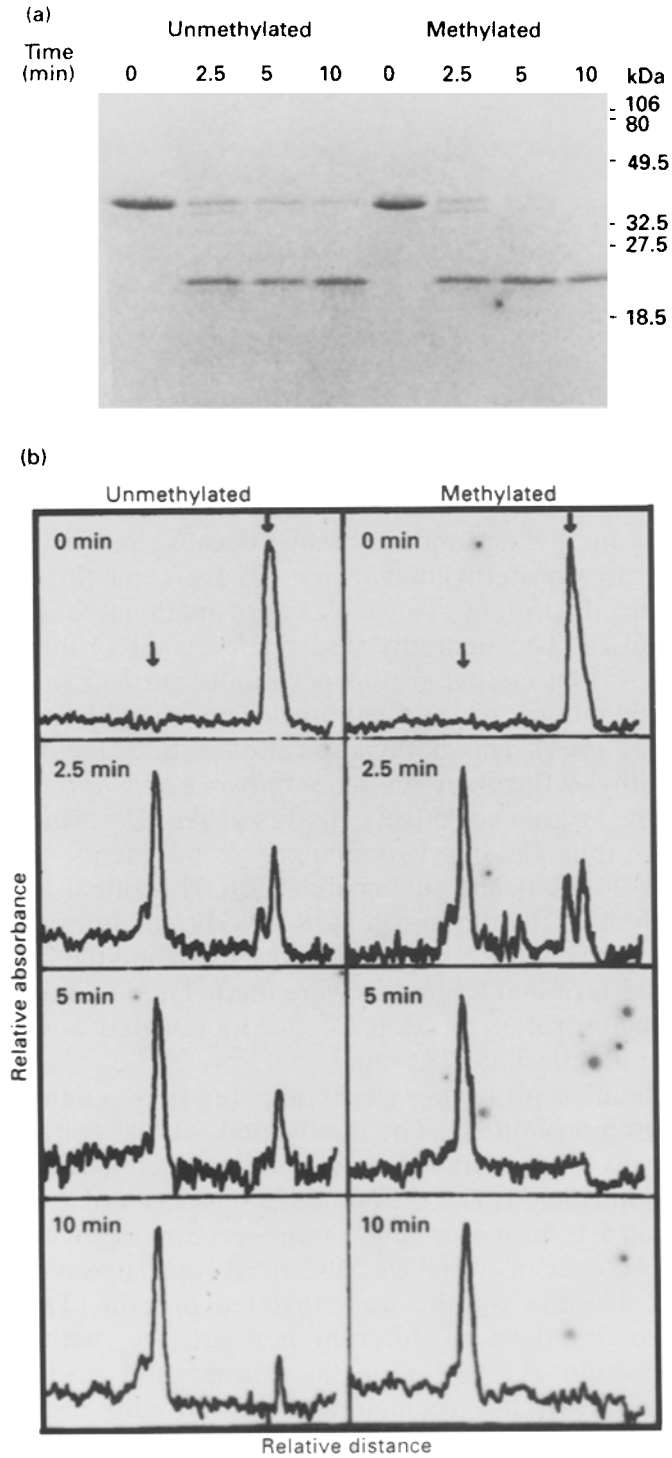

Fig. 5. Comparative trypsin sensitivity between the methylated and unmethylated protein A1 analyzed by SDS-PAGE. The 10-kDa COOH-terminal fragment which is extremely sensitive to tyrpsin and further degrades into smaller peptides, are not seen (Rajpurohit et al., 1994). **a** Coomassie Blue stained gel; **b** densiometric tracing

Table 3. Differences in physicochemical properties between recombinant unmethylated and arginine-methylate hnRNP Protein A1*

Property	Unmethylated	Methylated
pI	9.48	9.41
[NaCl] to release from ssDNA-cellulose	0.63 M	0.59 M
ssMS2-RNA binding:		
K_a	$5.75 \times 10^6 M^{-1}$	$4.63 \times 10^6 M^{-1}$
Binding size	54 nucleotides	58 nucleotides
[NaCl] to reverse 50% binding	235 mM	200 mM
Trypsin sensitivity [50% digestion (min)]		
No addition	1.63	1.31
+ssMS2-RNA	4.30	2.41
+ssDNA	2.74	2.00

* Data are from Rajpurohit et al. (1994).

presence of MS2-RNA, it was concluded that the methylated protein A1 had lower binding-capacity towards ss nucleic acid than the unmethylated. In other words, the enzymatic arginine-methylation rendered the protein A1 less basic and/or more hydrophobic than the unmethylated, thus making the N^G-methylarginine-containing protein A1 interact less tightly with nucleic acids than the unmethylated species. In this connection, it is noted that an introduction of a methyl-group to the guanidino nitrogen of arginine should interfer arginine-dependent hydrogen bonding between phosphate oxygen, as proposed with a model of "arginine fork" (Calnan et al., 1991): This notion is based on the finding that there is an essential arginine residue in the HIV Tat protein, a transactivator, which bind to a bulged region in TAR RNA.

