Protein Methylation : Enzymology and Biological Significance of Myelin Basic Protein Methylation

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Myelin is a membrane characteristic of the nervous tissue and functions as an insulator to increase the velocity of the stimuli being transmitted between a nerve cell body and its target. Myelin isolated from human and bovine nervous tissue is composed of approximately 80% lipid and 20% protein, and 30% of the protein fraction constitutes myelin basic protein (MBP). MBP has an unusual amino acid at Res-107 as a mixture of N^G-monomethylarginine and N^G, N^G-dimethylarginine. The formation of these methylarginine derivatives is catalyzed by one of the subtypes of protein methylase I which specifically methylates Res-107 of this protein. Several evidences are presented to demonstrate an involvement of this biological methylation in the integrity and maintenance of myelin

Key Words: Myelin bascic protein, MBP methylation, Protein-arginine methylation, Multiple sclerosis

PROLOGUE

Several amino acid side chains such as lysine, arginine, histidine, and aspartic/glutamic acid have been shown to be methylated at the polypeptide level[1~6]. The formation of these methylated amino acids involve various methyl-group transfer reactions utilizing S-adenosyl-L-methionine (AdoMet) as the methyl donor. It is now clear that each protein methyltransferase with a specificity for any given amino acid residue can be further subclassified based on the specific methyl acceptor protein. The significance of protein methylation covers from prokaryote to eukaryote and from structural/membrane proteins to other compounds such as carnitine[7] and calmodulin[8].

Protein methylase I[9] (PMI; S-adenosylmethionine: protein-arginine N-methyltransferase; EC 2.1.1.23) is one enzyme

with more than one subtype existed, however, an unequivocal molecular evidence for the presence of subclasses of the enzyme (myelin basic protein-specific and histone-specific) has been obtained only in recent years. In 1971, the occurrence of specific methylarginine in Res-107 of myelin basic protein (MBP), which is catalyzed by PMI, was independently reported by Baldwin and Carnegie[10], and Brostoff and Eylar[11]. Since this initial discovery, several laboratories have studied the MBP-arginine methylation in conjunction with myelination[12~18] because MBP is the major protein constituent of the myelin membrane. The modification of the side chain arginine is expected to play some role in the structure-function relationship of this membrane protein. Furthermore, this system is very suited for the study of the posttranslational modification, since membrane assembly is predominantly postsynthetic phenomenon. Here, we have reviewed two major areas of recent development in the study of protein-arginine methylation: (1) the molecular distinction between subclasses of PMI (namely, MBP-specific and histone-specific) of mammalian brain and other eukaryotic tissue; and (2) the biochemical significance of MBP-specific methylation as it relates to mye-

lination in the central nervous system.

ENZYMOLOGY OF PROTEIN-ARGININE METHYLATION

Reaction of protein methylase I

Enzymatic methylation on the guanidinium group of arginyl residue is catalyzed by PMI. The enzyme was initially discovered in 1968 in calf thymus[9], and the enzymatic reaction yields three methylated arginine derivatives[9,19]: N^Gmono-methylarginine [MMeArg], $N^G,N^{'G}$-dimethylarginine [Di(sym) MeArg] and N^G,N^Gdimethylarginine [Di(asym)MeArg], as shown in Fig. 1. The demethylated AdoMet, S -adenosyl-L-homocysteine (AdoHcy), which is a potent product inhibitor for all known AdoMet -dependent transmethylation reactions[20~22], also inhibits PMI with a Ki value of approximately 10^{-6} M^{23}.

The natural occurrence of these methylated amino acids in proteins is limited only to a number of highly specialized proteins such as MBP[10,11], and nuclear[24~26] and contractile proteins[27], and basic fibroblast growth factor[29]. The reaction is highly specific for the amino acid sequence around the methylation site : Among 18 arginine residues in MBP, only arginine at residue-107 in the sequence of Lys-Gly-Arg-Gly-Leu is present as a mixture of MMeArg and Di(sym)MeArg[10,11]. Additionally, several nuclear/nucleolar proteins and muscle proteins contain MMeArg and Di(asym)MeArg, however, Di(sym)MeArg is not detectable in these proteins. The presence of several Di(asym)MeArg residues in nonhistone nuclear proteins, such as nucleolin, 34 kDa nucleolar protein, HnRNP protein (protein A-1) and high mobility group proteins(HMG 1 and 2), are of interest since these proteins contain clusters of glycine and Di(asym)MeArg interspersed with phenylalanine[25,26].

Protein methylase I from mammalian organs

PMI activity is present widely in mammalian organs, in testis, brain, thymus, spleen, kidney, and liver in the decreasing order[3~5].

We have successfully purified two PMIs from calf brain cytosol to near homogeneity. It is remarkable to observe that although both enzymes methylate arginine residues in protein substrates, there are more differences than similarities in their
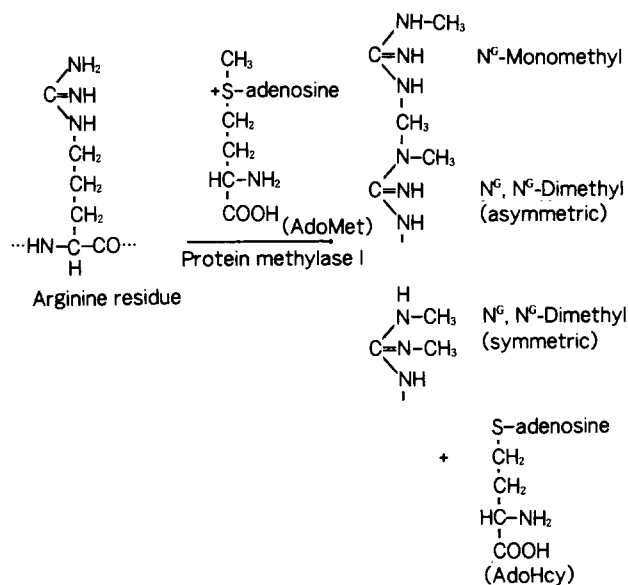

Fig. 1. Reaction of protein methylase I.

respective properties. The most notable differences are their molecular weights, affinities for protein substrate, and immunological recognition. The MBP-specific PMI (500 kDa) preferentially methylates MBP (K_m = 2×10^{-7} M) and histone to a much lesser extent (K_m = 1×10^{-4} M), while the histone-specific enzyme (275 kDa) methylates histone only. Both enzymes exhibit two major subunit bands on SDS-polyacrylamide gel electrophoresis; 100 and 75 kDa for the former, and 110 and 72 kDa for the latter Sensitivity toward various chemicals and higher temperatures are also different, as summarized in Table 1. Western immunoblot analysis of the purified PMIs following nondenaturing PAGE indicated that the corresponding enzyme band is only immunoreactive against its own respective antibodies, with no cross-reactivity between them.

