S-Adenosylmethionine and protein methylation

Review Article

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Summary. The enzymes responsible for protein methylation by S-adenosylmethionine, both at the carboxyl groups and at the nitrogen groups, are reviewed. The possibility that the reactions involved may be reversible is also considered.

Keywords: Protein methyltransferases – Isoaspartyl residues – Protein phosphatase 2A – Histones – Isoprenylcysteine

Introduction

S-adenosylmethionine (SAM) is one of the most commonly used and very versatile enzyme substrates. Its sulfonium group enables it to be employed as both a methyl donor (and it is recognized as the major methyl donor in all living organisms) and a monoalkyl donor in the synthesis of polyamines, and in the synthesis of ethylene in plants. It is also involved in the formation of 5'-deoxyadenosyl radicals, these being part of a family of iron-sulfur enzymes that utilize SAM to initiate the radical catalysis involved, for example, in the synthesis of lipoate and biotin.

As a methyl donor, SAM is used in many reactions to transfer the methyl group to the nitrogen or the oxygen of many different compounds. When proteins are methylated, too, methyltransferases transfer the methyl group to basic or acid amino acids, in this case forming an ester group. In either case, the function of the protein may be changed and the process is implicated, for instance, in signal transduction (Stock et al., 1992), regulation of transcription (Chen et al., 1999), heterogeneous nuclear ribonucleoprotein export (Shen et al., 1998) and possibly splicing (Friesen et al., 2001).

The protein methyltransferases currently known will now be described. Consideration will be mainly to those of animal origin.

Protein nitrogen groups methylation

Protein arginine methylation

Methylation of protein arginine has attracted particular attention because methylarginines are inhibitors of nitric oxide synthase (NOS), and therefore thought to participate in the regulation of the NO's many effects. Moreover, arginine methylation promotes specific protein–protein interactions (Friesen et al., 2001). In some cases, involvement in such interactions is due to inhibition of the binding of proline-rich ligands (Bedford et al., 2000). It has also been shown that DAL-1/4.1B, a tumor suppressor differentially expressed in adenocarcinoma of the lung, interacts with one of the arginine methyltransferases and inhibits its activity both in vitro and in vivo (Singh et al., 2004).

Distinct Protein Arginine (R) Methyl Transferases (PRMT) activities have been found in mammalian cells and divided into four types: type I activity gives rise to monomethylarginine (NMA) and asymmetric dimethylarginine (ADMA); type II to NMA and symmetric dimethylarginine; type III enzymes catalyze only the formation of NMA; type IV enzymes form δ-N8-monomethylarginine. PRMT 1, 3 and 4 are responsible for type I methylation, while PRMT5 catalyzes type II methylation.
A novel gene coding for a sixth human PRMT enzyme has been identified and called PRMT6. It resides in the nucleus, whereas PRMT3 and 5 are located in the cytoplasm. It has a type I activity, can methylate itself and has different substrate specificity (Frankel et al., 2002). A PRMT7 has also been described very recently. This, according to Miranda et al. (2004), is a type III enzyme, whereas according to Lee et al. (2005b) it synthesizes symmetric dimethylarginine and is thus of type II. These workers suggest that differences in the conditions of the assay are responsible for these results.

PRMT5 seems to have a role in cell cycle regulation.

Arginines which can be methylated have been shown in histones. Histone H3, for example, is methylated in vitro at Arg 2, 17 and 26 (Schurter et al., 2001) by PRMT4, now also called coactivator-associated arginine methyltransferase (CARM1), though it is not known which residues are methylated in vivo. PRMT4 is widely expressed in adult mouse tissues, whereas PRMT1 preferentially methylates histone H4 and thus facilitates its subsequent acetylation (Wang et al., 2001).

Methylarginines cannot be retransformed to arginine. However, protein-arginine-iminohydrolase (PAD), a Ca$^{++}$ dependent enzyme that catalyzes the conversion of protein arginine residues to citrulline residues in a post-translational modification (called citrullination), has been detected in several tissues. One form of this enzyme (PAD2) is widely distributed and particularly abundant in the muscles and brain (Moscarello et al., 2002). PAD4 is abundant in granulocytes (Arita et al., 2004; Hagiwara et al., 2002). It also converts histone methylarginine to histone citrulline and releases methylamine (Wang et al., 2004). It is thought that this reaction participates in gene expression.

When methylated proteins are degraded by proteases, methylarginines are formed. ADMA and NMA are potent endogenous inhibitors of NOS. Free methyl arginines are found in differing concentrations in the cell cytosol, plasma and tissues. Both ADMA and the symmetric isomer are eliminated by renal excretion, whereas ADMA alone is degraded to citrulline and methylamines by dimethylarginine dimethylaminohydrolase (DDAH), a zinc containing protein (Bogumil et al., 1998). Two DDAH isoforms with distinct tissue distribution and some relationship to NOS isoforms have been identified: DDAH I is found in tissues that express nNOS, whereas high levels of DDAH II are found in tissues with eNOS (Leiper et al., 1999). These results support the view that methyl arginine concentration is actively regulated. According to Tsikas et al. (2000) NOS inhibition by endogenous methylarginines, with ADMA as the most potent, is responsible for the so-called arginine paradox, i.e. the observation that excess arginine has no effect on NOS in vitro, but enhances its activity in vivo by competition with the inhibitor. Plasma ADMA levels are high in many disorders characterized by endothelial dysfunction (McCarty, 2004). According to Böger (2004), ADMA is a cardiovascular risk factor.

**Protein lysine methylation**

Lysine methylation mainly occurs on histone H3 and H4 and to a lesser extent on histone H1. Six lysines within histone H3 (4, 9, 14, 27, 36, 79) and two within histone H4 (20 and 59) are methylated by a specific histone methyltransferase (Lee et al., 2005a). Each residue can accept up to three methyl groups, resulting in mono-, di-, and trimethylated Lys. Moreover, Lys 9 in H3 can be both acetylated or methylated.

Initially only CARM1 appeared to display a stringent specificity for histone (Chen et al., 1999). Another enzyme has since been identified (Rea et al., 2000) as the mammalian homolog of a gene product encoded by the suppressor gene Su(var)3-9 of Drosophila and hence called SUV39H1. The SUV39HMTases are characterized by the presence of one invariant protein motif, namely the SET domain. The approximately 130-residue SET module is generally flanked by distinct pre-SET and post-SET domains at its N and C termini. The C-flanking domain is needed to generate the access channel that connects the cofactor-binding site on one surface of the domain with the substrate-binding site on the opposite surface. This access channel regulates whether the enzyme produces mono-, di- or tri-methylated lysine (Xiao et al., 2003). Dimethylated and trimethylated lysines on Lys9 or Lys4 of histone H3 have distinct roles (Tamaru et al., 2003; Santos-Rosa et al., 2002; Lee et al., 2005a).

Other enzymes, including G9a (Tachibana et al., 2002) and Set9 (Nishioka et al., 2002) have been shown later to methylate lysine in histones. Set9 contains a SET domain, but lacks the pre- and post-SET domains. Chuikov et al. (2004) have demonstrated that Set9 can methylate the tumor suppressor p53 in vivo, a process that seems necessary for its stabilization. The authors suggest that Set9 may also regulate the function of other factors. Another enzyme, SMYD3, has been postulated as a key factor in human carcinogenesis (Hamamoto et al., 2004).

We know now that lysine methylation regulates specific gene expression and organization of the chromosomal regions. However, the position of the methylated lysine within H3 and H4 marks a gene to be activated or repressed.