Homocysteine in Health and Disease

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S-Adenosylmethionine-dependent Methyltransferases

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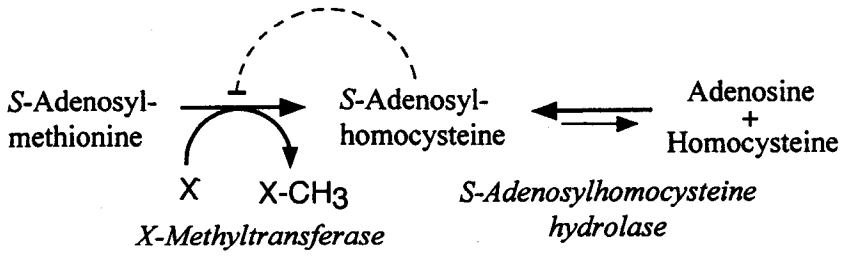
Mammalian *S*-adenosylmethionine-dependent methyltransferases each catalyze a reaction, giving rise to two products — *S*-adenosylhomocysteine and one of a variety of methylated biomolecules including nucleic acids, proteins, lipids, and small molecules. *S*-adenosylhomocysteine is subsequently broken down to adenosine and homocysteine by *S*-adenosylhomocysteine hydrolase (Figure 7.1). The homocysteine formed can be either remethylated to methionine or converted to cysteine via cystathionine (30, 72). As such, these methyltransferases are bifunctional; they make up an essential part of the conduit for the conversion of methionine to cysteine in addition to generating methylated products.

Recent attention has been drawn to methyltransferases to understand how elevated plasma total homocysteine levels are connected to disease. This is because higher intracellular homocysteine levels are expected to correlate with lower methyltransferase activities as described later. The inhibition of these enzymes may represent a specific biochemical mechanism that may explain at least some of the cellular toxicity associated with homocysteine accumulation. This chapter reviews the data on mammalian methyltransferases, allowing us to identify enzymes that might be particularly sensitive to inhibition and thus may be prime targets for homocysteine-associated effects.

Why would elevated intracellular free homocysteine levels necessarily result in the inhibition of cellular *S*-adenosylmethionine-dependent methyltransferases? This effect comes from two linked reactions. First, higher levels of homocysteine result in higher levels of *S*-adenosylhomocysteine by mass action effects on the *S*-adenosylhomocysteine hydrolase reaction, where the equilibrium favors the conversion of homocysteine and adenosine to *S*-adenosylhomocysteine (22). Second, *S*-adenosylhomocysteine is a potent product inhibitor of most *S*-adenosylmethionine-dependent methyltransferases, with K_i values in the submicromolar to low micromolar range (Figure 7.1). In fact, the K_i value for *S*-adenosylhomocysteine is often less than the K_m value for *S*-adenosylmethionine (10, 11, 46, 67, 97, 112). The potential loss of the body's ability to efficiently catalyze methyltransfer reactions thus may be linked to the higher risk of premature cardiovascular disease and the other clinical features, including neurological impairment, that results from total homocysteine accumulation in plasma and urine (52, 84).

The specific questions to be addressed are these: How many different types of *S*-adenosylmethionine-dependent methyltransferases occur in mammals? What reactions do they catalyze? How sensitive is each reaction to inhibition by *S*-adenosylhomocysteine? Are the known increases in plasma total homocysteine actually correlated with increases in intracellular *S*-adenosylhomocysteine? Can we begin to correlate the

Fig. 7.1. Metabolic relationships between *S*-adenosylmethionine, *S*-adenosylhomocysteine, and homocysteine in mammalian cells. *S*-adenosylmethionine-dependent methyltransferases using various methyl-accepting substrates (designated X) are inhibited by the product, *S*-adenosylhomocysteine, which can accumulate in the presence of homocysteine because the equilibrium of the *S*-adenosylhomocysteine hydrolase reaction favors *S*-adenosylhomocysteine formation.



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effects of their inhibition with the specific pathologies seen with elevated homocysteine levels?

Mechanistic Links Between Homocysteine Accumulation, Methyltransferase Inhibition, and Disease

The possible connection between elevated plasma total homocysteine and methyltransferase inhibition was apparent to the biochemists who first characterized homocystinuria (28, 29). Evidence was presented early on that elevated plasma total homocysteine can result in the appearance of *S*-adenosylhomocysteine in the urine, where it is otherwise not found (83). This hypothesis has only recently begun to be experimentally tested.

The clearest demonstration of a mechanistic connection between homocysteine accumulation, *S*-adenosylhomocysteine accumulation, and the inhibition of methyltransferases has been made by Perna and co-workers (77–82). Their interest has been in the mechanism of cellular injury in patients with chronic renal failure. Previously, investigators demonstrated that plasma total homocysteine is mildly elevated and may be associated with the premature occlusive arterial disease seen in these patients (14, 15). Perna and co-workers showed that erythrocyte *S*-adenosylhomocysteine levels are fourfold to eightfold higher in patients with renal failure, whereas little change in *S*-adenosylmethionine levels was observed (77). This change is accompanied by an up to 50% inhibition in the activity of the erythrocyte repair protein L-isoaspartate (D-aspartate) O-methyltransferase (77, 79, 80). Recently, it has been found that the plasma ratio of *S*-adenosylmethionine/*S*-adenosylhomocysteine (presumably reflecting the intracellular situation) is lowered in patients with end-stage renal failure (66). Additionally, a stable isotope study has shown that whole body transmethylation rates are decreased about 24% in these individuals (99).