More recently, an unmethylated recombinant hnRNP protein A1 became available and we, therefore, embarked to investigate the true nature of the substrate for the "histone-specific" PMI[30]. It was found that the K_m value for the recombinant protein A1 was two orders of magnitude lower than that of histone (1.9×10^{-7} M vs. 2.1×10^{-5} M) (Table 1), In addition, the maximal extent of methylation with protein A1 was 1.08 mol/mole of the protein, whereas only 0.04 mol into the histone. The greater capacity of the protein A1 methylation with higher affinity constant made it more

Table 1. Comparative properties of MBP-specific and nuclear protein/histone-specific protein methylase I from calf brain

Properties	MBP-specific	Nuclear protein/Histone-specific
M_r(by Sephadex G-200)	500 kDa	275 kDa
Subunit(by SDS-PAGE)	100 kDa, 72 kDa	110 kDa, 75 kDa
pI	5.09	5.68
Km values :		
MBP	2.3×10^{-7} M	
Histone	1.0×10^{-4} M	1.1×10^{-5} M
Protein A1		1.9×10^{-7} M
AdoMet*	4.4×10^{-6} M	8.0×10^{-6} M
Ki values :		
MBP	—	3.42×10^{-5} M
AdoHcy*	1.8×10^{-6} M	2.3×10^{-6} M
Sinefungin	7.0×10^{-6} M	6.6×10^{-6} M
Dialysis	Easily inactivated	Not inactivated
50% inactivation :		
p-chloromercuribenzoate	0.46 mM	0.15 mM
Guanidine.HCl	3.1 mM	0.3 mM
At 50℃ for 5 min	99% activity remained	60% activity remained

* AdoMet represents S-adenosyl-L-methionine and AdoHcy S-adenosyl-L-homocysteine.

relevant to rename the enzyme as "nuclear protein/histone-specific" protein methylase I[30]. In order to investigate the substrate specificity, several synthetic peptides were prepared for methyl-acceptability for the nuclear protein/histone-PMI [31,32]. The hexapeptide containing Gly-Arg-Gly- motif was found to be the minimum essential structure to be methylated.

Protein methylase I from cultured mammalian cells

Several mammalian culture cells have been shown to contain a significant amount of PMI activity. Casellas and Jeanteur[33] partially purified PMI from Krebs II ascites cell nuclei by lysing the cells in 0.4 M NaCl followed by phosphocellulose, DE-52, and hydroxyapatite chromatography. Histones were the only efficient substrates. The reaction was very sensitive to the strength of ion, 50% of the activity being inhibited at 0.1 M NaCl. The molecular weight of the enzyme was approximately 500 kDa determined by gel filtration, indicating a possible multimeric structure or aggregation, however, all attempts to dissociate the complex into smaller enzymatically active molecules have failed. The methylated products were identified as MMeArg and Di(asym)MeArg.

In order to probe AdoMet binding site, the inhibition kine-

tics of ascites cell PMI were carried out using various analogs of AdoHcy, and S-isobutyladenosine(SIBA)[34~36] was found to be the most potent inhibitor[37]. Similar studies were also carried out by Lederer and co-workers[31~33] with PMI isolated from chick embryo fibroblast. The most significant observation in this study was that there existed a correlation between the inhibition of PMI and that of the virus-induced chick embryo fibroblast transformation by AdoHcy analogs; i.e. all good inhibitors of PMI were also good inhibitors of the fibroblast transformation.

Protein methylase I from other eukaryotes

PMI has also been partially purified from wheat germ[38] and Euglena gracilis[39]. The former enzyme is specific for histone and the latter for cytochrome c. Both methylases do not methylate MBP. An unique feature of wheat germ PMI is that intracellular adenosine acts as an endogenous inhibitor of the enzyme. Thus, the recovery of the enzyme activity was 160% of the total whole homogenate activity after 90-fold purification. The enzyme requires a low molecular weight, dialyzable and heat-labile cofactor present in cell extract, but its identity is not yet clarified. The molecular weight of the en-

zyme is 28 kDa and the optimum pH of the reaction is 9.0, which is unlike that of mammalian PMI which is pH 7.2. The in vitro methylation site of the histone H4 by the enzyme was identified as the Res-35 arginine yielding only N^G-monomethylarginine[38]. The AdoMet analogs such as AdoHcy, 9145C, sinefungin and Sinosyl(2-hydroxy-4-methyl-thio)-butyrate on wheat germ PMI showed the K_i value of about 10^{-6} M. The PMI that specifically methylates horse heart cytochrome c at the Res-38 arginine has also been identified and purified from Euglena gracilis cytosol[40]

METHYLATION OF MYELIN BASIC PROTEIN AND MYELINATION

Myelin Basic Protein(MBP)

Myelin is a membrane characteristic of the nervous tissue laid down in segments along the selected nerve fibers and functions as an insulator to increase the velocity of the stimuli being transmitted between a nerve cell body and its target[41]. The myelination process can be broadly divided into two stages of development[42,43]. In the mouse, the first stage is between 8 to 15 d of postnatal period, and the second stage between 16 to 30 d of age. In the first stage, oligodendrocytes proliferate, enlarge, and plasma membrane is formed which then starts to wrap loosely around the nerve axon to form a multilamellar structure. In the second stage, an active deposition of different proteins takes place for the compaction of the lamellar structure.

The cytoplasmic surfaces of these bilayers interact to form a major dense line of myelin where MBP is appositioned, while the extra cytoplasmic surfaces interact to form the intraperiod line where proteolipid proteins are located. Thus, structurally, myelin is recognized as a lipid bimolecular leaflet with proteins sandwiched between the two layers which is wrapped in a spiral fashion around a segment of axon. A model, based on available data, illustrating the molecular organization of myelin macromolecules, indicates that proteolipid protein is a transbilayer component in monomeric/polymeric form, whereas MBP is an extrinsic protein having one or more domains in contact with hydrophobic interior of the bilayer[44]. Thus, the basic protein is exclusively located at the cytoplasmic layer, probably as a dimer, a head-to-tail (i.e., antiparallel) orientation.