IV. Enzymatic arginine-methylation of synthetic oligopeptides

Structural specificity in terms of amino acid sequence and minimum chain length of methylatable substrate for protein-arginine methyltransferase have been investigated using several chemically synthesized polypeptides whose sequences are identical to the region surrounding the methylatable arginine of the natural substrate proteins. A hexapeptide, Gly-Lys-Gly-**Arg**-Gly-Leu which corresponds to the residues 104–109 of bovine MBP was found to be the shortest methyl-accepting peptide, while a tetrapeptide Gly-**Arg**-Gly-Leu was inactive as a substrate (Ghosh et al., 1990). Having found the hexapeptide as the shortest methyl acceptor, several analogs of the hexapeptide with substitutions only on the n − 1 Gly were tested: When replaced with Asp and Phe, the methyl-accepting capacity was found to be much reduced, and the activity was completely nil with His and Leu substitution on the n − 1 (Rawal et al., 1995). On the other hand, none of the hexapeptide with substitution at n + 1 had any methyl-accepting activity, indicating that the n + 1 Gly is the essential structural feature to be recognized by the methyltransferase.

protein	residues	relative position							ref
		$n-3$	$n-2$	$n-1$	n	$n+1$	$n+2$	$n+3$	
human A1 hnRNP	190–196	Ser	Ser	Gln	Dma	Gly	Arg	Ser	(Kim et al., 1997)
	202–208	Gly	Gly	Gly	Dma	Gly	Gly	Gly	
	214–220	Asn	Phe	Gly	Dma	Gly	Gly	Asn	
	221–227	Phe	Ser	Gly	Dma	Gly	Gly	Phe	
CHO nucleolin	652–658	Phe	Gly	Gly	Dma	Gly	Gly	Gly	(Lapeyre et al.,
	656–662	Gly	Gly	Gly	Dma	Gly	Gly	Phe	1986)
	662–668	Phe	Gly	Gly	Dma	Gly	Gly	Gly	
	666–672	Gly	Gly	Gly	Dma	Gly	Gly	Gly	
	670–676	Gly	Gly	Gly	Dma	Gly	Gly	Phe	
	676–682	Phe	Gly	Gly	Dma	Gly	Dma	Gly	
	678–684	Gly	Dma	Gly	Dma	Gly	Gly	Phe	
	684–690	Phe	Gly	Gly	Dma	Gly	Gly	Phe	
	688–694	Gly	Gly	Phe	Dma	Gly	Gly	Dma	
	691–697	Dma	Gly	Gly	Dma	Gly	Gly	Gly	
rat nucleolin	656–662	Phe	Gly	Gly	Dma	Gly	Gly	Gly	(Lischwe et al.,
	660–666	Gly	Gly	Gly	Dma	Gly	Gly	Phe	1985)
	666–672	Phe	Gly	Gly	Dma	Gly	Gly	Gly	
	670–676	Gly	Gly	Gly	Dma	Gly	Gly	Dma	
	673–679	Dma	Gly	Gly	Dma	Gly	Gly	Phe	
	679–685	Phe	Gly	Gly	Dma	Gly	Dma	Gly	
	681–687	Gly	Dma	Gly	Dma	Gly	Gly	Phe	
	687–693	Phe	Gly	Gly	Dma	Gly	Gly	Phe	
	691–697	Gly	Gly	Phe	Dma	Gly	Gly	Dma	
	694–700	Dma	Gly	Gly	Dma	Gly	Gly	Gly	
rat fibrillarin	5–11	Phe	Ser	Pro	Dma	Gly	Gly	Gly	(Lischwe et al.,
	12–18	Phe	Gly	Gly	Dma	Gly	Gly	Phe	1985)
	18–24	Phe	Gly	Asp	Dma	Gly	Gly	Dma	
	21–27	Dma	Gly	Gly	Dma	Gly	Gly	Gly	
	25–31	Gly	Gly	Gly	Dma	Gly	Gly	Dma	
	28–34	Dam	Gly	Gly	Dma				
physar. fibrillarin	2–8	Phe	Glu	Gly	Dma	Gly	Gly	Phe	(Christensen &
	8–14	Phe	Gly	Gly	Dma	Gly	Gly	Gly	Fuxa, 1988)
	13–18	Gly	Gly	Asp	Dma	Gly	Gly	Dma	
	16–22	Dma	Gly	Gly	Dma	Gly	X	Gly	
Artemia P38	Frag 6	Pro	Pro	Met	Dma	Gly	Gly	Dma	(Pype et al., 1994)
	Frag 6	Dma	Gly	Gly	Dma	Gly	Gly	Leu	
	Frag 12	Gly	Pro	Ala	Dma	Gly	Gly	Lys	
	Frag 13	Gly	Pro	Thr	Dma	Gly	Gly	Lys	
frequency		G(14)	G(28)	G(29)	Dma(38)	G(38)	G(33)	G(14)	
		F(14)	S(3)	F(2)			Dma(2)	F(11)	
		Dma(7)	P(3)	D(2)			R(1)	Dma(7)	
		X(3)	Dma(2)	X(5)				K(2)	
			X(2)					X(3)	
preferred		G/F	G	G	Dma	G	G	G/F	
% preferred residues		74%	74%	76%	100%	100%	87%	74%	