In a second type of approach, Wang and co-workers used a cell culture system to provide evidence that increased homocysteine concentrations in the medium in the physiological range may specifically inhibit the methyltransferase that catalyzes the "capping" of protein C-terminal isoprenylcysteine residues (62, 107). Wang et al. (107) reported that the incubation of human aortic endothelial cells with 50 μmol/L homocysteine resulted in an eightfold decrease in the ratio of *S*-adenosylmethionine to *S*-adenosylhomocysteine. This decrease was accompanied by the inhibition of DNA synthesis and a 50% inhibition in the C-terminal methylation of one or more members of the small G-protein ras family, presumably as a result of the inhibition of isoprenylcysteine methyltransferase.

It is clear that a similar mechanism of inhibition can generally take place with other types of methyltransferases, and it would be expected that increased *S*-adenosylhomocysteine would affect many of these enzymes in the cell (8). It is therefore useful to briefly examine some of the relevant features of these enzymes. However, much more must be learned about the correlation of levels of extracellular total homocysteine and intracellular *S*-adenosylhomocysteine in different tissues. Indeed, it has been pointed out that elevated plasma total homocysteine may be a result of the *increased* activity of intracellular methyltransferases (D. E. Vance, personal communication). Additionally, the intracellular concentration of adenosine will also affect the position of equilibrium between homocysteine and *S*-adenosylhomocysteine, and it is important to know how these adenosine levels change under various physiological conditions.

Mammalian Methyltransferases Catalyze a Variety of Reactions

Table 7.1 shows all of the known types of mammalian *S*-adenosylmethionine-dependent methyltransferases. These 39 species were identified by selecting AdoMet-dependent mammalian species from the Enzyme Commission list (E.C. 2.1.1.XX) using the "Enzyme" database of the Swiss Institute for Bioinformatics (http://www.expasy.ch/enzyme/) as well as literature searches using the Medline data base from 1966 to the present. We have also been aided by the recent compilation of methyltransferases prepared by Blumenthal (5).

Table 7.1 lists a total of two DNA methyltransferases, 10 RNA methyltransferases, 3 lipid methyltransferases, 11 protein methyltransferases, and 13 small molecule methyltransferases. In many cases, the gene and enzyme have been well described. In other cases, the presence of a methyltransferase is only inferred from the presence of a well-characterized methylated product. Variants created by RNA splicing reactions and other processes further enlarge the diversity of these methyltransferases. Although Table 7.1 aims to include all well-characterized enzymes, new methyltransferases will certainly be added as more is learned about methylation pathways. Efforts have been made previously to use conserved sequence motifs in a large family of these methyltransferases to identify new methyltransferases in the output of genomic sequencing projects (54, 73). In the yeast *Saccharomyces cerevisiae,* where the complete genome sequence is known, it has been possible to identify 26 potentially novel methyltransferases (73). With a nearly complete sequence of the human genome released in the year 2000, it will be possible to extend this approach to identify new human methyltrans-

Table 7.1 Mammalian *S*-Adenosylmethionine-dependent Methyltransferases

E. C. #	Enzyme Name	Gene Designation	Methyl-accepting Substrate	Function	Notes	Reference
DNA						
2.1.1.37	DNA (cytosine-5-)-methyltransferase 1	*DNMT1*	DNA	Regulation of gene expression, chromosome inactivation, genomic imprinting	Major activity	50, 101
2.1.1.37	DNA (cytosine-5-)-methyltransferase 3	*DNMT3*	DNA		Minor activity	50, 101
RNA						
2.1.1.56	mRNA (guanine-*N* 7-)-methyltransferase	*RNMT*	$GpppN_1N_2$	mRNA capping	Three alternatively spliced forms	6
	Cap I-mRNA 2′-*O*-methyltransferase		$N7mGpppN_1N_2$	mRNA capping	Nuclear enzyme	6
	Cap II-mRNA 2′-*O*-methyltransferase		$N7mGppp^mN_1N_2$	mRNA capping	Cytoplasmic enzyme	6
	(2′-*O*-methyladenosine-*N* 6-)-methyltransferase		$N7mGppp^mA_1{}^mN_2$	mRNA capping	Cytoplasmic enzyme	6
	mRNA *N* 6-adenosine methyltransferase		Internal adenosine residues	Affects efficiency of pre-mRNA splicing or transport to cytoplasm		6
2.1.1.29	tRNA (cytosine-5-)-methyltransferase		tRNA	Alters flexibility of tRNA for codon recognition		47
2.1.1.31	tRNA (guanine-*N* 1-)-methyltransferase		tRNA	Alters flexibility of tRNA for codon recognition		47
2.1.1.32	tRNA (guanine-*N* 2-)-methyltransferase		tRNA	Alters flexibility of tRNA for codon recognition		47
2.1.1.36	tRNA (adenine-*N* 1-)-methyltransferase		tRNA	Alters flexibility of tRNA for codon recognition		47
	rRNA (2′-ribose-O)-methyltransferase		Internal adenosine residues in rRNA	rRNA maturation and ribosome assembly?		26
LIPIDS						
2.1.1.17	Phosphatidylethanolamine *N*-methyltransferase	*PEMPT*	Phosphatidyl-ethanolamine	Phosphatidylcholine synthesis	PEMT1, PEMT2 splice variants	98, 105
	Dihydroxynonaprenylbenzoate *O*-methyltransferase	*COQ3*	Pathway precursors	Ubiquinone synthesis	Rat gene cloned, human ESTs* present	69, 53a
	COQ5 *C*-methyltransferase	*COQ5*	Pathway precursors	Ubiquinone synthesis	Yeast gene cloned, human ESTs* present	3
PROTEIN Carboxyl						
2.1.1.77	Protein L-isoaspartate (D-aspartate) *O*-methyltransferase	*PCMT1*	L-isoaspartate, D-aspartate residues	Protein repair		20