Myelin isolated from human and bovine nervous tissue is composed of approximately 80% lipid and 20% protein. The protein fraction coustitutes 30% of MBP, 50% proteolipid and 20% high molecular weight Wolfgram protein[45]. The protein is highly conserved and occurs in many species as a single polypeptide chain with a molecular weight of approximately 18,000 (170 amino acids)[46,47] In rodents, however, several different molecular species of MBP are reported and they are encoded by distinct mRNA formed by alternative RNA splicing pathways acting on a common primary transcript[48,49]. MBP has several unusual properties. It is highly basic (pI=10.5) with a high proline content and lacks of tertiary structure[50]. It undergoes rapid proteolysis[51,52] particularly when it is dissociated from the membrane structure[53]. Baldwin and Carnegie[10] and Brostoff and Eyla[11] found a presence of an unusual amino acid at Res-107 and identified it as methylarginine derivatives.

MBP contains a single tryptophan residue near the midpoint of the sequence[45~47] which has been implicated as a focus for the immunological properties of MBP[56]. When injected with this protein, the animal develops neurological symptoms known as "experimental allergic encephalomyelitis", a model often used for the study of multiple sclerosis in the human[45,50,57]. An another unique feature of the protein is the presence of a relatively rare triproline sequence (Res 99 to 101) in the region close to the tryptophan residue. It has been suggested that the triproline sequence bends the protein into a double chain configuration and that the methylation of arginine at Res-107 could provide stabilization of the "hair-pin" conformation(Fig. 2), either by interaction with lipids or in conjugation with an adjacent phenylalanine side chain, thereby helping the insertion of MBP into myelin sheath[11,50].

MBP methylation in myelination

An involvement of biological methylation in the integrity and maintenance of myelin has been suggested early through the animal experiments of subacute combined degeneration (SCD). SCD is also found in man with untreated vitamin B_{12} deficiency and is characterized by the degeneration of the myelin sheath. When mice[58] or monkeys[58,59] were exposed to an atmospheric environment containing 15% nitrous oxide, the animal became ataxic and neurological disorder developed
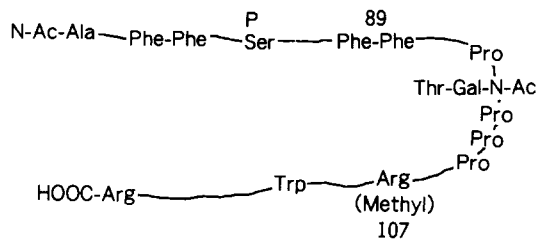
Fig. 2. Schemtic representation of myelin basic protein (MBP) in the hair-pin configuration.

Fig. 3. Biological transmethylation pathway.

over a period of 2 to 3 weeks until the animals were moribund. Microscopic examination of the nitrous oxide-treated spinal cord and peripheral nervous system showed the classical changes of SCD[60]. Nitrous oxide oxidizes cobalamin (vitamin B_{12}), thus blocking the formation of methylcobalamin which is an essential intermediate for the biosynthesis of methionine in animals (the overall biological transmethylation pathway is depicted in Fig. 3). However, when the experimental diet was supplemented with methionine, the animals were free of any detectable clinical changes[60].

Jacobson and co-workers[61] and Flipp and Holder[62] further demonstrated that a neurological syndrome similar to vitamin B_{12} deficiency can be produced in mice by injecting cyclo-leucine[63]. Cycloleucine is an analog of methionine which inhibits the biosynthesis of AdoMet, a biological methyl donor for a variety of compounds including proteins and other macromolecules[64]. Indeed, Crang and Jacobson[18,61] found that cycloleucine inhibited the activity of S-adenosylmethionine synthetase (EC 2.5.1.6) as well as methylation of MBP in the brain. These results thus argue against the previously suspected phospholipid methylation hypothesis[59,65] which involves the synthesis of methylmalonyl-CoA[64]: Cycloleucine does not block CoA biosynthesis. Utilizing cycloleucine to produce myelopathy in mice, Small et al[66]. suggested that the inhibition of MBP methylation might be responsible for this condition, since cycloleucine strongly depressed the formation of N^G-methylarginine in vivo whereas the incorporation of methionine directly into myelin protein was not affected, suggesting that MBP-methylation but not the synthesis of the protein was inhibited
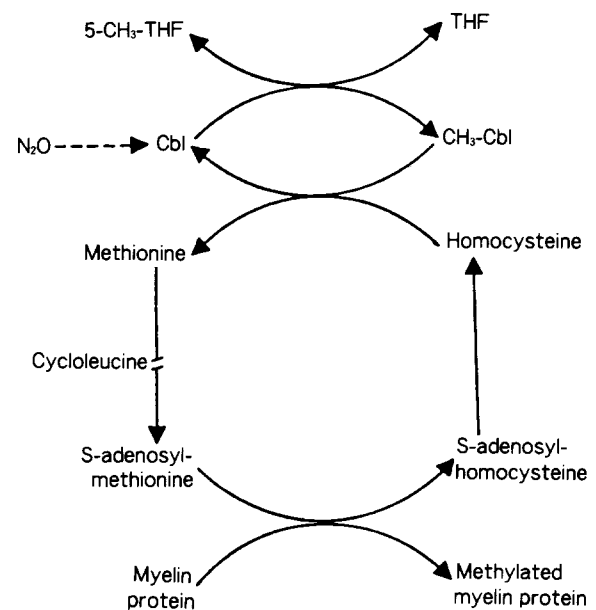
In vivo biosynthesis and methylation of myelin basic

Protein: The developmental expression of the mouse MBP was studied by Campagnoni and his coworkers using intra-cerebral injection of L-[3H]lysine[43]. The studies showed that the biosynthesis of MBP paralleled with the myelination process with maximal at 18 d of the mouse brain, with higher rate of synthesis of the 14-kDa synthesis than that of the 18.5-kDa species. As shown by several investigators[67-69] unlike bovine and human MBP (Mr =18.0 kDa), the MBP from rodents displays polymorphism, with apparent molecular weights of 34, 30, 29, 23.6, 21, 18.5, 17, and 14.5K, which are all immunoreactive against MBP antibody. We have also demonstrated that differences in the rate of accumulation of each MBP species correlated with brain development[70]; the higher molecular weight species were predominant in younger brain while the smaller MBPs were the major species in older brain. Although the genetic basis of this developmental MBP polymorphism is not clear, it has been speculated that each species may play a particular role during the myelination process : Different rates of incorporation into myelin, their turnover rates, and/ or their functional relationship within the membrane.

