Purification and kinetic mechanism of *S*-adenosylmethionine:myelin basic protein methyltransferase from bovine brain

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The enzyme *S*-adenosylmethionine (AdoMet):myelin basic protein (MBP) methyltransferase was purified 250-fold from bovine brain with an overall yield of 130 %, relative to crude supernatant. The purification involves acid–base and $(\text{NH}_4)_2\text{SO}_4$ precipitation, chromatography over Sephadex G-100 and DEAE-cellulose, followed by preparative isoelectric focusing. The enzyme has a pI of 5.60 ± 0.05 , and the M_r is estimated to be between 71 000 (from SDS/polyacrylamide-gel electrophoresis) and 74 500 (from gel filtration). The enzyme is stable at 37 °C for over 2 h, is stable frozen and does not require metal ions or reductants. The enzyme shows a high specificity for MBP and does not accept polyarginine as a substrate; F1 histone is methylated at 37 % of the rate of MBP. Methylation occurs on an arginine residue in a single h.p.l.c.-resolvable peptide from the tryptic cleavage of MBP. Simple saturation kinetics are observed with respect to both substrates, with K_m values of 18 μM and 32 μM for MBP and AdoMet respectively. The simplest kinetic mechanism that is consistent with the data requires ordered rapid-equilibrium binding, with AdoMet as the first substrate. The enzyme isolated in this work is different, both physically and kinetically, from the histone-specific arginine methyltransferases described by other workers. A new, simple, assay system for the methylation of MBP is described.

INTRODUCTION

It is well established that myelin basic protein (MBP) is partially methylated on an arginine residue *in vivo* by an enzyme which utilizes *S*-adenosylmethionine (AdoMet) as methyl donor. Amino acid analysis clearly shows that this residue (arginine-108) exists in un-, mono- and di-methylated forms (Carnegie, 1971; Eylar *et al.*, 1971; Deibler & Martenson, 1973; Young & Grynspan, 1987). For bovine MBP, monomethylarginine comprises about 61 % of Arg-108, with 31 % being dimethylated, leaving about 8 % as unmethylated (Young & Grynspan, 1987).

Enzymes which methylate the guanidino group of protein arginyl residues have been described by many workers. Paik & Kim (1971), Lee *et al.* (1977) and Park *et al.* (1986) have described an enzyme, denoted protein methylase I (PM-I) [*S*-adenosylmethionine:protein-(arginine) *N*-methyltransferase; EC 2.1.1.23], which is widely distributed in various organs and is especially rich in brain, testis and thymus. The enzyme methylates a variety of protein acceptors, including histone and MBP, with the greatest activity being towards histones. Histone-specific enzymes from wheat germ (Gupta *et al.*, 1982) and from bovine brain (Park *et al.*, 1986) have been described, as well as an enzyme which methylates an arginine residue in cytochrome *c* (Farooqui *et al.*, 1985).

An important question concerning protein methylation within any one class of methyltransferases concerns the origin of the specificity of the enzyme for the protein substrate. Are there specific methyltransferases for each

protein, or do the enzymes simply recognize and methylate certain peptide sequences, with one enzyme servicing many separate proteins (Paik & Kim, 1980; Lischwe *et al.*, 1985)? A problem in approaching this question is finding appropriate substrate proteins; proteins which are methylated *in vivo* are seldom available unmethylated, hence protein methylation must often be studied with substrates which are ‘unnatural’ and possibly non-specific. Paik and co-workers have circumvented this problem somewhat by combining cytochrome *c* methyltransferases from *Neurospora crassa* and *Euglena gracilis* with unmethylated cytochrome *c* from horse heart as methyl acceptor (DiMarid *et al.*, 1979; Farooqui *et al.*, 1985). The sequence homology is apparently sufficient between the cytochromes, so that methylation is observed on a single specific residue, and the specific activities of the enzymes are quite high, relative to the activities observed with other, non-specific, substrates. The fact that myelin basic protein is only partially methylated *in vivo* makes MBP an almost unique case where a natural methyl-accepting protein can be examined with a methyltransferase enzyme from the same source. Further, since MBP is reported to be largely unstructured in solution, mechanisms of site specificity (i.e. sequence or ‘microscopic’ domains of secondary structure) potentially can be probed within this system.