(continues)

Table 7.1 *Continued*

E. C. #	Enzyme Name	Gene Designation	Methyl-accepting Substrate	Function	Notes	Reference
2.1.1.100	Protein *S*-isoprenylcysteine *O*-methyltransferase	*ICMT*	C-terminal isoprenyl cysteine residues	Protein stability? G-proteins and Ras processing and localization		115, 3a
	Protein phosphatase 2a *O*-methyltransferase	*LCMT*	C-terminal leucine residues on PP2A	Regulation of metabolism?		114, 21a
Lysine						
2.1.1.43	Histone-lysine *N*-methyltransferase		Histones	Unknown		65
2.1.1.60	Calmodulin-lysine *N*-methyltransferase	*CLNMT*	Calmodulin	Unknown		113
Arginine						
2.1.1.126	[Myelin basic protein]-arginine *N*-methyltransferase		Arginine residues on myelin basic protein	Unknown		59
	Protein arginine *N*-methyltransferase 1	*PRMT1*	Arginine residues	Signal transduction, nuclear transport?		64
	Protein arginine *N*-methyltransferase 3	*PRMT3*	Arginine residues	Signal transduction, nuclear transport?		92
	Coactivator-associated arginine methyltransferase-1	*CARM1*	Arginine residues	Transcriptional control?		16
Histidine						
	Protein histidine *N*-methyltransferase		Histidine residues on actin and myosin			76
Other						
	Diphthine synthase	*DPR5(Y)*	α-Amino group of a modified histidine residue	Diphthamide synthesis in EF-2	Homologous human ESTs* of yeast DPH5 enzyme	70

SMALL MOLECULES						
***N*-Methyltransferases**						
2.1.1.1	Nicotinamide *N*-methyltransferase	*NNMT*	Nicotinamide and other pyridines	Metabolism of nicotinamide and other pyridines		91
2.1.1.2	Guanidinoacetate *N*-methyltransferase	*GAMT*	Guanidoacetate	Creatine synthesis		51, 74
2.1.1.8	Histamine *N*-methyltransferase	*HNMT*	Histamine	Histamine inactivation		100
2.1.1.20	Glycine *N*-methyltransferase	*GNMT*	Glycine	Sarcosine synthesis, regulation of homocysteine pathway		41, 17a
2.1.1.28	Phenylethanolamine *N*-methyltransferase	*PNMT*	Phenylethanol-amine	Epinephrine synthesis		63
	β-Carboline-2-*N*-methyltransferase	*β C-2-NMT*	β-carbolines (norharman and harman)	Unknown	Similar to INMT but highly expressed in brain	35
	Indolethylamine *N*-methyltransferase	*INMT*	Tryptamine, others	Unknown	Similar to βC-2-NMT but expressed in non-neural tissue	94
***O*-Methyltransferases**						
2.1.1.4	Hydroxyindole *O*-methyltransferase	*HIOMT*	Serotonin	Melatonin synthesis		23
2.1.1.6	Catechol *O*-methyltransferase	*COMT*	Catechols (epinephrine, norepinephrine, dopamine and others)	Epinephrine, norepinephrine and dopamine inactivation		102
***S*- and *As*-Methyltransferases**						
2.1.1.9	Thiol *S*-methyltransferase	*TMT*	Thiols	Metabolism of endogenous and xenobiotic compounds		9
2.1.1.67	Thiopurine *S*-methyltransferase	*TPMT*	Thiopurines	Metabolism of endogenous and xenobiotic compounds		27
	Thioether *S*-methyltransferase		Thioethers (sulfur, selenium, tellurium)	Metabolism of endogenous and xenobiotic compounds		108
	Arsenite methyltransferase		Arsenite	Metabolism of endogenous and xenobiotic compounds		93

*EST, expressed sequence tag.