Employing chicken as an experimental animal, Small and

Carnegie studied the in vivo methylation of MBP[70]. The incorporation of methyl groups of injected [^{3}H-methyl]methionine into methylarginines in myelin was found to occur readily in 2-d-old chickens at a ratio of Di(sym)MeArg : MMeArg : Arg of 1.3:0.9:1.0, and these arginine derivatives were confined only in MBP. Di(asym)MeArg was not detected in MBP, and could only be found in other brain proteins, occurrence of Di(sym)MeArg being exclusively in MBP. DesJardins and Morell[71] further studied the metabolic relationship between MBP backbone and the methyl groups of methylarginine using [methyl-^{3}H]-methionine as a precursor. Turnover rates of the incorporated [^{3}H]-labels into MBP and the methylarginine were shown to have similar half lives, indicating the parallel synthesis and methylation of MBP. Emplying a double-labelling technique with L-[35-S]-methionine for backbone synthesis and L-[methyl-3H]methionine for both backbone and methylation via Ado[methyl-3H]Met, Chanderkar et al[69] found that the methylation of MBP was the highest at 17 d of age when myelination and protein synthesis were at maximum and decreased thereafter. These results demonstarate strongly that MBP-arginine methylation is closely coupled with myelination process.

In vitro methylation of MBP embedded in membranes of different degrees of compaction has been investigated to understand the effect of membrane structure on their methylation[72]. Myelin fractions of different compactions were first isolated [myelin, P$_3$A and P$_3$B[73]], and the methyl-acceptability of MBPs embedded in each fractions were compared for their methyl-acceptability. The result showed that MBP from the most compact myelin had higher methyl-acceptability compared to those from the less compact fractions, P$_3$A and P$_3$B, and suggested possible sturctural and/or topographical differences in their methyl-acceptabilities between MBPs associated with different membrane subfractions. Apparently, a highly methylatable MBP structure is bound to compact myelin.

Protein methylase I activity during brain development

Miyake[17], and Crang and Jacobson[18], employing developing rat brain and the mouse spinal cord, respectively, have demonstrated a parallel increase of the MBP-PMI during active myelination, whereas histone-specific PMI activity decreased during this period. In 1986, Chanderkar et al[69] have also

confirmed the temporal correlation between MBP-PMI and myelination in mouse brain; the enzyme activity increased during brain development and reached its peak level at the age of 17 d when myelination and MBP-synthesis is maximal. Employing primary embryonic brain cells, often used for the study of differentiation of oligodendroglia, Amur et al[74] further demonstrated an effect of thyroid hormone as well as temporal correlation between expression of MBP-PMI and myelin marker enzymes[75] such as 2',3'-cyclic nucleotide 3'-phosphohydrolase and 5'-nucleotidase during the culture period.

Protein methylase I activity in dysmyelinating brain

There are a number of dysmyelinating mutant mice which possess abnormalities in the structure, composition, and/or metabolism of myelin. These animal models are useful tools in studying biochemical lesions which are associated with molecular defects of myelination, since the pathology is limited to the myelinated fiber tracts. These include jimpy (jp) which is a sex-linked recessive, and quaking (qk), myelin deficient (mld), twitcher mouse(Twi), and shiverer (shi), which are autosomal recessive mutations. These mutants are characterized by reduction of myelin in their brains, although the reasons for its deficit are different in each case[76].

The jimpy mouse is the most severely affected dysmyelinating mutant and is characterized by failure to incorporate MBP into myelin sheath, manifested by the fact that the deficit of MBP in myelin is greater than the total reduction of the basic protein in whole brain[77,78] In 1984, using both histone and MBP as the methyl acceptor substrates, Kim et al[79] studied PMI activity in the jimpy brain as a function of age. The histone-specific PMI activity in the jimpy brain exhibited an age -dependent decrease as normal brain does. However, the MBP-specific PMI activity in 15-, 18-, and 21-d-old hemizygous jimpy mice (jp/y) brains decreased by 20, 50, and 75%, respectively, from those of normal littermates (Fig. 4). The heterozygous jimpy mice (jp/+) which are phenotypically normal showed unaltered normal enzyme levels.

Another hypomyelinating mutant mouse, shiverer, also displayed an altered pattern of PMI activity[80], however, significantly differed from that of jimpy mouse. The activity in homozygous shiverer (shi/shi) mutant mouse brain is significantly higher at the onset of myelination than in the nor-
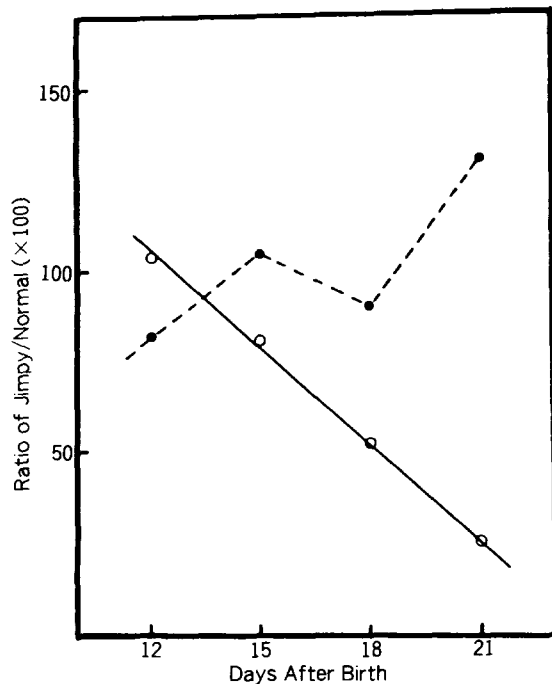
Fig. 4. Iteration of protein methylase I activity in jimpy(jimpy/y) mice. ●—● represents the ratio of enzyme activities using histone as the substrate, and ○—○ using MBP as the substrate. Jimpy/normal represents the ratio of protein methylase I activity in jimpy (hemizygous) to that of normal.

mal littermate brain but decreases rapidly during the period of myelination. Since there is no difference either in the weight or protein concentration between the normal and shiverer mutant brains, the decrease in enzyme activity is not a simple reflection of changes in the protein concentration in the mutant brain. Although the reason for this anomalously high MBP-PMI activity in the younger shiverer brain is not known, it may be related to the fact that the number of oligodendrocytes in shiverer brain was found to be high (Knobler, unpublished observation).

Among the mutant mice, myelin deficient quaking is known to have a normal life span[76] and is characterized by the reduction of 14-kDa MBP as compared to the 18.5-kDa species in the brain. In homozygous quaking mutant (qk/qk) brain, both histone-specific and MBP-specific PMI activities were shown to be normal[79]. Finally, when the methylation of an arginine residue of MBP was examined in slices of the brain stem and spinal cord, using [³H]-methionine as the donor of the methyl groups, no difference was found between

Twi and the controls[81]. Radioactivity of the [³H] methionine residue of MBP of Twi was also similar to that of the control.