There has been a considerable amount of evidence suggesting that the enzyme that methylates myelin basic protein is different from the histone-methylating activity in the central nervous system (Miyake & Kakimoto, 1973; Miyake, 1975; Kim *et al.*, 1984; Amur *et al.*, 1984). Park *et al.* (1986) have reported the separation of the

Abbreviations used: MBP, myelin basic protein; AdoMet, *S*-adenosylmethionine; PM-I, protein methylase I.

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MBP- and histone-methylating PM-I activities from calf brain supernatant, and have purified the histone-methylating activity approx. 50-fold, to a specific activity of 54.1 pmol/min per mg. Further, they have found that MBP is actually an inhibitor of the histone-methylating PM-I activity isolated from bovine brain supernatant (Park *et al.*, 1986).

In order to begin a characterization of the process, importance and mechanism of the methylation of MBP, we have purified and kinetically characterized the MBP-methylating PM-I activity from bovine brain. The enzyme preferentially methylates MBP rather than histone, and is physically and kinetically different from the histone-methylating PM-I described by previous workers (Park *et al.*, 1986).

MATERIALS AND METHODS

Materials

All chemicals used were readily available commercial products and were used without further purification unless otherwise noted. [*Me*-¹⁴C]- and [*Me*-³H]AdoMet were from Amersham (sp. radioactivity 52 mCi/mmol and 59 Ci/mmol respectively) and were diluted to give 100–150 d.p.m./pmole. Bovine brains were obtained at a local slaughterhouse approx. 30 min after death. Whole brain was dissected to be essentially free of grey matter and circulatory debris. The enriched white matter was cut into small pieces, frozen in liquid N₂ and stored at –25 °C. Glass-distilled water was used throughout. Liquid-scintillation counting was performed in a Packard Tri-Carb model B-2450 system; internal standards were used to calculate quench corrections; external standards were run with every set of samples, and observed radioactivity (c.p.m.) was corrected for quenching and ³H decay. The pH values of solutions were determined with a Corning pHM 130 pH-meter with a combined glass electrode and a calomel junction. H.p.l.c. was performed in a Rainin isocratic apparatus, with a reversed-phase C₁₈ column; effluent was monitored at 210 nm in a continuous u.v. flow cell. Nitrocellulose membrane filters impregnated with CM-cellulose were from Schleicher and Schuell.

Isolation methods

MBP was prepared by the procedure of Young *et al.* (1982), based on slight modifications of the methods described by Deibler *et al.* (1972) and Eylar & Oshiro (1970). Electrophoresis on 5%-polyacrylamide gels, 8 M in urea and 1 M in acetic acid (Deibler *et al.*, 1972), and on SDS/10.5%-acrylamide gels (2.5%-acrylamide stacking gel) indicated a homogeneous protein. Tryptic peptides from [¹⁴C]methylated MBP were obtained by the procedure of Eylar *et al.* (1971). After digestion, the solution was freeze-dried and the peptides were solubilized in 5 mM-NaH₂PO₄ buffer, pH 7.2, containing 0.1 M-NaClO₄ and 15% (v/v) CH₃CN (h.p.l.c. buffer). Peptides were analysed by isocratic h.p.l.c. (Deibler *et al.*, 1985) with h.p.l.c. buffer; peptide elution was monitored at 210 nm, and fractions were collected for scintillation counting. A sample of the radioactive peptide was hydrolysed, converted into the phenylthiocarbonyl derivative and analysed by h.p.l.c. by using the 'Pico Tag' elution system (Young & Grynspar, 1987). A purified 'non-specific' adenosine deaminase was obtained from *Aspergillus oryzae* by the method of Sharpless &

Wolfenden (1967), purified 250-fold relative to crude extract, and stored frozen in 10 mM-Mops buffer, pH 7.2, containing soya-bean trypsin inhibitor (1 mg/ml; added to inhibit proteolysis of MBP by an impurity in the deaminase preparation). Activity of the non-specific deaminase was measured spectrophotometrically (Sharpless & Wolfenden, 1967). Oligodendroglia were isolated from fresh bovine brains by the method described by Poduslo & Norton (1975).