Table 7.2 Possible Mammalian Methyltransferases

	Enzyme Name	Gene	Substrate	Source	Notes	Ref.
DNA						
	DNA (cytosine-5-)-methyltransferase 2	*DNMT2*	DNA	Human	No enzymatic activity demonstrated	50
RNA						
	tRNA 2′-*O*-ribose methyltransferase		Ribose of tRNA	Yeast	Human homolog identified	12
PROTEIN						
Arginine						
	Protein arginine *N*-methyltransferase 2	*PRMT2*	Arginine residues	Human	No enzymatic activity demonstrated	34
Lysine						
	Hsp68, Hsc70 and BiP protein lysine *N*-methyltransferase		Hsp68, Hsc70, BiP	Mouse	Distinct enzyme not identified	106
	Myosin lysine *N*-methyltransferase		Myosin lysine residues	Rabbit	Distinct enzyme not identified	96
	EF-1α lysine *N*-methyltransferase		EF-1α	Rabbit	Distinct enzyme not identified	24
	Citrate synthase lysine *N*-methyltransferase		Lysine residues	Pig	Distinct enzyme not identified	4
	Myosin N-terminal alanine *N*-methyltransferase		N-terminal alanine residue of myosin	Human	Distinct enzyme not identified	48

ferases as well as to link newly described genes to known methyltransferases.

There are cases where the presence of a mammalian methyltransferase is either less certain or where it is unclear which enzyme catalyzes the reaction. Table 7.2 includes potential methyltransferases encoded by genes with amino acid sequence homology to other methyltransferases but where no enzyme activity has yet been demonstrated. Also included are species where the methylation chemistry is well established, but it is unknown whether a distinct enzyme is involved or the reaction can be catalyzed by a previously known methyltransferase. With further research, some of these species can be added to the group in Table 7.1, whereas others may turn out not to be *S*-adenosylmethionine-dependent methyltransferases at all.

Functions of *S*-Adenosylmethionine-dependent Methyltransferases

From the data in Table 7.1, we can divide the known or postulated functions of most of these enzymes into five broad and sometimes overlapping categories: small molecule biosynthesis; inactivation and elimination of small molecules and xenobiotics; stabilization of DNA, RNA, and proteins; cellular signaling pathways; and protein synthesis. It is notable that several of these enzymes have been shown to be involved with neurochemical processes.

Small Molecule Biosynthesis

Six methyltransferases from Table 7.1 catalyze biosynthetic steps. The phosphatidylethanolamine *N*-methyltransferase catalyzes the conversion of phosphatidylethanolamine to phosphatidylcholine in the liver in an alternate pathway of phosphatidylcholine synthesis and is responsible for about 15% of the total biosynthesis of this major membrane structural building block (104). In mice whose gene encoding this activity is disrupted, their overall physiology is not apparently affected (103); however, when the animals were stressed by a choline-deficient diet, their liver function was severely compromised (104). This pathway may thus be important when organisms are under nutritional deprivation or are pregnant (104).

Two methyltransferases are involved in the synthesis of coenzyme Q or ubiquinone, the COQ3 O-methyltransferase and COQ5 C-methyltransferase (3, 18, 53a, 69). Ubiquinone is an essential electron carrier in mitochondrial oxidative phosphorylation and has antioxidant roles as well (18). The loss of ubiquinone would be expected to severely impact the ability of cells to produce adenosine triphosphate (ATP) for their

energy needs. Another enzyme, the guanidinoacetate methyltransferase, catalyzes a crucial step in the biosynthesis of creatine phosphate, which serves as a buffer for ATP and an additional source of cellular energy. Loss of this activity might also be reflected in diminished cellular energy metabolism. The reaction may account for the bulk of hepatic *S*-adenosylmethionine use and thus represents a major potential source of homocysteine for cysteine biosynthesis (72).

Two distinct methyltransferases, the hydroxyindole and the phenylethanolamine methyltransferases, catalyze steps in the biosynthesis of the neurotransmitter/hormones melatonin and epinephrine. The loss of activity of these methyltransferases might be expected to result in abnormal neurotransmitter levels and aberrant neurological function. As discussed later, at least two methyltransferases are also involved in inactivation pathways of neurotransmitters, and the loss of these activities would also be expected to affect the function of the brain.

Inactivation and Elimination of Small Molecules and Xenobiotics

Seven methyltransferases listed in Table 7.1 have crucial roles in inactivating hormones and neurotransmitters and in preparing other types of endogenous and xenobiotic small molecules for elimination from the body. These enzymes can be characterized by their ability to remove molecules that have served their function or may be intrinsically toxic to the body.

Two methyltransferases catalyze the inactivation of neurotransmitters/hormones. The catechol O-methyltransferase acts on dopamine, norepinephrine, and epinephrine; the histamine *N*-methyltransferase acts on histamine (111). Knockout mice with a homozygous disruption in the gene for catechol O-methyltransferase display some behavioral changes but otherwise appear to be fairly normal (40).