Hormonal effect on myelination and protein methylase I

The functional maturation of the mammalian central nervous system depends on the presence of the thyroid hormone. It has been shown that both neonatally induced hypo- and hyperthyroidism cause marked reduction in the number of oligodendroglia[82,83], and also impairment of myelination in rat brains[84]. In 1981, Walters and Morell[85] studied the developmental pattern of myelin proteins in both hypo- and hyperthyroid status by intracranial injection of [³H]glycine. The results indicated that hypothyroidism retards the developmental program for myelinogenesis, whereas myelin synthesis is initiated earlier and also terminated earlier in hyperthyroid state. Employing myelinogenic cultures of cells dissociated from embryonic mouse brain, the effect of thyroid hormone on the expression of MBP-PMI was studied by Amur et al[74]. Whereas the MBP-PMI activity in the cell was highly dependent on the presence of the thyroid hormone, such dependency was not seen for histone-PMI expression. These investigators have also studied several adrenergic effectors and neurotransmitters as a potential regulator for MBP- and histone-specific PMI[86]. Both enzymes were stimulated by β-adrenergic agonists (propranolol) via an increase in the cAMP/adenyl cyclase system. However, the agonist did not block the stimulation of MBP-enzyme triggered by thyroid hormone, indicating that the effect of the hormone is not mediated via β-adrenergic-dependent system. Therefore, the methylation of MBP seems to be regulated by the thyroid hormone and/or by neurotransmitter, whereas the methylation of histone is regulated by an adrenergic system.

Effect of MBP-methylation on myelin structure

A direct approach to elucidate a possible involvement of MBP-arginine methylation in the formation of compact myelin has been carried out at an ultrastructural level; the myelin-like membranes isolated from sinefungin inhibitor for PMI-treated and -untreated myelogenic cells in culture were compared using a transmission electron microscope[87]. In the myelogenic cells, MBP-PMI activity was inhibited by 50% at 25 μM of the inhibitor, although the ratio of MBP to the total protein concentration was not significantly altered. The sine-
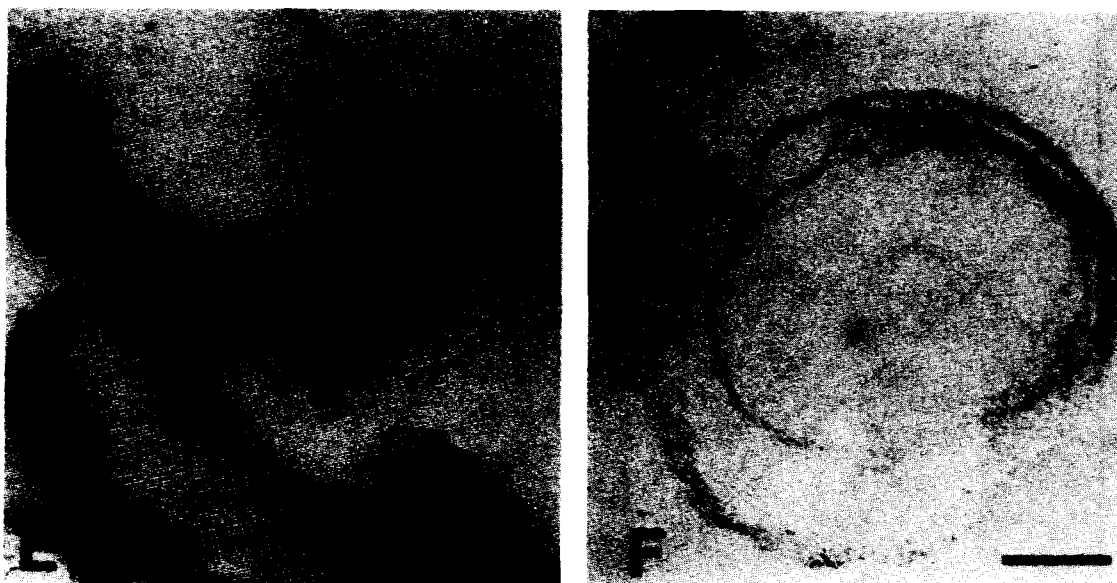
Fig. 5. Electron micrographs of myelin-like membranes obtained from brain cell cultures. E is from normal controlcultureand F from sinefungin (30μM)-treated culture. Bar=100nm

fungin-treated membrane exhibited a ring-like structure that was devoid of multilamellar periodicity and compactness characteristic of normal myelin, resembling vacuolated myelin observed in vitamin B_{12}-deficient membrane(Fig. 5). In support of these findings, Young et al[88]. have studied an effect of MBP-methylation in the dimer formation of unilamellar vesicle; the fully methylated bovine MBP was shown to be more efficient in inducing the dimerization of the vesicle than by the carp MBP which is not in vitro methylated.

Urinary excretion of methylated arginines in demethylating human diseases

Multiple sclerosis is one of the demethylating diseases in human. Under the conditions, MBP is dissociated from the membrane, and MBP-fragments formed by intracellular proteolysis[89,90] and free amino acids find their way into the body fluids[91~94]. Futhermore, Park et al[95] showed the presence of MBP-specific PMI as well as its endogenous substrate in human cerebrospinal fluid. Since methylarginines are not reutilized for protein synthesis in vivo, it appeared to be quite feasible to assess myelin-associated abnormalities by analyzing the urinary concentrations of these methylarginine. Thus, Whitaker et al[96,97], attempted to correlate clinical features and urinary excretion of MBP-like material in multiple sclerosis

patients. Employing highly sensitive HPLC post-column o-phthaldialdehyde derivatization method, we have also analyzed the urinary samples from multiple sclerosis patients and found approximately 20% lower level of DiMeArg from all the patients and 33% decrease from the chronic-progressive conditions[98].

CONCLUDING REMARKS

Among the enzymatic post-/cotranslational methylation of arginine in proteins, the modification of MBP-arginine in the central nervous system is one of the best-studied biological systems thus far. There are several evidences which point out strongly possible involvement of MBP-arginine methylation in the formation of compact myelin sheath. (1) Historically, the methyl-deficiency in animals is known to induce myelopathy[99], and studies indicate that the methylation of MBP, not that of phospholipid, is linked to the biochemical lesion associated with the methyl-deficiency. (2) The specific location of methylarginine in MBP (residue- 107) together with the chemical nature of hydrophobicity enhanced by the methylation has been implicated to help in stabilizing the proper myelin structure. (3) In several experimental systems, the MBP-specific PMI activity, but not histone-specific PMI,

is shown to be temporarily correlated with myelination during brain development. Finally, (4) cells treated with transmethylation inhibitor (sinefungin) yielded membrane structure which lacks the compactness, similar to vitamin B_{12}-deficient myelin with concurrent reduction in the MBPspecific PMI.