Assay methods

The methylation of MBP by AdoMet was assayed by adsorbing MBP from the assay mixture on to nitro-cellulose filters coated with CM-cellulose. This assay system relies on the selective removal of MBP from the assay mixture by adsorption to the resin; MBP has a high affinity for CM-cellulose, whereas the enzyme mixture, having a pI < 7.0, is not adsorbed. AdoMet slightly adsorbs to the resin, but can be quantitatively removed by extensive washing (400 ml) on a vacuum manifold.

The standard assay contained MBP (1.0 mg), methyltransferase enzyme preparation, 50 μM-AdoMet (typically 110 d.p.m./pmol) and sufficient Mops buffer (10 mM, pH 7.2) to give an assay volume of 0.5 ml. Assays were performed in 1.5 ml polyethylene micro-centrifuge tubes suspended in a thermostatically controlled water bath at 37 °C for 20 min. The reaction was terminated by the addition of 1.0 ml of ice-cold Mops buffer, followed by filtration of the mixture through the CM-cellulose filter on a vacuum manifold. The filter was washed with 400 ml of buffer to remove retained AdoMet and then transferred to a scintillation vial. The protein was eluted from the membrane by the addition of 1.0 ml of 1 M-HCl. Finally, 15 ml of scintillation fluid was added to the vial to dissolve the membrane coating. Radioactivity incorporation due to the formation of methyl esters by contaminating protein methylase II was assayed by the procedure of Terwilliger *et al.* (1983) and corrected for the partitioning of methanol between the assay mixture and the scintillation fluid (72:28 respectively). Average values for kinetic constants were obtained by standard non-linear least-squares analysis of the data; representative plots of these data (Figs. 3 and 4) are constructed by using these average values.

Purification of AdoMet:MBP methyltransferase

Frozen enriched white matter was thawed and homogenized in 2 vol. of pre-cooled (4 °C) 0.25 M-sucrose/1 mM-EDTA/10 mM-Mops, pH 7.2, in a Waring Blender for 2 min. The crude material was centrifuged at 10000 g for 20 min (to give 'white-matter homogenate') and the supernatant was centrifuged again at 105000 g for 3 h. The precipitate was discarded and the supernatant passed through a glass-wool plug. The supernatant was acidified to pH 5.0 by the careful dropwise addition of 1 M-HCl. The acidified solution was stirred for 12 h at 4 °C and then adjusted to pH 7.2 by the careful dropwise addition of 1 M-NaOH. The neutralized solution was stirred for 3 h at 4 °C and the supernatant collected by centrifugation (10000 g for 20 min). Crystalline (NH₄)₂SO₄ was slowly added to the supernatant to give a final concentration of 60% saturation (4 °C). The solution was left in the cold for 12 h and the precipitate then collected by centrifugation (10000 g for 20 min). The (NH₄)₂SO₄ precipitate (650 mg) was applied to a

Sephadex G-100 column (4 cm × 69 cm) and eluted with 10 mM-Mops buffer, pH 7.2. Fractions were collected and monitored for methyltransferase activity, which was eluted as a tail to the major protein peak after approx. 300 ml of eluent had passed through the column. Combined Sephadex G-100 fractions displaying activity were applied to a DEAE-cellulose column (4 cm × 5 cm) and washed with 125 ml of 10 mM-Mops buffer, pH 7.2. Enzyme protein was eluted with a linear gradient [0–1.0 M-(NH₄)₂SO₄ in 10 mM-Mops buffer]; activity was concentrated in the 0.25–0.35 M-(NH₄)₂SO₄ fractions. Preparative isoelectric focusing was carried out in a LKB 8100 Ampholine 110 ml column, with a 5–50 % (w/v) sucrose gradient containing 5 % (v/v) Ampholine (pH range 2.5–8) and 88 mg of protein. Focusing was allowed to proceed at 4 °C for 48 h at 640 V, after which time fractions were eluted, adjusted to pH 7.2 and assayed for activity. Fractions containing activity were precipitated with (NH₄)₂SO₄ and analysed by SDS/polyacrylamide-gel electrophoresis.