The nicotinamide methyltransferase appears to be involved in the elimination of excess pyridine compounds from cells by fixing a positive charge on its aromatic ring (1, 88). Four other methyltransferases in Table 7.1 appear to be involved in the metabolism of sulfur, selenium, and arsenic compounds in the cell. The methyl acceptors for these enzymes can either represent normal products of metabolism (such as hydrogen sulfide) or xenobiotic compounds. The membrane-bound thiol methyltransferase and the soluble thiopurine methyltransferase recognize a variety of sulfhydryl-containing species (37, 111). The thioether products of these enzymes can then be recognized by the thioether methyltransferase that methylates these products as well as selenoethers to generate positively charged molecules that can be more readily excreted in the urine (108). A related species can also methylate arsenite and represents a detoxification pathway for this toxin (116).

Finally, two similar methyltransferases are present that modify tryptamine and related compounds and may serve similar functions. One of these enzymes is most active in non-neuronal tissues (94, 95), and a second activity from brain appears to activate the precursor form of neurotoxins such as the β-carbolines (35). It will be of interest to see if endogenous substrates for these enzymes exist.

Stabilization of DNA, RNA, and Proteins

At least seven types of methyltransferases appear to function in ensuring nucleic acid and protein stability. In humans, two genes have been described that catalyze the methylation of cytidine residues in CpG contexts in reactions that appear to both activate and inactivate transcription (Table 7.1). Null mutants have been prepared of the *DNMT1* gene encoding the major mammalian activity in mouse embryonic stem cells (17). These cells have elevated mutation rates, suggesting the importance of the methylation reaction in maintaining the genome. RNA molecules are also modified by a variety of methyltransferases, as described in the section on protein synthesis. One of these enzymes, the mRNA cap methyltransferase that forms 7-methylguanine, is essential to yeast cells and may stabilize mRNAs against exonucleolytic degradation in addition to playing roles in translation initiation and efficiency (6, 68).

Protein stability also appears to be dependent on methyltransferase action. The widely distributed protein L-isoaspartyl (D-aspartyl) methyltransferase specifically recognizes polypeptides that have been spontaneously damaged in the aging process by the conversion of normal L-aspartyl and L-asparaginyl residues to L-isoaspartyl and D-aspartyl residues (20). The methylation of theses species can initiate a pathway that leads to their reconversion to L-aspartate residues (20). This pathway has been specifically studied in relation to the elevated homocysteine levels in patients with renal disease, as discussed earlier (77–82). The elevated homocysteine levels are directly correlated with elevated erythrocyte *S*-adenosylhomocysteine levels and the inhibition of the protein L-isoaspartate (D-aspartate) methyltransferase. Notably, it has been demonstrated that increased plasma total homocysteine is a general feature of aging (42).

Knockout mice that totally lack the protein L-isoaspartate (D-aspartate) methyltransferase demonstrated both a slow growth phenotype and a fatal seizure phenotype that resulted in death at 20 to 60 days after birth (58). The seizure phenotype was characterized as a persistent defect in the electrical activity of the brain and led directly to the slow growth phenotype (57). Interestingly, seizures are found in some patients with

homocystinuria, although it is possible that these can result from homocysteine or one of its metabolites acting directly as an excitatory amino acid (2).

Proteins can also be stabilized by methylation reactions against untimely proteolytic degradation. The best example of this is the action of the protein isoprenylcysteine methyltransferase that "caps" the C-terminal isoprenylcysteine residue of a class of proteins including the small and large G proteins involved in signal transduction, the cGMP phosphodiesterase involved in visual information processing in the retina, and the nuclear lamins (19, 49, 115). Another possible set of examples are the two known methyltransferases that catalyze the sequential trimethylation of lysine residues to give species more resistance to proteolysis (113), but may affect the functions of the proteins as well (21). Other proteins contain trimethyllysine residues at specific sites and at least one protein contains an N-terminal trimethylalanine residue, but the methyltransferase or methyltransferases responsible for these modifications have not been identified (Table 7.2) (4, 24, 48, 96, 106).

Cellular Signaling

Four of the methyltransferases in Table 7.1 appear to be involved in signal transduction pathways. The catalytic subunit of protein phosphatase 2A is carboxyl methylated at its C-terminal leucine residue in a reversible reaction that may control the activity of this enzyme (21a, 60). This phosphatase is a major player in the regulation of amino acid and lipid metabolism by reversible protein phosphorylation/dephosphorylation reactions (21a, 60).

Recently, mammalian genes encoding the three type I protein arginine *N*-methyltransferases have been described that appear to be involved in the modulation of cellular metabolism (16, 34, 92). These enzymes all catalyze the asymmetrical dimethylation of the side chain guanidino group nitrogen atoms of a variety of eukaryotic proteins. Several roles have been described for them including modulating the interaction of proteins with RNA, regulation of signaling pathways, and the regulation of nuclear protein transport.