In addition to the above, a possible alteration in the susceptibility of the protein toward intracellular proteases by arginine-methylation should also be considered in relation to membrane structure-function. While the maintenance of compact myelin sheath requires an intact maintenance of its membrane components, MBP is known to be easily metabolized by the MBP-associated neutral and acidic proteases. Since the methylated arginine residue in MBP is partially resistant to trypsin[11], it is tempting to speculate that the rate of MBP degradation may be influenced by the level of MBP-arginine methylated, inducing the stability of myelin.

Recent advances in enzymology on PMI have clarified the earlier confusion concerning the identity of MBP-specific and histone-specific PMI. Although both enzymes methylate arginine residues, they are completely different proteins in terms of molecular, catalytic, and immunological properties. In light of these findings, it is now possible to study the molecular mechanism by which PMI activities are regulated, since MBP-specific PMI is low at postnatal and increases during myelination, while histone-specific enzyme is high in early postnatal and decreases progressively thereafter. In this regard, one interesting finding should be pointed out in which MBP was shown to be an inhibitor for histone-specific PMI in vitro[28]. What role, if any, MBP plays on the in vivo expression of two coexisting PMI activities during myelination is not known.

ACKNOWLEDGEMENT

REFERENCES

1. Ambler RP and Rees MW: e-N-Methyl-L-lysine in bacterial

flagellar protein. Nature 184: 56, 1959

2. Kim S and Paik WK: Studies on the origin of e-N-methyl-L-lysine in protein. J Biol Chem 240: 4629, 1965

3. Paik WK and Kim S: Protein methylation. Science 174: 114, 1971

4. Kim S and Paik WK: Protein methylation: perspectives in central nervous system, in biochemical and pharmacological roles of adenosylmerhionine and the dentral nervous system, Zappia Z, Usdin E and Salvatore F. Eds. Pergamon Press Oxford 1978, 37

5. Paik WK and Kim S: Protein methylation, in Biochemistry: A Series of Monographs, Vol. I, John Wiley & Sons. New York 1980, 112

6. Paik WK and Kim S: Methylation and demethylation of protein, in the enzymology of post-translational modifeation of proteins, Vol 2, Freedman RB and Hawkins HC, Eds. Academic Press London 1985, 187

7. Paik WK, Nochumson S and Kim S: Carnitine biosynthesis via protein methylation. TIBS 2: 159, 1977

8. Sitaramayya A, Wright LS and Siegel FL: Enzymatic methylation of calmodulin in rat brain cytosol. J Biol Chem 255: 8894, 1980

9. Paik WK and Kim S: Protein methylase 1. J Biol Chem 243: 2108, 1968

10. Baldwin GS and Carnegie PR: Specific enzymic methylation of an arginine in the experimental allergic encephalomyelitis protein from human myelin. Science 171: 579, 1971

11. Brostoff S and Eylar EH: Localization of the methylated arginine in the A I protein from myelin Proc. Natl Acad Sci USA 68: 765, 1971

12. Paik WK and Kim S: Protein methylases during the development of rat brain, Biochim Biophys Acta 313: 181, 1973

13. Paik WK and Kim S: Protein methylation during the development of rat brain, Biochem. Biophys Res Commun 46: 993, 1972

14. Jones GM and Carnegie PR: Methylation of myelin basic protein by enzymes from rat brain. J Neurochem 23: 1231, 1974

15. Small DH and Carnegie PR: In vivo methylation of an arginine in chicken myelin basic protein. J Neurochem 38: 184, 1982

16. Miyake M and Kakimoto Y: Protein methylation by cerebral tissue. J Neurochem 20: 859, 1973

17. Miyake M: Methylases of myelin basic protein and histone in rat brain. J Neurochem 24: 909, 1975

18. Crang AJ and Jacobson W: The relationship of myelin basic protein (arginine) methyltransferase to myelination in mouse spinal cord. J Neurochem 39: 244, 1982

19. Nakajima T, Matsuoka Y and Kakimoto Y: Isolation and identification of NG-monomethyl. NG,NGdimethyl- and N Nt6-dimethylarginine from the hydrolysate of proteins of

bovine brain. Biochim Biophys Acta 230: 212, 1971

20. Zappia V, Zydek-Cwiek CR and Schlenk F: The specificity of S-adenosylmethionine derivatives in methyl transfer reactions. J Biol Chem 244: 4499, 1960

21. Deguchi T and Barchas J: Inhibition of transmethylations of biogenic amines by S-adenosylhomocysteine. J Biol Chem 246: 3175, 1971

22. Borchardt RT and Pugh CSG: Analogues of S-adenosyl-L-homocysteine as inhibitors of viral mRNA methyltransferases, in Transmethylation, Developments in Neuroscience, Vol 5, Usdin E, Borchardt RT and Creveling CR, Eds. Elsvier/North-Holland New York 197, 1979

23. Lee HW, Klm S and Palk WK: S-adenosylmethionine: protein-arginine methyltransferase, purification and mechanism of the enzyme. Biochemistry 16: 78, 1977

24. Karn J, Vidali G, Bona L and Allfrey VG: Characterization of the non-histone nuclear proteins associated with rapidly labeled heterogeneous nuclear RNA. J Biol Chem 252: 7307, 1977

25. Lischwe MA, Cook RG, Ahn YS, Yeoman LC and Busch H: Clustering of glycine and NG,NGdimethylarginine in nucleolar protein C23. Biochemistry 24: 6025, 1985

26. Lischwe MA, Ochs RL, Reddy R, Cook RG, Yeoman LC and Busch H: Purification and partial characterization of a nucleolar scleroderma antigen (M, = 34,000; pl, 8.5) rich in NG,NG-dimethylarginine, J Biol Chem 260: 14304, 1985

27. Reporter M and Corbin JL: N^G, N^G-Dimethylarginine in myosin during muscle development, Biochem Biophys Res Commun 43: 644, 1971

28. Park GH, Chanderkar LP, Paik WK and Kim S: Myelin basic protein inhibits histone-specific protein methylase 1. Biochim Biophys Acta 874: 30, 1986

29. Burgess WH, Blizik J, Mehlman T, Quarto N and Rifkin DB: Direct evidence for methylation of arginine residues in high moleculae weight forms of basic fibroblast growth factor. Cell Regulation 2: 87, 1991

30. Rajpurohit R, Lee SO, Park JO, Paik WK and Kim S: Enzymatic methylation of recombinant heterogeneous nuclear RNP protein A1. Dual substrate specificity for S-adenosylmethionine: histon-arginine methyltransferase. J Biol Chem 269: 1075, 1994

31. Ghosh SK, Syed SK, Jung S, Paik WK and Kim S: Substrate specificity for myelin basic protein-specific protein methylase I. Biochem Biophys Acta 1039: 142, 1990