RESULTS AND DISCUSSION

Isolation of AdoMet:MBP methyltransferase

The general class of enzymes which methylate the guanidino group of protein arginine residues, with AdoMet as the methyl donor, have been denoted as PM-I and have been described by many workers (Paik & Kim, 1971; Miyake & Kakimoto, 1973; Sunderraj & Pfeiffer, 1973; Jones & Carnegie, 1974; Miyake, 1975; Lee *et al.*, 1977; Crang & Jacobson, 1982; Gupta *et al.*, 1982; Kim *et al.*, 1984; Amur *et al.*, 1984; Farooqui *et al.*, 1985; Park *et al.*, 1986). Initial steps in the isolation of the activity from bovine brain in the present work paralleled those of Lee *et al.* (1977), and the enzyme activity was first noted in the clear supernatant from ultracentrifugation. The purification scheme utilized is somewhat similar to that reported by Park *et al.* (1986), who have described the separation of MBP- and histone-methylating enzymes from calf brain supernatant and the partial purification of the histone methylase (approx. 50-fold, to a specific activity of 54 pmol/min per mg). Briefly, the purification involves acid and (NH₄)₂SO₄ precipitations, chromatography over Sephadex G-100 and DEAE-cellulose and isoelectric focusing. The enzyme had no affinity for Blue Dextran covalently linked to Sepharose, which serves as an affinity gel for many AdoMet enzymes. The increase in activity which is observed in the early steps of the purification, and again on isoelectric focusing, most likely involves the removal of inhibitory endogenous proteins during the purification steps. A similar gain in activity has also been described by previous workers (Miyake, 1975; Lee *et al.*, 1977; Durban *et al.*, 1978; Gupta *et al.*, 1982) in the purification of other arginine methyltransferase enzymes.

The *M_r* of the enzyme was estimated to be 71 000 and 74 500 by interpolation on linear replots of log *M_r* versus *R_f* for SDS/polyacrylamide-gel electrophoresis and for elution from a calibrated Sephadex G-100 column, respectively. The correlation between *M_r* values under native and denaturing conditions suggests that the enzyme is a single polypeptide chain. The enzyme isolated in this work is smaller and much more stable to incubation and manipulation than was the histone-specific methylase (*M_r* 1 500 000) described by Lee *et al.* (1977).

Table 1. Protein-substrate specificities for bovine brain AdoMet:MBP methyltransferase and for calf brain PM-I

Protein substrate	Activity (%)	
	AdoMet:MBP methyltransferase*	PM-I†
MBP	100	30
Histone F1	37 ± 0.5	100
Lysozyme	9 ± 2	0
Polylysine	7 ± 3	0
Bovine serum albumin	5 ± 2	0
Polyhistidine	2 ± 1	—
Polyarginine	0.5 ± 0.5	25

* Assay in 10 mM-Mops buffer, pH 7.2, 37 °C; 20 min incubation. The assay mixture included 1 mg of substrate protein, 10 μM-AdoMet and 0.6 mg of enzyme protein.

† From Lee *et al.* (1977).

Table 2. Purification of AdoMet:MBP methyltransferase from bovine brain

Activity is expressed in terms of pmol of labelled methyl groups transferred/min per mg of total protein. Incubations were carried out in 10 mM-Mops buffer, pH 7.2, 37 °C, for 20 min.