Protein Synthesis

The modification of a histidine residue in elongation factor 2 to diphthamide includes the trimethylation of an α-amino group (70). This modification is apparently crucial for the function of this factor because the ADP-ribosylation of this residue by diphtheria toxin inhibits protein synthesis. There are also a variety of methylation reactions to the sugars and bases of ribosomal, messenger, and transfer RNA. The function of these modifications is poorly understood, but it has been hypothesized that they are important in the processing and transport of these species to the cytosol (6,47).

Methyltransferases with Other Types of Functions

Methyltransfer reactions offer an opportunity to expand the pool of amino acids that can be incorporated into proteins. Here, post-translational methylation can create new amino acid residues that can endow proteins with new functions. The role of methyltransferases in diphthamide synthesis was discussed previously. In many cases, however, the role of the modification is not so clear.

The type II protein, arginine methyltransferase, specifically catalyzes the symmetrical dimethylation of arginine-107 of myelin basic protein (59). Although the precise role for the latter enzyme is unknown, it is interesting to note that demyelination in brain is associated with depletion of *S*-adenosylmethionine (13). Another highly specific protein methyltransferase modifies the histidine-73 residue on actin and possibly myosin. Interestingly, this modification is present in actins from lower eukaryotes to vertebrates with the notable exception of the yeasts (55).

Finally, the glycine methyltransferase appears to have multiple roles in the cell. It appears to be the species responsible for allowing the pathway from methionine to cysteine to occur even when the rate of the other methyltransferases is insufficient to provide adequate levels of *S*-adenosylhomocysteine. The conversion of glycine to sarcosine and the subsequent oxidative metabolism of sarcosine in the mitochondrial electron transport pathway can effectively remove excess methyl groups from the cell and in the process control the ratio of *S*-adenosylmethionine to *S*-adenosylhomocysteine (72). This function requires that it be relatively resistant to product inhibition by *S*-adenosylhomocysteine and this is reflected in the relatively high K_i value of 35 μmol/L in Table 7.3.

Assessment of the Inhibition of Specific Methyltransferases by Elevated S-Adenosylhomocysteine Levels

Table 7.3 shows the values for the kinetic constants (K_m value for *S*-adenosylmethionine and K_i value for *S*-adenosylhomocysteine) that are known for the mammalian methyltransferases described in Table 7.1. It is immediately clear that there is a range of these values such that some methyltransferases will be much more susceptible to the inhibitory effect of *S*-adenosylhomocysteine than others. Because *S*-adenosylhomocysteine is generally a competitive inhibitor, the catalytic activity of these methyltransferases in vivo is also a function of the concentration of *S*-adenosylme-

Table 7.3 Sensitivity of Methyltransferases to Inhibition by *S*-Adenosylhomocysteine

Methyltransferase	K_m for AdoMet (μmol/L)	K_i for AdoHcy (μmol/L)	Source	Fraction of Maximal Methyltransferase Activity			Percentage of activity at 100 μmol/L relative to 1 μmol/L AdoHcy	Fraction of Maximal Methyltransferase Activity		Percentage activity at 6.7 μmol/L relative to 0.8 μmol/L AdoHcy	Reference
				AdoMet 30 μmol/L AdoHcy 1 μmol/L	AdoMet 30 μmol/L AdoHcy 10 μmol/L	AdoMet 30 μmol/L AdoHcy 100 μmol/L		Control	Uremic		
								AdoMet 2.7 μmol/L AdoHcy 0.8 μmol/L	AdoMet 2.7 μmol/L AdoHcy 6.7 μmol/L		
DNA											
DNA (cytosine-5-) 1	1.4	1.4	Mouse erythroleukemia cells	0.93	0.72	0.23	25%	0.55	0.25	45%	32
RNA											
tRNA (cytosine-5-)	0.5	0.9	Human HeLa cell	0.97	0.83	0.35	36%	0.74	0.39	53%	56
tRNA (guanine-*N* 1-)	3	0.11	Rat liver	0.50	0.10	0.01	2%	0.10	0.01	15%	38
tRNA (guanine-*N* 2-)	2	23	Rat liver	0.93	0.91	0.74	79%	0.57	0.51	90%	38
tRNA (adenine-*N* 1-)	0.3	0.85	Rat liver	0.98	0.89	0.46	47%	0.82	0.50	61%	39
rRNA (2′-ribose-*O*)	0.24 (K_d AdoMet)	0.17 (K_d AdoHcy)	Mouse nucleoli	0.95	0.68	0.18	18%	0.66	0.22	33%	26
LIPIDS											
Phosphatidylethanolamine	18.2	3.8	Rat liver	0.57	0.31	0.06	10%	0.11	0.05	47%	43, 44, 45
PROTEIN											
Carboxyl											
Protein-L-isoaspartate (D-aspartate)	2	0.08	Bovine brain	0.53	0.11	0.01	2%	0.11	0.02	14%	53
Protein *S*-isoprenylcysteine	2.1	9.2	Rat liver	0.93	0.87	0.55	59%	0.54	0.43	79%	90
Lysine											
Calmodulin-lysine *N*-	2	15.2 (IC_{50} value)	Rat testes	0.93	0.90	0.66	71%	0.56	0.48	86%	113