32. Rawal N, Rajpurohit R, Lischwe MA, Williams KS, Paik WK and Kim S: Structural specificity of substrate for S-adenosyl-methionine: protein-arginine N-methyltransferase. Biochem Biophys Acta 1248: 1391, 1993

33. Casellas P and Jeanteur P: Protein methylation in animal cells. 1. Purification and properties of S-adenosylL-methionine; pro-

tein (arginine) N-methyltransferase form Krebs 11 ascites cells, Biochim. Biophys Acta 519: 243, 1978

34. Casellas P and Jeanteur P: Protein methylation in animal cells. II. Inhibition of S-adenosyl-Lmethionine:protein (arginine) N-methyltransferase by analogs of S-adenosyl-L-homocysteine. Biochim Biophys Acta 519: 255, 1978

35. Vedel M, Lawrence F, Robert-Gero M and Lederer E: The anti-fungal antibiotic sinefungin as a very active inhibitor of methyltransferases and of the transfommation of chick fibroblasts by Rous sarcoma virus, Biochem. Biophys Res Commun 85: 371, 1978

36. Enouf J, Lawrenee F, Tempete C, Robert-Gero M and Lederer E: Relationship between inhibition of protein methylase I and inhibition of Rous sarcoma virus-induced cell transformation. Cancer Res 39: 4497, 1979

37. Robert-Gero M, Lawrenee F, Farrugia G, Berneman A, Blanehard P, Vigier P and Lederer E: Inhibition of virus- induced cell transfommation by synthetic analogues of S-adenosyl homocysteine, Biochem. Biophys. Res Commun 65: 1242, 1975

38. Gupta A, Jensen D, Kim S and Paik WK: Histone-specific protein-arginine methyltransferase from wheat gemm, J Biol Chem 257: 9677, 1982

39. Farooqui JZ, Tuck M and Paik WK: Purification and characterization of enzymes from Euglena gracilis that methylate methionine and arginine residues of cytochrome c. J Biol Chem 260: 537, 1985

40. Disa SG, Gupta A, Kim S and Paik WK: Site specificity of histone H4 methylation by wheat gemm protein-arginine N-methyltransferase. Biochemistry 25: 2443, 1986

41. Raine CS: Morphology of myelin and myelination, in Uyelin, 2nd ed., Morell P Ed. Plenum Press New York 1984, 1

42. Norton WT and Podeslo SE: Myelination in rat brain: changes in myelin composition during brain maturation. J Neurochem 21: 759, 1973

43. Campagnoni CW, Carey GD and Campagnoni AT: Synthesis of myelin basic proteins in the developing mouse brain. Arch Biochem Biophys 190: 118, 1978

44. Braun PE: Molecular organization of myelin, in Myelin, 2nd ed, Morell P Ed. Plenum Press New York 1984, 97

45. Eylar EH: The structure and immunological properties of myelin. Ann NY Acad Sci 195: 481, 1972

46. Carnegie PR: Amino acid sequence of the encephalitogenic basic protein from human myelin. Biochem J 123: 57, 1971

47. Eylar EH: Amino acid sequence of the basic protein of the myelin membrane. Proc Natl Acad Sci USA 67: 1425, 1970

48. Takahashi N, Roach A, Teplow DB, Prusiner SB and Hood L: Cloning and characterization of the MBP gene from mouse: one gene can encode both 14 kd and 18.5 kd MBPs by alternate use of axons. Cell 42: 139, 1985

49. Campagnoni AT: Molecular biology of myelin proteins from the central nervous system. J Neurochem 51: 1, 1988

50. Carnegie PR: Properties, structure and possible neuroreceptor role of the encephalitogenic protein of human brain. Nature 229: 25, 1971

51. Kornguth ML, Tomasi ML, Keyes DL and Kornguth SE: The proteolytic degradation of a punfied pit brain protein. Biochim Biophys Acta 229: 167, 1971

52. Sato S, Quarles RH and Brady RO: Susceptibility of the myelin-associated glycoprotein and basic protein to a neutral protease in highly purified myelin from human and rat brain. J Neurochem 39: 97, 1982

53. Glynn P, Chantry A, Groome N and Cuzner ML: Basic protein dissociating from myelin membranes at physiological ionic strength and pH is cleaved into three major fragments. J Neurochem 48: 752, 1987

54. Deibler GE and Martenson RE: Detemlination of methylated basic amino acids with the amino acid analyzer. J Biol Chem 248: 2387, 1973

55. Dunkley PR and Carnegie PR: Amino acid sequence of the smaller basic protein from rat brain myelin. Biochem J 141: 243, 1974

56. Brostoff SW: Immunological responses to myelin and myelin components, in Myelin, 2nd ed., Morell P, Ed. Plenum Press New York 1984, 405

57. Suckling AJ, Kirby JA, Wilson NR and Rumsby MG: The generation of the relapse in chronic relapsing experimental allergic encephalomyelitis and multiple sclerosis - parallels and differences, in ExperimentalAllergicEncephalomyelitis: A Useful Model for Multiple Sclerosis. Alvord JEC, Kies MW and Suckling AJ, Eds. Alan R Liss New York, 1984, 7

58. Jacobson W, Gandy G and Disman RL: Experimental subacute combined degeneration of the cord in mice. J Pathol 109: 243, 1973

59. Dinn JJ, Weir DG, MeCann S, Reed B, Wilson P and Scott JM: Methyl group deficiency in nerve tissue: a hypothesis to explain the lesion of subacute combined degeneration. Irish J Med Sci 149: 1, 1980

60. Scott JM, Wilson P, Din JJ and Weir DG: Pathogenesis of subacute combined degeneration: a result of methyl group deficiency. Lancet 2: 334, 1981

61. Crang AJ and Jacobson W: The methylation in vitro of myelin basic protein by arginine methylase from mouse spmal cord. Biochem Soc Trans 8: 611, 1980

62. Flippo TS and Holder WD, jr: Neurological degradation associated with nitric oxide anesthesia in patients with vitamin B12 deficiency. Arch Surg 128: 1391, 1993

63. Lombardini JB, Coulter JB and Talalay P: Analogues of methionine as substrates and inhibitors of the methionine adenosyl-transferase reaction: Deductions concerning the con-

formation of methionine. Mol Pharmacol 6: 481, 1970

64 Frenkel EP, Kitchens RL and Johnson JM: The effect of vitamin B,2 deprivation on the enzyme of fatty acid synthesis. J Biol Chem 248: 7540, 1973