	Fraction of activity recovered*	Specific activity
Crude extract	1.00	~ 9
105 000 g supernatant	1.00	12
pH 5.0 supernatant	1.01	32
(NH ₄) ₂ SO ₄ ppt.	1.01	80
Sephadex G-100	0.95	280†
DEAE-cellulose	0.98	840
Isoelectric focusing	1.30	2270

* Fraction of the activity recovered from the previous step. The recoveries and changes in activity which are shown are representative of several trials.

† Specific activities of up to 800 with a fraction recovery of 0.35 can be obtained in this step.

The enzyme seems to be largely associated with white matter, since crude supernatant obtained from enriched white matter had 2.3 times the specific activity of supernatant obtained from whole brain; no activity was detected in the microsomal fractions. Oligodendrocyte cell suspensions which had been disrupted by sonication showed a specific activity similar to that of white matter, consistent with the suggestion that the methyltransferase is an enzyme which is associated with normal myelin metabolism (Crang & Jacobson, 1982; Amur *et al.*, 1984; Kim *et al.*, 1984).

Substrate specificity (Table 1)

For calf brain PM-I, described by Lee *et al.* (1977), MBP is only 30 % as efficient a methyl acceptor as is the F1 histone fraction, and polyarginine is equally as effective as MBP. For the AdoMet:MBP methyltrans-

Fig. 1. Dependence on enzyme concentration of the rate of methyl-group incorporation from AdoMet into MBP

Assay mixtures contained 0.5 mg of MBP, 10 μ M-[3 H]-AdoMet and 0.02 mg of adenosine deaminase in 0.5 ml of Mops buffer, pH 7.2, 37 °C; 20 min assay.

Fig. 2. Effect of adenosine deaminase concentration on the rate of methyl-group incorporation from AdoMet into MBP

Assay mixtures contained 0.5 mg of MBP, 10 μ M-[3 H]-AdoMet and 0.6 mg of enzyme in 0.5 ml of Mops buffer, pH 7.2, 37 °C; 20 min assay. The rate of incorporation is expressed as that in the presence of adenosine deaminase minus that at zero adenosine deaminase.

ferase isolated in the present work, the substrate specificities are significantly different; polyarginine is not a substrate at all, and the F1 histone is only 37% as effective a substrate as MBP. The relative efficiencies of histone and MBP as substrates for the present methyltransferase are essentially independent of the extent of purification [0.37/1.0 at the '(NH₄)₂SO₄ precipitate' stage and 0.31/1.0 for 'G-100' protein], suggesting that a site exists on the histone protein that can function as a methyl acceptor with this enzyme under the assay conditions. Significant carboxymethylation was not observed with 'G-100' enzyme samples; a low carboxymethylase activity was, however, detected in crude enzyme preparations (~35%, normalized for efficiency; Terwillinger *et al.*, 1983). Spin-echo 1 H n.m.r. difference spectra of MBP before and after methylation with the methyltransferase enzyme clearly show an enhancement in the absorbance at 2.778 p.p.m. which arises from the methylarginine resonance, supporting the contention that arginine methylation is being observed; no evidence for lysine or carboxylate methylation is observed in the n.m.r. (P. R. Young & F. Grynspan, unpublished work).

The question of whether the enzyme isolated in this work displays a site specificity within MBP was addressed by the procedures described by Baldwin & Carnegie (1971). Tryptic cleavage of the [14 C]methylated protein, followed by h.p.l.c. analysis (Deibler *et al.*, 1985) of the resulting peptides, showed the vast majority (85%) of the peptide-associated radioactivity to be concentrated in a single peptide, consistent with the suggestion that a single site is being methylated by the enzyme. This peptide was hydrolysed, and its derivative with phenyl isothiocyanate was prepared and analysed by the 'Pico-Tag' h.p.l.c. method; the amino acid composition of this peptide (Gly_{1.0}Ser_{0.7}Arg_{1.1}Leu_{0.9}) was essentially identical with that of peptide T23, as reported by Baldwin & Carnegie (1971). This peptide was identified by those

authors as consisting of residues 107–114, with the methylation occurring on Arg-108.

