MYELIN BASIC PROTEIN ARGININE METHYL TRANSFERASE: WIDE DISTRIBUTION AMONG BOTH NEUROGENIC AND NON-NEUROGENIC TISSUES

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Received April 23, 1973

SUMMARY

A methyl transferase catalyzing the transfer of methyl groups from S-adenosyl-methionine specifically to an arginine residue at position 107 of the nervous system specific myelin basic protein from beef brain was detected not only in neurogenic, but also in non-neurogenic rat organs and tumor cell lines. The products of methylation were identified as N-monomethylarginine and N,N'-dimethylarginine. The specific activity of crude enzyme preparation towards myelin basic protein was greater than that towards histones type III or type IV. Studies on competition of the substrates for the enzyme(s) indicated that at least part of the methylation of histones type III and type IV was probably catalyzed by the enzyme(s) methylating myelin basic protein.

Enzymes catalysing the transfer of methyl groups from S-adenosyl-methionine to arginine residues, C-terminal carboxyl groups, and ε-amino groups of lysine residues have been isolated from calf thymus and designated protein methylases I, II, and III respectively (1,2,3,4). Protein methyl transferase activity is present in many organs, among which brain is one of the richest sources (5,6,7). In brain extracts, in addition to histones, the encephalitogenic myelin basic protein is also methylated, with methylation occurring specifically at arginine-107 (8,9). The possibility that the enzyme catalysing the methylation of this particular arginine residue in myelin basic protein might be specific to the cells which make myelin was investigated. This report presents evidence that this methyl transferase is widely distributed among cell types.

MATERIALS AND METHODS

S-adenosyl-L-[methyl-^{14}C] methionine (specific activity 52 mCi/mM) and S-adenosyl-L-[methyl-^{3}H] methionine (4.54 Ci/mM) were purchased from New England Nuclear (Boston). Histone type III and type IV, S-adenosyl-L-methionine and trypsin (treated with L-tosylamido-2-phenyl-ethyl chloromethyl ketone) were obtained from Sigma Chemical Co.

Preparation of beef brain myelin basic protein - Beef-brain basic protein was prepared according to Oshiro and Eylar (10). Homogeneity was examined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate according to Fairbanks et al. (11), and at pH 5.3 in 7.5% polyacrylamide gels using ß-alanine buffer (12).

Animals - BD-9 rats (13) (2-months-old) were used for the experiments.

Tissue Culture Conditions - Clonal lines were grown as described by Pfeiffer and Wechsler (14).

Preparation of Crude Enzyme - All operations were performed between 0 and 4°. Cultured cells in early stationary phase were washed and harvested in phosphate buffer-saline (pH 7.2) and pelleted at 1000 x g for 10 min. Fresh rat organs or cell pellets were homogenized in 0.25 M sucrose containing 5 mM $CaCl_2$ (1) using a tissue homogenizer. The homogenates were centrifuged at 37,000 x g for 30 min., and supernatant fractions collected for the assays. In some experiments arginine methylase was partially purified by its precipitation from the supernatant fractions by the addition of $(NH_4)_2SO_4$ to 40% saturation (1).

Protein Methyl Transferase Assay - Protein methyl transferase was assayed according to Paik and Kim (1) with slight modifications. The reaction mixtures, containing 0.05 to 0.2 ml enzyme preparations (1 to 3 mg/ml), 0.1 ml of 1 M phosphate buffer (pH 7.2), 4 nmoles of methyl labelled S-adenosyl-L-methinine (0.21 µc ^{14}C or 3.64 µc ^{3}H) and 1.0 mg of methyl acceptor protein in a total volume of 0.5 ml, were incubated at 37° for 15 min. Preliminary experiments showed that S-adenosyl-methionine and methyl acceptor substrates were present at saturating concentration. The reaction was stopped by adding an equal volume of 30% CCl_3COOH. Terminal carboxyl methyl groups (formed from the activity of methylase II) were hydrolyzed as described by Paik et al (5). The residual protein fractions were then collected on fiberglass pads, dried, and transferred to toluene-based scintillation fluid (Liquifluor, New England Nuclear) for detection of radioactivity in a Beckman liquid scintillation system.

