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CHEMICAL METHYLATION OF PHOSPHATIDYLETHANOLAMINE BY S-ADENOSYLMETHIONINE

Vinay S. Bansal and J. N. Kanfer

Department of Biochemistry, Faculty of Medicine, University of Manitoba
770 Bannatyne Avenue, Winnipeg, Canada R3E 0W3

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The chemical methylation of phosphatidylethanolamine (PE) by S-adenosyl methionine (SAM) is most active when carried out at alkaline pH's. Phosphatidylmonomethylethanolamine (PMME) and phosphatidyldimethylethanolamine are less effective reactants. The PE present in the microsomal and myelin membrane can serve as an acceptor in this reaction. Thin layer chromatography indicates the formation of the expected products.

The enzyme system which catalyzes the methylation of phosphatidylethanolamine (PE) to phosphatidylcholine (PC), termed phospholipid-N-methyltransferases (PMT) was initially described with rat liver microsomes (1). This activity is less than 1% in non-hepatic tissues of that observed with hepatic microsomes (2). It has been proposed that the stepwise transfer of three methyl groups to PE producing PC is directly involved in signal transmission mediated by surface receptors (3) and as mediators of several other membrane functions (4). However, this functional role has been controversial and this proposal that the methylation of PE could account for these physiological responses has been challenged (5,6).

We have recently observed a significant incorporation of [^{3}H] SAM into methylated lipids of rat brain microsomes that had been kept at 100° C for 30 minutes and subsequently incubated either in the absence or presence of varying PE concentrations. This communication provides data about the nonenzymatic methylation of PE with S-adenosyl-L-methionine as the methyl donor.

Abbreviations: MES, 2[N-Morpholino] ethanesulfonic acid;
HEPES, N-2-Hydroxyethylpiperazine N'-2-ethanesulfonic acid;
CAPS, cyclohexylaminopropane sulfonic acid;
SAM, S-adenosyl methionine

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MATERIALS AND METHODS

S-Adenosyl-L-methionine, [methyl-^{3}H] SAM (15 Ci/nmol) was purchased from New England Nuclear, Mass., U.S.A. Phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PMME) and phosphatidyldimethylethanolamine (PDME) were obtained from GIBCO. Most other biochemicals were purchased from Sigma Co., St. Louis, Mo., U.S.A. The remaining reagents were of analytical 1 1 Rat brain microsomes were prepared as described earlier (7). Purified myelin was obtained according to the procedure of Ledeen (8). Lipids were extracted by the method of Folch, et al. (9) and the PE content of myelin and microsomes was estimated by determining the phosphorus content (10) after separation by thin layer chromatography employing chloroform-methanol-acetone-acetic acid-water (50:15:15:10:5 v/v).

The experimental procedures are similar to those described previously utilized for studies on the enzyme catalyzed reactions (11,12,13). The standard incubation mixture for the nonenzymatic methylation is a slight modification of the earlier procedures (11,12,13). Either PMME or PDME (25 mg) was suspended in 2.5 ml of 10mM N-2-hydroxy ethylpiperazine-N-2-ethanesulfonic acid (HEPES) and 1 mM EDTA, pH 8.0, sonicated for 5 min. to obtain an homogeneous micellar dispersion and then stored at 4°C for not more than 7 days. The reaction mixtures contained 100 mM CAPS buffer, pH 11.0, 0 to 200 nmoles of [^{3}H] SAM and where indicated different concentrations of the phospholipid acceptors were added in a total volume of 0.2 ml and then processed essentially as described earlier (12,13). Details of each experiment are described in the legends of respective figures. Aliquots of the final chloroform phase were removed for radioactivity determination by liquid scintillation spectrometry. The remaining sample was applied to a TLC plate with appropriate phospholipid standards and developed employing a solvent system of n-propanol-proprionic acid -chloroform -water (3:2:2:1) (14). Iodine vapour was employed to visualize standards and the components present in each sample. The plates were sprayed with ENHANCE (N.E.N) Boston) and exposed to Fuji x-ray film to locate the labeled products.

RESULTS

pH

A single pH optima of 11 was obtained (Fig. 1) with incubations containing 330 nmoles of PE, 40 nmoles of S-adenosyl-L-[methyl-^{3}H] methionine and buffers at various pH's ranging from 5 to 12 in the absence of protein.