In the best case, the specific activity of the MBP-methyltransferase has been increased by approx. 700-fold, relative to crude supernatant; more typical values are shown in Table 2. The purification observed in the chromatography steps (notably the Sephadex G-100) is highly variable, and seems to be a delicate function of protein concentration and flow rate. Enzyme with a specific activity of ~300 pmol/min per mg was routinely obtained at this step and was used for the determination of the overall kinetic mechanism. This is justified, since the DEAE-cellulose and isoelectric-focusing steps, although yielding enzyme of higher activity, were difficult to scale up so that kinetic experiments (requiring very large amounts of enzyme) could be completed with the same batch of enzyme. Further, the state of purity of the enzyme preparation (from the G-100 step onward) seems to have essentially no effect on the observed Michaelis parameters, strongly suggesting that these samples are not contaminated with significant amounts of lysine- or histone-specific methyltransferases, which are detected in the assay system used.

Kinetic characterization

The rate of [3 H]methyl incorporation from AdoMet into MBP is a simple linear function of enzyme concentration, even with relative crude enzyme preparations (Fig. 1; specific activity 75 pmol/min per mg). The absence of upward curvature in this plot indicates that there are no endogenous protein substrates that are components of the enzyme mixture (even at this relatively crude stage) which are detected by the filter assay. The lack of downward curvature, even at high enzyme concentrations, means that the slopes that are measured

Fig. 3. Double-reciprocal plots of $1/v_0$ versus $1/[\text{AdoMet}]$ for methyl-group incorporation from AdoMet into MBP

The following fixed concentrations of MBP were used: ●, 11.1 μM ; □, 16.6 μM ; ▲, 22.2 μM ; ○, 33.3 μM ; ■, 55.5 μM ; △, 111.1 μM . Assay mixtures contained enzyme and 0.02 mg of adenosine deaminase in 0.5 ml of Mops buffer, pH 7.2, 37 °C; 20 min assay.

Fig. 4. Double-reciprocal plots of $1/v_0$ versus $1/[\text{MBP}]$ for methyl-group incorporation from AdoMet into MBP

The following fixed concentrations of AdoMet were used: ●, 5.0 μM ; □, 7.0 μM ; ▲, 10.0 μM ; ○, 20.0 μM ; ■, 30.0 μM ; △, 40.0 μM . Assay mixtures contained enzyme and 0.02 mg of adenosine deaminase in 0.5 ml of Mops buffer, pH 7.2, 37 °C; 20 min assay.

in the assay are true initial velocities and are valid for kinetic analysis.

In order to avoid complications arising from product accumulation during assays, *S*-adenosylhomocysteine was removed from the system by using the enzyme adenosine deaminase from *Aspergillus oryzae*. The enzyme from this source has been termed 'non-specific', in that it accepts a wide range of adenosine derivatives and converts them into the corresponding inosine derivatives (Sharpless & Wolfenden, 1967). Thus *S*-adenosylhomocysteine is converted by the enzyme into *S*-inosylhomocysteine, a compound which is a poor competitive inhibitor for most AdoMet-requiring enzymes. AdoMet itself is not a substrate for the deaminase, probably because of unfavourable binding interactions at the cationic sulphur atom. Varying the concentration of the

deaminase in the assay (Fig. 2) results in an increase in the initial velocity up to a limiting value. On the basis of this plot, 0.01 mg of deaminase was added to standard assay mixtures. Assays conducted without MBP, but with adenosine deaminase present, showed no methyl incorporation into the deaminase, suggesting that the enzyme preparation contains no protein substrates for the methyltransferase which are detected by the filter assay.