Methylation of endogenous protein acceptors was determined in reactions

TABLE I

SPECIFIC ACTIVITY OF PROTEIN METHYL TRANSFERASE IN VARIOUS TISSUES††

Source of Enzyme	Methyl Acceptor			
Rat Organs	No Exogenous Protein	Beef Brain Myelin Basic Protein	Histone Type III	Histone Type IV
Cerebrum	0.7	7.1	1.8	3.2
Cerebellum	1.0	6.8	1.8	2.9
Sciatic Nerve	0.7	2.7	1.3	1.4
Kidney	0.25	0.9	0.4	0.4
Spleen	0.1	1.0	0.2	0.7
Liver	0.7	1.0	1.1	0.8
Heart	0.4	2.6	0.7	0.7
Lung	0.5	2.2	0.7	1.2
Testis	0.2	3.9	0.6	2.1
Cell Lines†				
RN_2-Rat Schwann Cells (14)	0.7	3.2	1.0	1.7
C_6-Rat Astrocytes (15)	0.3	1.5	0.4	1.1
NB41A-Mouse Neuroblastoma (16)	0.5	2.8	0.6	1.6
Y_1-Mouse Adrenal (17)	0.3	1.5	0.5	1.5
L-Fibroblast (18)	0.6	2.5	1.0	2.2
3T6-Fibroblast (19)	0.3	2.7	3.7	1.2

† Literature references in parentheses.

†† pmoles of ^{14}C or ^{3}H transferred/min/mg protein.

run under identical conditions without any added acceptor protein. Background levels were determined from reaction mixtures similarly run with heat-inactivated homogenates.

Peptide Mapping of Methylated Myelin Basic Protein - Myelin basic protein was methylated using $(NH_4)_2SO_4$ precipitated enzyme (100 µg) as described above

except that incubation was extended to 1 hour and the acceptor concentration was increased twofold. Residual protein fractions (see above) were washed several times with ether to remove CCl_3COOH, suspended in 0.2 M NH_4HCO_3 buffer (pH 8.2), digested with trypsin for 2 hours at 37° (the ratio of residual protein to trypsin was 50:1 w/w), the tryptic digests lyophilized and the residues were resuspended in small amounts of water, spotted on Whatman No. 3 mm paper for separating the peptides.

Amino Acid Analysis of Methylated Peptides - Labelled peptides were eluted from chromatograms, hydrolysed in 6 N HCl at 107° for 22 hours and analysed by chromatography followed by electrophoresis using the same conditions as those used for peptide mapping.

RESULTS AND DISCUSSION

Levels of Methyl Transferases in Different Tissues - The levels of methyl transferase activity in cell lines and rat organs are presented in Table 1. Most of the observed methylation is attributed to arginine methyl transferase (Methylase I) since lysine methyl transferase (Methylase III) has been shown to be located exclusively in nuclei (4) and has an optimum pH around 9.0, in contrast to the pH 7.2 used for the assays, and carboxyl methyl groups formed by methylase II were hydrolysed at high pH as described above. In all cases, there was detectable methylation of endogenous proteins in crude enzyme preparations. The addition of myelin basic protein, histone III or histone IV, resulted in increases in methylase activities. The extent of increase varied markedly among the organs examined, but not significantly among various cell lines tested. Beef brain myelin basic protein was a better substrate than either histone III or IV for methyl transferase activity from both the cell lines and rat organs. Only in liver was there no marked difference in methylase activity among histone III, histone IV or myelin basic protein as methyl acceptors.

Competition for Methyl Transferase by the Acceptor Proteins - The addition to reaction mixtures of 1 mg of either histone III or histone IV along with myelin basic protein resulted in specific activities less than the sum of specific

TABLE 2

COMPETITION FOR METHYL TRANSFERASES BY ACCEPTOR SUBSTRATES

Substrate	Specific activity of methyl transferase pmoles methyl groups transferred/min/mg protein						
	Cerebrum		Liver		Heart	Testis	RN_2 Cell Line
	a	b	a	b	a	a	a
---	0.5	1.1	0.8	1.5	0.4	1.6	1.9
Myelin Basic Protein	6.1	10.4	1.3	2.0	3.5	6.4	3.2
Histone III	2.0	3.3	1.2	2.0	0.7	2.7	2.3
Histone III + Myelin Basic Protein	6.0 (7.6)	10.7 (12.6)	1.2 (1.7)	2.2 (2.5)	2.6 (3.8)	5.4 (7.5)	2.3 (3.6)
Histone IV	1.8	3.3	3.8	3.8	1.0	3.1	4.0
Histone IV + Myelin Basic Protein	4.9 (7.7)	7.9 (12.6)	1.4 (4.3)	2.7 (3.3)	2.5 (4.1)	5.1 (7.9)	2.6 (5.3)

a = crude homogenate b = 40% $(NH_4)_2 SO_4$ fraction

Figures in parentheses indicate the expected specific activity if different enzymes were catalyzing the methylation of histones and myelin basic protein

activities obtained with these acceptor substrates added singly (Table 2). The decreased specific activities in mixed substrate reactions suggests that at least a part of the methylations of histones III and IV was catalysed by the enzyme(s) catalysing methylation of myelin basic protein.