Acceptor Specificity

The effect of varying the concentration of PE or PMME or PDME on the incorporation of [^{3}H] methyl groups was examined and the results are presented in Fig. 2. PE was chemically methylated to a significantly greater extent compared to either PMME or PDME at all the concentrations tested. Increased methylation was obtained with increasing concentrations of both PE and PMME acceptors. The equilibrium constants calculated for the reaction with PE, PMME and PDME were 1.5×10^{-6}, 2×10^{-7} and 1.5×10^{-8} respectively.

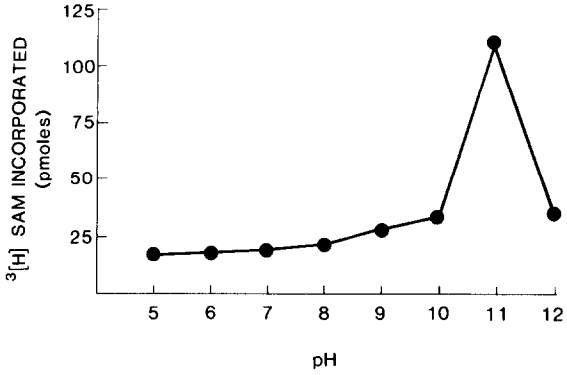

Fig. 1. Effects of varying pH on [^{3}H] SAM incorporation into methylated lipids. Assays were carried out as described under "Materials and Methods" except that buffers of different pH were used in the absence or presence of 330 nmoles exogenous PE (●—●) incubated with 40 nmoles of [^{3}H] SAM (250 nCi/nmole) in a total volume of 0.2 ml. The buffers used were: 100 mM MES at pH 5.0 and 6.0: 100 mM HEPES at pH 7.0 and 8.0: 100 mM sodium hydroxide-glycylglycine at pH 9.0 and 10.0 and 100 mM CAPS buffer at pH 11.0 and 12.0.

Effect of varying SAM concentrations

The extent of chemical methylation as a function of varying SAM concentration from 0.2 to 200 nmoles was determined in the presence of the three phospholipid acceptors (Fig. 3). A linear increase in amount of radioactivity incorporated with increasing SAM concentrations was observed with both PE and PMME. The chemically methylated lipids were separated by TLC plates, autoradiograms were prepared and the radioactivity associated with various areas were quantitated. Under these experimental conditions, in addition to the formation of three expected methylated products PC, PDME,

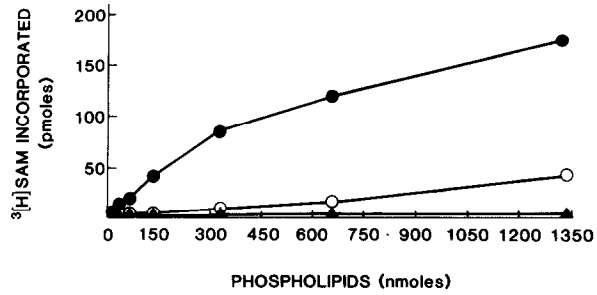

Fig. 2. Effect of varying exogenous PE (●—●) or PMME (○—○) or PDME (▲—▲) concentration on [^{3}H] SAM incorporation into methylated lipids. Assays were carried out in the presence of 40 nmoles [^{3}H] SAM (250 nCi/nmole), 100 mM CAPS, pH 11.0 in the presence of different concentrations of exogenous phospholipids from 0 to 1330 nmoles in a total volume of 0.2 ml.

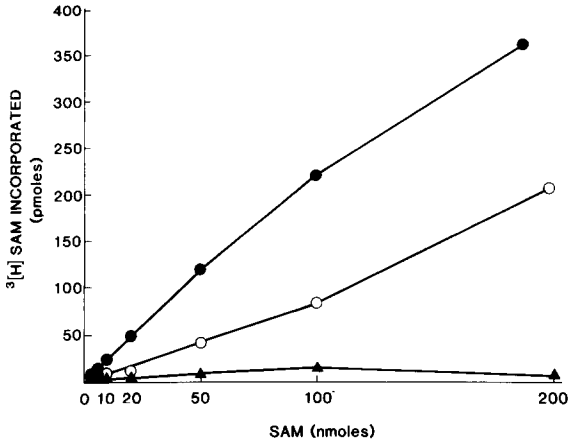

Fig. 3. Effects of varying [^{3}H] SAM concentrations on incorporation of radioactivity into methylated lipids. Assays were carried out under standard conditions as described in "Materials and Methods" with different concentrations of [^{3}H] SAM from 0.2 to 200 nmoles in the presence of 330 nmoles of exogenous PE (●—●) or PMME (○—○) or PDME (▲—▲) in a total volume of 0.2 ml. The specific radioactivity of [^{3}H] SAM was adjusted to 10 uCi/nmole between 0.2 and 2 nmoles; 2 uCi/nmole between 5 and 20 nmoles, 200 nCi/nmole between 50 and 200 nmoles. The buffer used was 100 mM CAPS, pH 11.0.

