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Phosphatidylethanolamine-N-methyltransferase in Rat Brain: Subcellular Distribution and High Specific Activity in Nuclear Envelopes

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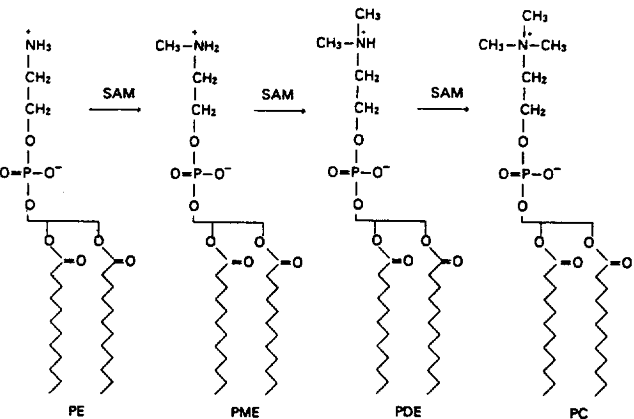
Phosphatidylethanolamine N-methyltransferase (PMT) is a membrane bound enzyme which catalyzes the methylation of phosphatidylethanolamine (PE) to phosphatidyl-choline (PC) by way of phosphatidyl-N-monomethylethanolamine (PME) and phosphatidyl-N,N-dimethylethanolamine (PDE) with S-adenosylmethionine (SAM) serving as the methyl donor. the presence and characteristics of this enzyme have been reported in various sources such as rat brain,[1,2] rat hepatocytes,[3] bovine adrenal medulla,[4] and yeast.[5] The physiological role of this enzyme has been studied in many ways.[6-8] It is thought that this enzyme plays a role related to signal transmission.[6] The sequential methylation reactions increase membrane fluidity,[7] which aids the coupling of receptor, transducer, and amplifier. For example, when D-isoproterenol binds to the β-adrenergic receptor, PMT is stimulated and



subsequent increase of membrane fluidity facilitates the coupling of β-receptor and adenylate cyclase.[8] In view of paramount importance of PMT as a possible modulator for certain biological signal transduction, many reports relating various membrane phenomena to the phospholipid methylation in brain tissue have been forwarded, inc'ıding effects of aldosterone,[9] nerve growth factor,[10] histamine,[11] and protein kinase C.[12] As part of our effort for studying signal transduction system, we are also investigating enzymological aspect of PMT in rat brain tissue.

Initially Crews *et al.*[1] described the presence of phospholipid methylating enzymes in the brain. They identified two methyltransferases, PMT I and PMT II. The first enzyme (PMT I) was found to methylate PE to form PME and to have a high affinity to the methyl donor SAM. The second enzyme (PMT II) methylated PME twice to form PC and had a low affinity to SAM. They also reported that the most of methyltransferase activity in the brain was localized in particulate fraction, especially highly localized in synapatosomal plasma membranes. However, when the total activity recovered in each particulated subfraction was compared, nuclear fraction (P_1) had almost one half of the whole homogenate, meanwhile crude mitochondrial fraction (P_2) which contained synaptosomes had only a one-third and microsomal fraction (P_3) had less than a one-fifth of the total activity. In view of this report of a large activity found in P_1 fraction, we looked for the PMT activity in the nuclear subfraction in detail. We now present a revised subcellular distribution of PMT activity in rat brain, in which the highest specific activity is localized in nuclear envelopes. Recently we published a report on the characterization of PMT activity in rat brain myelin.[2]

Upon reinvestigating the subcellular distribution of PMT, we reoptimized the assay condition of PMT in nuclear fraction extensively. The methylation of phospholipid was measured by the incorporation of radioactive methyl group from SAM into lipids extracted by chloroform-methanol. Preliminary experiment revealed, however, that added exogenous substrates improved markedly the incorporation of methyl group into the products. Therefore the standard assay condition included exogenous substrate PME and PDE. The incubation medium was consisted of 100 μl of solution containing 10 mM carbonate buffer (pH 10.0), 10 mM MgCl, 100 μg of PME, 100 μg of PDE, 0.2-0.4 mg protein of nuclear suspension, and 100 μM of [H-methyl]-SAM (specific activity, 300 μCi/μmol). After maintaining uniform lipid environment

Table 1. Subcellular Distribution of PMT Activiies in Rat Brain

Fraction	Protein amount (mg)[a]	PME			PDE			PC		
		Total[b]	%[c]	Specific[d]	Total	%	Specific	Total	%	Specific
H_1	162.8	391.7	100	2.41	135.6	100	0.83	681.7	100	4.19
P_1	20.3	99.1	25	4.88	40.5	30	1.99	217.5	32	10.71
P_2	66.1	118.1	30	1.79	29.4	22	0.44	222.6	33	3.37
P_3	20.6	84.5	22	4.11	16.0	12	0.78	197.8	29	9.62
S_3	41.9	22.4	6	0.54	70.1	51	1.67	91.8	14	2.19

[a] Total amount of protein in each fraction from 1 rat brain of 1.5 g. [b] Total activity (CH_3-incorporation, pmol/hr). [c] Percent activity, the proportion to the activity of whole homogenate. [d] Specific activity (CH_3-incorporation, pmol/mg protein/hr).

Table 2. Distribution of PMT Activities in Neuronal Nuclear Subfraction

Fraction	PME[a]	PDE[a]	PC[a]
N1	6.0	20.6	14.1
N2	5.1	22.7	19.3
N1 envelopes	8.6	102.7	63.4

[a] Specific activity (CH_3-incorporation, pmol/mg protein/hr).

in assay system, the nuclear activity of PMT could be divided into three types instead of two enzymes previously reported.[1,2] That is each methylation step was assayed separately and represented by different types (activites) of enzymes, type I for PME formation, type II for PDE, and type III for PC formation, the obseved K_m values of type I, II, and III were 15.1, 14.9, and 49.0 μM, and the maximum velocities were 7.7, 21.1, and 18.0 pmol/mg protein/hr, respectively.