Localization of the Site of Methylation in Myelin Basic Protein Methylated In Vitro - Tryptic digests were prepared from myelin basic protein methylated by $(NH_4)_2SO_4$ precipitated enzymes from either cerebrum, cerebellum, liver, testis, heart or tissue culture cells. Peptide maps of these tryptic digests showed the presence of two labelled peptides (T_1 and T_2) which appeared at the same positions regardless of the source of methylase (Fig. 1). T_2 was a product of the methylation of an endogenous acceptor as judged from its presence as a

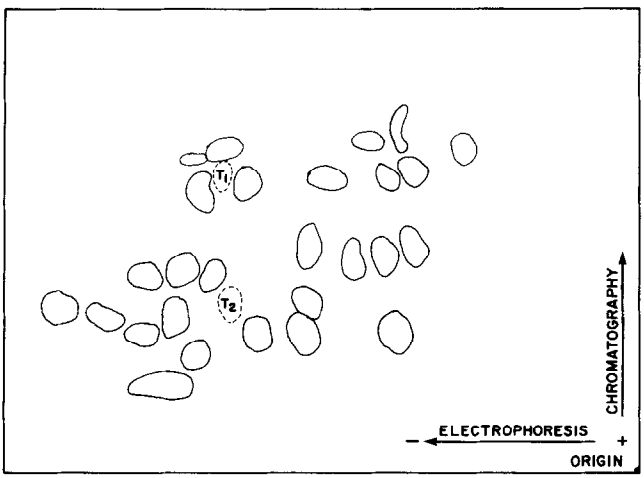

Fig. 1. Peptide map of a tryptic digest of myelin basic protein methylated using an enzyme preparation from cerebrum. Ascending chromatography was carried out using n-butanol:glacial acetic acid:pyridine:water (122:38:139:151) in the first direction followed by electrophoresis at (3500 V) 40 voltes/cm for 1 hour in pyridine:glacial acetic acid: H_2O (6.6:66:2928) at pH 3.5 in the second direction the peptide maps were placed in contact with x-ray film for 8-15 days to identify the labelled fragments. Duplicate peptide maps were stained with cadmium acetate-ninhydrin reagent (20).

single labelled peptide on peptide maps of tryptic digests of the crude enzyme reactions run in the absence of added acceptor.

The presence of T_1 at the same position in all the peptide maps suggests that the methylation of myelin basic protein occurred at the same site in every case. Acid hydrolysis and chromatographic analyses of this peptide showed the presence of leucine, arginine, serine, glycine, monomethyl arginine and dimethyl arginine. Eighty to eighty-six % of the label incorporated into peptide T_1 was present as N-monomethyl arginine, while the rest was present as N-N'-dimethyl arginine. By virtue of its position on peptide maps and its amino acid composition, peptide T_1 appears to be identical to peptide $T_{16}A$ described by Eylar et al. (21).

In summary, a methyl transferase(s) catalysing the transfer of a methyl group from S-adenosyl-methionine to only one of nineteen arginine residues (that at position 107) in beef myelin basic protein found in comparable activity not

$$\begin{array}{c} (\text{Methyl})_x \quad (x = 1 \text{ or two}) \\ | \\ \text{-Gly-Arg-Gly-Leu-Ser-Leu-Ser-Arg} \\ 107 \end{array}$$

Fig. 2. Amino acid sequence of peptide T 16A described by Eylar, et al. (26).

only in rat cerebrum, cerebellum and tumor cell lines of neural origin, but also in liver, heart, testis and several non-neural tumor cell lines.

ACKNOWLEDGEMENTS

We wish to acknowledge the expert technical assistance of B. Glastris and A. Morse. We thank Dr. R. Church for running SDS-acrylamide gels in the initial stage of these investigations. This work was supported by grant No. NS CA 10861-01 from the National Institutes of Health.

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