PMME, significant radioactivity was present at the solvent front and with areas corresponding with standards of sphingomyelin and lysophosphatidylcholine.

Chemical methylations of heated myelin and microsomes

Myelin and microsomes were maintained in a boiling water bath for 30 minutes and various aliquots were incubated with 200 nmoles of [^{3}H] SAM to measure the chemical methylation and the results are shown in Fig. 4. The PE content of the microsomes and myelin were determined to be 180 nmole /mg protein and 740 nmole /mg protein respectively employing standard procedures described in Materials and Methods. An increase was seen in the chemical methylation with an increase in the PE present in the increasing quantity of both heated myelin and microsomes added. Myelin yielded a slightly higher methylation than the microsomes. There was no significant difference in radioactivity incorporation between equivalent amounts of heated and untreated microsomes after the incubation with [^{3}H] SAM.

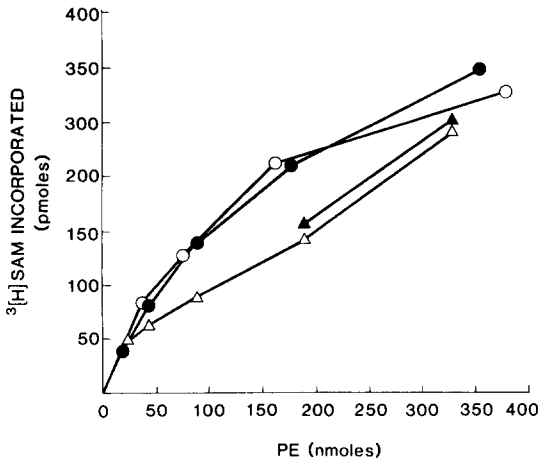

Fig. 4. Incorporation of [^{3}H] SAM into methylated lipids in the presence of PE (●—●) or boiled myelin (○—○) or boiled microsomes (△—△) or untreated microsomes (▲—▲). Assays were carried out with 100 mM CAPS buffer pH 11.0 in the presence of 200 nmoles of [^{3}H] SAM (70 nCi/nmole) in a total volume of 0.2 ml. The various concentrations of PE used were between 0 to 3330 nmoles, or aliquots of membranes containing an equivalent amount of PE.

DISCUSSION

S-Adenosylmethionine (SAM) is the methyl donor for many transmethylation reactions of various compounds including porphyrins, fatty acids, phospholipids, polysaccharides, proteins, nucleic acids and biogenic amines (15). SAM has been reported to chemically methylate both DNA and proteins (16, 17). The chemical methylation of DNA produced the same putative promutagenic and procarcinogenic lesions as was formed by carcinogenic chemical methylating agents. Methylation of DNA was more pronounced in acidic environment at pH 5.0. The increased stability of SAM at lower pH (18) was not considered responsible for this increase in chemical methylation (16). The chemical methylation of proteins exhibited two pH optima, one at pH 6.3 and the other at pH 12.3. Egg white globulin and other commercially available proteins were found to be better acceptors for radioactive SAM at pH 12.5 than at pH 6.5 (17). The present results strongly suggest a chemical methylation of PE occurs at an alkaline pH.

PE is a major phospholipid in all the biomembranes and our results indicated that this membranebound form can be chemically methylated either in myelin or microsomal membranes as effectively as free PE (Fig. 4). Employing

the conditions of incubation at pH 8.0 as described by Crews et al. (19), chemical methylation of the brain P_3 fraction represented about 65% of the total and enzymatic methylation about 35%. Chemical methylation represented 85% of the total methylation with a P_2 fraction under these conditions. Therefore, the possibility exists that some of the previous observations using a variety of cells or membranes incubated with [methyl-^{3}H] methionine or SAM might reflect the continuation of both chemical and enzymatic methylation.

ACKNOWLEDGEMENT

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