(continues)

Table 7.3 *Continued*

Methyltransferase	K_m for AdoMet (μmol/L)	K_i for AdoHcy (μmol/L)	Source	Fraction of Maximal Methyltransferase Activity: AdoMet 30 μmol/L AdoHcy 1 μmol/L	AdoMet 30 μmol/L AdoHcy 10 μmol/L	AdoMet 30 μmol/L AdoHcy 100 μmol/L	Percentage of activity at 100 μmol/L relative to 1 μmol/L AdoHcy	Fraction of Maximal Methyltransferase Activity — Control: AdoMet 2.7 μmol/L AdoHcy 0.8 μmol/L	Uremic: AdoMet 2.7 μmol/L AdoHcy 6.7 μmol/L	Percentage activity at 6.7 μmol/L relative to 0.8 μmol/L AdoHcy	Reference
Histone-lysine *N*-Arginine	12.5	5.9	Rat brain nuclei	0.67	0.47	0.12	18%	0.16	0.09	57%	25
[Myelin basic protein]-arginine	4.4	1.8	Calf brain	0.81	0.51	0.11	13%	0.30	0.12	39%	36
Protein arginine I	8	2.3	Calf brain	0.72	0.41	0.08	11%	0.20	0.08	40%	36
SMALL MOLECULES											
***N*-Methyltransferases**											
Guanidinoacetate	49	16	Pig liver	0.37	0.27	0.08	21%	0.05	0.04	75%	51
Histamine	1.7	11.8 (IC_{50} value)	Mouse kidney	0.94	0.91	0.65	69%	0.60	0.50	84%	89
Glycine	100	35	Rabbit liver	0.23	0.19	0.07	32%	0.03	0.02	86%	41
Phenylethanolamine	10	1.4	Rabbit liver	0.64	0.27	0.04	6%	0.15	0.04	30%	23
β-Carboline-2	81	14.8 (IC_{50} value)	Bovine brain	0.26	0.18	0.05	18%	0.03	0.02	73%	35
Indolethylamine	54.3	8.65	Rabbit lung	0.33	0.20	0.04	13%	0.04	0.03	63%	7
***O*-Methyltransferases**											
Hydroxyindole	14	2.1	Rabbit liver	0.59	0.27	0.04	7%	0.12	0.04	36%	23
Catechol	3.1	1	Human brain	0.83	0.47	0.09	11%	0.33	0.10	31%	87
***S*-Methyltransferases**											
Thiopurine	3	5.8 (IC_{50} value)	Mouse Liver	0.90	0.79	0.35	40%	0.44	0.29	67%	75
Thioether	1	40 (IC_{50} value)	Mouse lung	0.97	0.96	0.90	93%	0.73	0.70	96%	71

thionine (11). Assuming competitive inhibition, the Michaelis-Menton expression for the rate of the transmethylation reaction is:

$$\text{Fraction of maximal velocity} = [\text{AdoMet}]/(K_m + K_m\,[\text{AdoHcy}]/K_i + [\text{AdoMet}])$$

Here, [AdoMet] and [AdoHcy] are the concentrations of *S*-adenosylmethionine and *S*-adenosylhomocysteine, respectively, K_i is the inhibition constant for *S*-adenosylhomocysteine, and K_m is the Michaelis constant for *S*-adenosylmethionine. Cantoni et al. (11) initially made calculations for a group of 10 methyltransferases and calculated the percentage of maximal activity at ratios of AdoMet/AdoHcy of 4 and 1.6 and assumed concentrations of AdoMet and AdoHcy. Those enzymes with low K_i values relative to K_m values were more markedly inhibited at the lower AdoMet/AdoHcy ratio. In Table 7.3, we calculated relative enzyme activities using the preceding equation, assuming an approximate physiological level of 30 μmol/L *S*-adenosylmethionine and 1 μmol/L *S*-adenosylhomocysteine in nonliver cells (46). These values are also calculated for 10-fold and 100-fold increased concentrations of *S*-adenosylhomocysteine, which represent approximate concentrations that may occur in mild and severe homocystinuria, respectively. In the calculations for nonliver cells, the level of *S*-adenosylmethionine is assumed to stay constant at about 30 μmol/L (46).