Kinetics

Simple saturation kinetics are observed for the AdoMet:MBP methyltransferase with each substrate variable at a fixed concentration of the other substrate. Linear double-reciprocal plots are observed for both substrates (Figs. 3 and 4), indicating a first-order dependence on each substrate. This linearity also suggests that there are no significant complications arising from the large changes in total protein concentration as MBP is varied from 0.2 to 4.0 mg/ml. MBP occurs partially dimethylated *in vivo*; therefore two molecules of AdoMet are potentially involved in the methylation of each MBP molecule. We have reported previously, however, that extended incubation of MBP with this enzyme preparation results in a limiting methyl incorporation of about 0.11 mol/mol of protein. This correlates very well with the fraction of MBP that is unmethylated (~8%; Young & Grynspan, 1987), strongly suggesting that the present enzyme is capable of methylating only unmethylated arginine residues and that the subsequent dimethylation is accomplished by a second enzyme.

Double-reciprocal plots for variable AdoMet concentrations at several fixed concentrations of MBP, and the corresponding plots for variable MBP at several fixed concentrations of AdoMet, are shown in Figs. 3 and 4 respectively. A classical mixed-type pattern is observed for variable AdoMet; however, with MBP as the variable substrate, only a slope effect is observed. A slope effect such as this is only observed in an ordered rapid-equilibrium mechanism, and only for that substrate which is added last to the enzyme (Segal, 1975). In other words, the addition must be ordered, in rapid equilibrium, and AdoMet must bind to the enzyme first. A

Fig. 5. Replot of $1/K'_{\text{AdoMet}}$ versus $1/[\text{MBP}]$ from the data in Fig. 3

Scheme 1

basic kinetic model and the corresponding rate equations are shown in Scheme 1 and eqn. (1). Consistent with these rate equations, appropriate replots of apparent K_m (K'_{AdoMet}) values from Figs. 3 and 4 are linear, giving K_m values of 18 and 32 μM for MBP and AdoMet respectively (see Fig. 5).

$$v_0 = \frac{V_{\text{max}} [\text{AdoMet}] [\text{MBP}]}{K_{\text{AdoMet}} K_{\text{MBP}} + K_{\text{MBP}} [\text{AdoMet}] + [\text{AdoMet}] [\text{MBP}]} \quad (1)$$

The simplest kinetic model which is consistent with the data is a sequential rapid-equilibrium mechanism with AdoMet binding to the enzyme first, followed by unmethylated MBP. A sequential steady-state mechanism has been suggested by Lee *et al.* (1977) for the methylation of histone H4 by calf brain PM-I; again, AdoMet was suggested to bind first to the enzyme, and the K_m values observed for AdoMet and MBP are 7.6 and 71 μM respectively. The smaller K_m value for MBP observed in the present work possibly reflects an increased specificity of this enzyme for MBP. The major problem in interpreting the present K_m values is the very real possibility that the 60% or so of the MBP in the assay system that is already monomethylated may function as a competitive inhibitor of the methyltransferase enzyme, making the apparent K_m values artificially large. The synthesis of unmethylated peptides having sequence homology with MBP in the region of Arg-108, and determination of their kinetic parameters, should address this problem. Although the product inhibition will affect the observed Michaelis parameters, the overall kinetic mechanism of the enzyme should be independent of these concerns. The fact that simple equilibrium binding steps are observed in this methylation means that substrate binding and product release are fast, that the chemical aspects of transmethylation are rate-limiting and that this enzyme system may allow a direct probe into the chemistry of protein methylation.

The role of the methylated arginine in MBP remains unknown (Martenson, 1980), although it has been suggested that methylation might affect the conformation of MBP or the interaction between MBP and the myelin membrane (Baldwin & Carnegie, 1971). We have reported (Young *et al.*, 1987) that methylation of MBP by this enzyme increases the apparent equilibrium constant for the MBP-myelin association by about 4.2 kJ/mol, approx. 10% of the total free-energy change observed for the MBP-myelin interaction. The relatively small contribution of the methyl group to the overall interaction, the relatively high K_m values which are observed in the present work (with the natural mixture of methylated substrates), coupled with the requirement for two enzymes to complete the dimethylation, might all combine with the dynamic nature of the assembly of the lamellar process to explain the very unusual partial methylation which is observed on MBP *in vivo*.

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