Fig. 6. Surrounding amino acid sequences of N^G,N^G-dimethylarginine in RNA binding proteins (Kim et al., 1997)

A list of known N^G,N^G-dimethylarginine sites among the naturally occurring nucleic acid binding proteins have been compiled. Figure 6 displays a symmetrical Gly/Phe-Gly-Gly-**DMA**-Gly-Gly-Gly-Phe to be preferred sequence with an absolute requirement for a COOH-terminal flanking glycine. The latter fact may explain why arginine 193 in protein A1 is methylated while the nearby arginine 195 which is followed by serine is not. Although glycine is certainly preferred in the n − 1 position, there are instances where phenylalanine, aspartic acid, proline, and glutamine are found in this position.

Lack of methylation of 20 arginine residues out of a total 24 in protein A1 may be either due to the effect of the n − 1 amino acid or due to highly ordered structure of the RBDs in the NH_2-terminal domain, thus precluding access to methylase. The natural occurrence of N^G-dimethylarginines in nucleic acid binding proteins (Fig. 6), therefore, well agrees with the studies carried out with synthetic oligopeptides.

V. Concluding remarks

Enzymatic methylation on the guanidino nitrogen of arginine residues in a protein is catalyzed by protein methylase I (S-adenosylmethionine:protein-arginine N-methyltransferase; EC. 2.1.1.23) utilizing a high energy methyl donor compound, S-adenosyl-L-methionine, to yield N^G-methylated arginines. The enzyme recognizes not only specific arginine residue to be methylated, but also substrate protein on a whole. Thus, subclasses of protein methylase I are present in mammalian organs. The molecular and catalytic properties of MBP- and nuclear protein/histone-specific protein methylase I have been presented. To understand biological implication of the methylation, the unmethylated recombinant heterogeneous nuclear RNP protein A1 (one of the most highly *in vivo* methylated proteins) was stoichiometrically methylated *in vitro* by purified nuclear protein/histone-specific protein methylase I. The difference in their properties such as binding to ssDNA-cellulose, pI value and trypsin sensitivity in the presence and absence of MS2-RNA between the methylated and the unmethylated proteins indicates that the methylation of arginine residues decreased the binding capacity of protein to single stranded nucleic acid. Compilation of known nucleic acid binding proteins containing N^G-methylated arginine indicates that Gly/Phe-Gly-Gly-*__Arg__-Gly-Gly-Gly-/Phe is a preferred recognition motif. These results together with the synthetic hexapeptide sequence recognized by protein-arginine methyltransferase and altered physichochemical properties of the methylated hnRNP protein A1 all point out that the side chain N^G-guanidino arginine methylation is a potentially modulatory modification of the nucleic acid binding *in vivo*.

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Authors' address: Dr. Sangduk Kim, Department of Biochemistry, Korea University Medical School, 126, 5-Ga Anam-Dong, Sung Buk-Gu, Seoul, 136-701, Korea, Fax 82-2-928-4853

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