65. Siddons RC, Spence JA and Dayan AD: Experimental vitamin B,2 deficiency in the baboon. Adv Neurol 10: 239, 1975

66. Small DH, Carnegie PR and Anderson R McD: Cycloleucine-induced vacuolation of myelin is associated with inhibition of protein methyla'tion. Neurosci Lett 21: 287, 1981

67. Yu YT and Campagnoni AT: In vitro synthesis of the four mouse myelin basic proteins: evidence for the lack of a metabolic relationship. J Neurochem 39: 1559, 1982

68. Carson JH, Nielson ML and Barbarese E: Developmental regulation of myelin basic protein expression in mouse brain. Dev Biol 96: 485 1983

69. Chanderkar LP, Paik WK and Kim S: Studies on myelin basic protein methylation during mouse brain development. Biochem J 240: 471, 1986

70. Small DH and Carnegie PR: In vivo methylation of an arginine in chicken myelin basic protein. J Neurochem 38: 184, 1982

71. DesJardins KC and Morell P: Phosphate groups modifying myelin basic proteins are metabolically labile: methyl groups are stable. J Cell Biol 97: 438, 1983

72. Ghosh SK, Rawal N, Syed SK, Paik WK and Kim S: Enzymatic methylation of myelin basic protein in myelin. Biochem J 275: 381, 1991

73. Cruz TF and Moscarello MA: Characterization of myelin fractions from human brain white matter. J Neurochem 44: 1411, 1985

74. Amur SC, Shanker G and Pieringer RA: Regulation of myelin basic protein (arginine) methyltransferase by thyroid hormone in myelinogenic cultures of cells dissociated from embryonic mouse brain. J Neurochem 43: 494, 1984

75 Bhat NR, Rao GS and Pieringer RA: Investigations of myelination in vitro, regulation of sulfolipid synthesis by thyroid hormone in cultures of dissociated brain cells from embryonic mice. J Biol Chem 256: 1167, 1981

76. Hogan EL: Animal models of genetic disorders of myelin, m Myelin, Morell P, Ed. Plenum Press New York 1977, 489

77. Campagnoni AT, Campagnoni CW, Huang AL and Sampugna J: Developmental changes in the basic proteins of normal and jimpy mouse brain. Biochem Biophys Res Commun 46: 700, 1972

78. Mattjieu JM, Windmer S and Herschkowitz N: Jimpy, an anomaly of myelin maturation: biochemical study of myelination phases. Brain Res 55: 403, 1972

79. Kim S, Tuck M, Kim M, Campagnoni AT and Paik WK: Studies on myelin basic protein-protein methylase I m various dysmyelinating mutant mice. Biochem Biophys Res Commun 123: 468. 1984

80. Kim S, Tuck M, Ho LL, Campagnoni AT, Barbarese E, Knobler RL, Lublin FD, Chanderkar LP and Paik WK: Myelin basic protein-specific protein methylase I activity in shiverer mutant mouse brain. J Neurosci Res 16: 357, 1986

81. Yoshimura T, Kobayashi T, Shinnoh N and Goto I: Accumulation of galactosylsphingosine (psychosine) does not interfere with phosphorylation and methylation of myelin basic protein in the twitcher mouse. Neurochem Res 15: 963, 1990

82. Bass NH and Young E: Effects of hypothyroidism on the differentiation of neurons and glia in developing ratcerebrum. J Neurol Sci 18: 155, 1973

83. Pelton EW and Bass NH: Adverse effects of excess thyroid hormone on the maturation of rat cerebrum. Arch Neurol 29: 145, 1973

84. Dalal KB, Valcana T, Timiras PS and Einstein ER: Regulatory role of T4 on myelinogenesis in the developing rat Neurobiology 1: 211, 1971

85. Waters SN and Morell P: Effect of altered thyroid states on myelinogenesis. J Neurochem 36: 1792, 1981

86. Amur SG, Shanker G and Pieringer RA: ~-Adrenergic stimulation of protein (arginine) methyltransferase activity in cultured cerebral cells from embryonic mice. J Neurosci Res 16: 377, 1986

87 Amur SG, Shanker G, Cochran JM, Ved HS and Pieringer RA: Correlation between inhibition of myelin basic protein (arginine) methyltransferase by sinefungin and lack of compact myelm formation m cultures of cerebral cells from embryonic mice. J Neurosci Res 16: 367, 1986

88. Young PR, Vacante DA and Waickus CM: Mechanism of the interaction between myelin basic protein and the myelin membrane: the role of arginine methylation. Biochem Biophys Res Commun 145: 1112, 1987

89. Whitaker JN and Heinemann MA: The degradation of human myelin basic protein peptide 43-88 by human renal renal protease. Nuerology 33: 744, 1983

90. Bever CT and Whitaker JN: Proteinases in inflammatory demyelinating disease, Springer Semin. Immunopathol 8: 235, 1985

91. Kakimoto Y and Akazawa S: Isolation and identification of N^G, N^G-and N^G, $N^{,G}$-dimethylarginine, N^ϵ-mono, di, and trimethyllysine, and glucosylgalactosyl- and galactosyl-hydroxylysine from human urine. J Biol Chem 245: 5751, 1970

92. Carnegie PR, Fellows FC and Symington GR: Urinary excretion of methylarginine in human disease. Metabolism 26: 531, 1977

93. Lou MF: Human muscular dystrophy: elevation of urinary dimethylarginines. Science 203: 668, 1979

94. Yudkoff M, Nissium I, Pereira G and Segal S: Urinary excretion of dimethylarginines in premature infants. Biochem Med 32: 242, 1984

95. Park J, Greenstein JI, Paik WK and Kim S: Studies on protein methyltransferase in human cerebrospinal fluid. J Mol Neurosci 1: 151, 1989

96. Whitaker JN, Layton BA, Herman PK, Kachelhofer RD, Burgard S and Bartolucci AA: Correlation of myelin basic protein-like material in cerebrospinal fluid of multiple sclerosis patients with their response to glucocorticoid treatment. Ann Neurol 33: 10, 1993

97. Whitaker JN, William PH, Layton BA, McFarland HF, Stone LA, Smith EM, Kachelhofer RD, Bradley EL, Burgard S, Zaho G and Paty DW: Correlation of clinical features and findings on cranial magnetic resonance imaging with urinary myelin basic protein-like material in patients with urinary myelin basic protein-like material in patients with multiple sclerosis. Ann Neurol 35: 577, 1994

98. Rawal N, Lee YJ, Paik WK and Kim S: Studies on N^G-methylarginine derivatives in myelin basic protein from developing and mutant mouse brain. Biochem J 287: 929, 1992

99. Russell JSR, Batten FE and Collier J: Subacute combined degeneration of the spinal cord. Brain 23: 39, 1900