In nucleated mammalian cells, there is little information on the actual levels of intracellular *S*-adenosylmethionine and *S*-adenosylhomocysteine as a function of plasma total homocysteine. However, Perna and co-workers have shed light on the situation in human red blood cells (78). They correlated changes in control patients and patients with chronic renal disease of plasma total homocysteine concentrations with changes in red cell adenosine, *S*-adenosylmethionine, and *S*-adenosylhomocysteine levels (78). Plasma total homocysteine was 12 μmol/L in the control group and 41 μmol/L in the patient group. This difference was also reflected in the erythrocyte levels of 8 and 32 μmol/L, respectively (78). Adenosine levels were about 1 μmol/L in each group. If the portion of the total homocysteine in the free state was known, the erythrocyte concentration of *S*-adenosylhomocysteine could be calculated from the equilibrium constant of the *S*-adenosylhomocysteine hydrolase reaction of 1.4×10^{-6} (22). However, the direct measurement of the *S*-adenosylhomocysteine concentration revealed values of 0.8 μmol/L in the control group and 6.7 μmol/L in the uremic patient group, whereas the concentration of *S*-adenosylmethionine was about 2.7 μmol/L in both groups (78). These results suggest that the concentration of *S*-adenosylhomocysteine, at least in erythrocytes, may be a linear function of plasma total homocysteine, a result consistent with the equilibrium relationship given the unchanging adenosine concentration. Table 7.3 shows data for the level of inhibition expected for each of the methyltransferases under the conditions of mildly elevated plasma total homocysteine found in patients with chronic renal disease.

There may be significant tissue differences in the way in which *S*-adenosylhomocysteine levels respond to increases in plasma total homocysteine. Cell culture experiments have shown that 50 μmol/L homocysteine has little or no effect in vascular smooth muscle cells or fibroblasts while dramatically increasing the concentration of *S*-adenosylhomocysteine in vascular endothelial cells (107). Additionally, increased extracellular homocysteine resulted in a parallel increase in *S*-adenosylmethionine and *S*-adenosylhomocysteine concentrations in liver (31, 44), lessening the potential degree of inhibition of methyltransferases.

More Likely and Less Likely Targets for Homocysteine-linked Methyltransferase Inhibition and Pathogenesis: Comparing the Clinical Features of Homocysteine Accumulation with Effects of Methyltransferase Loss

The results of the calculations in Table 7.3 make clear that even at relatively high levels of intracellular *S*-adenosylhomocysteine, some methyltransferases can retain most of their activity. The function of these species, therefore, would not be expected to be markedly changed by elevated plasma total homocysteine. By contrast, several other methyltransferases appear to be particularly sensitive to *S*-adenosylhomocysteine inhibition. At the mildly elevated homocysteine levels seen in uremic patients, the guanine-*N*-1 tRNA methyltransferase and the protein repair L-isoaspartate (D-aspartate) methyltransferase are inhibited more than 70% (15% and 14% residual activity, respectively) (Table 7.3). At higher levels, significant inhibition is also seen with two protein arginine methyltransferases and three enzymes involved in the metabolism of neurotransmitters and related compounds — phenylethanolamine *N*-methyltransferase involved in the synthesis of epinephrine, catechol *O*-methyltransferase involved in its degradation (as well as that of dopamine and norepinephrine), and the β-carboline-2-*N*-methyltransferase with an unknown physiological function (Table 7.3). Because mice totally deficient in the catechol *O*-methyltransferase show only mild behavioral effects (40), this activity may not be important with respect to possible homo-

cysteine-induced methyltransferase inhibition. However, it is possible that an additive effect of the inhibition of multiple enzyme activities drives the phenotypic expression of the pathology.

It will be interesting to see if the inhibition of any of these latter enzymes in humans with elevated homocysteine levels may be correlated with seizures and types of disturbances linked to altered brain metabolism (33, 61). In at least one case study, homocystinuria and schizophrenic behavior have been tightly linked (33), and in a more recent study it has been reported that hyperhomocysteinemia can often be found in schizophrenic patients (86). However, as mentioned previously, homocysteine has neuromodulator properties itself (2). These studies suggest that homocysteine-induced loss of these methyltransferase activities may result in an abnormal balance of these neurotransmitters. The knowledge that deficiency of the protein L-isoaspartate (D-aspartate) methyltransferase can lead to seizures in mice (57, 58), and that limitation of the type II protein arginine methyltransferase action resulting from *S*-adenosylmethionine-depletion may be linked to demyelinization (13), strengthens the possible link between homocysteine-induced methylation loss and neurological pathology.

It will also be of interest to examine the role of the protein repair methyltransferase in relation to vascular disease. This enzyme has been shown to be trapped in the extracellular space when blood vessels are injured and may play a role in the repair of damaged collagen molecules (109, 110).

Conclusion

The clinical features resulting from homocysteine accumulation may be linked, at least in part, to the inhibition of cellular methyltransferases by changes in the cellular ratio of *S*-adenosylmethionine and *S*-adenosylhomocysteine. To begin to address this problem, this chapter has detailed the present state of knowledge of mammalian methyltransferases and the effects of inhibiting their activities. Humans have at least 39 distinct methyltransferases that catalyze a broad range of reactions for a broad range of cellular functions. Although it is difficult at this point to specifically link the inhibition of any one of the methyltransferases to the pathology resulting from homocysteine buildup, the partial loss of many of these enzymes and the cumulative effect of the loss of function of this large group of enzymes may in fact be reflected in cardiovascular damage, neurological pathology, and the other clinical features that are described elsewhere in this book. Further work is warranted to study the function of individual methyltransferases and to identify new methyltransferases.

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