In order to examine the distribution of PMTs in the subcellular fractions of rat brain, whole homogenate (H_1) was fractionated into nuclear pellet (P_1), crude mitochondrial pellet (P_2), microsome (P_3), and cytosolic supernatant (S_3) as described by Wittaker.[13] The PMT activities were assayed in each fraction and the result is summarized in Table 1. Because the lipid environments of PMT in each subcellular fraction were maintained uniformly as much as possible by saturating with exogenous phospholipids, it could be assume that full activities of PMTs were expressed in each fraction. Therefore the possible influences on PMT activities due to the different lipid environments can be excluded and the specific activities calculated here could be regarded to be proportional to the protein amounts of PMTs among the total protein in each fraction. The results revealed that most of the three PMT activities were rather localized in pellet fractions except PDE synthesizing activity which showed 50% of total activity in S_3 fraction (Table 1). Each of three specific activity was the highest in P_1 among the other fractions and the total activity of P_1 occupied about 30% of whole homogenate activity.

The neuronal nuclear pellet P_1 was further fractionated according to the procedure of Thompson.[14] The pellet was resuspended in 3 m*l* of 2.4 M sucrose and 1 m*l* of 1.8 M sucrose was overlaid. After 30 min centrifugation at 85,000 g, there was a thin pellet of nonastrocytic glial nuclei (N_2) at the bottom of the tube, and above this N_2 pellet, neuronal nuclei (N_1) diffused but rather accumulated at the top of the 2.4 M layer. After the 1.8 M layer was removed, 2.4 M layer was decanted and diluted by mixing with two volumes of 0.32 M sucrose. Additionally nuclear envelopes was isolated by treating the N_1 fraction wtih DNase and centrifusing discontinuous gradient of 0.25 M/2.2 M sucrose layers. Comparison of PMT activities in the isolated N_1, N_2, and nuclear envelopes showed that PMT was highly localized in nuclear envelopes (Table 2). N_1 and N_2 had similar specific activities while the N_1 envelopes contained about 5 fold higher PDE and 3 to 4 fold higher PC synthesizing activity than those of N_1 or N_2. However in the case of PME synthesizing activity, the localization in the envelopes was not so different from the other fractions. The difference was around 30% among the nuclear subfractions. These data provides another evidence that there could be three types of PMT activities present in N_1 fraction. If PMT were a single enzyme, the distribution or the degree of localization should be similar for all the three activities.

In this study, only one third of total activity of PMT is recovered from P_1 nuclear fraction. This recovered activity is much less than the previous report of one half recovery.[1,2] However the current result is more reliable since the reoptimized assay condition maintained uniform lipid environment for each of the subcellular fractions. The one third of total activity observed in nuclear fraction means that the phospholipid-methylation could play a significant role in neurobiological process involving nuclei. Particularly, the highest specific activity found in nuclear envelopes (3 times over the synaptosomal plasma membranes) implies that the PMT might be connected to a certain regulation of gene expression. Therefore it is desirable to investigate further the molecular properties of PMTs in nuclear fraction and the factors governing their activities.

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Samarium(II) Iodide Catalyzed Addition of CCl_3Br and CBr_4 to Olefinic Compounds: Synthesis of the Key Intermediates for Synthetic Pyrethroids

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The addition reaction of polyhaloalkanes to olefins is catalyzed by free-radical initiators such as dibenzoyl peroxide or azoisobutyronitrile (AIBN).[1] A range of transition metal (*e.g.*, Cu, Fe, Co, V, Pd, Cr, Ru, and Re) complexs and salts has also been found to act as catalysts for these addition reactions.[2] Recently, samarium(II) iodide is known to be a useful reducing reagent. Its synthetic utility has been studied [3] and found to be an effective initiator in the addition of fluoroalkyl iodide to terminal alkenes and alkynes.[4] As the regioselective addition of polyhaloalkanes to terminal olefins is of interest in wide synthetic application, we describe here the samarium(II) iodide catalyzed regioselective addition of bromotrichloromethane and tetrabromomethane to olefinic compounds.

We have investigated the reaction of several termainal olefins with CCl_3Br and CBr_4 by using SmI_2 as a catalyst (0.1 equiv) in dimethylformide at 80-90℃. The results are summarized in Table 1. The terminal olefins **1a-c** were treated with bromotrichloromethane and tetrabromomethane in DMF at 80℃ or 90℃ to produce the adducts **2a-c** or **3a-c**, respectively (Entries 1-3). The adducts **2d** and **3d** (Entry 4) thus obtained by this method are the key intermediates in the synthesis of synthetic pyrethroids, such as cypermethrin and decamethrin.[5] In the case of terminal 1,6-dienes, initial formation of radical followed by cyclization by 5-*exo-trig* mode afforded the cyclized products **4e-g** and **5e-f** (Entry 5-7). The reaction of diallyl ether (**1e**) with CCl_3Br and CBr_4 gave the tetrahydrofuran (**4e** and **5e**) with low *cis* (1 : 1) selectivties (Entry 5). In the addition of CCl_3Br and CBr_4 to 1,6-heptadiene (**1f**), the regioselective radical cyclization proceeded more stereoselectively for *cis*-isomers (2 : 1) (Entry 6). The addition reaction of **1f** with CBr_4 afforded cyclopentane (**5f**) and the simple addition product as the minor and major product, respectively. This could imply that the simple addition and cyclization are competing in the radical chain propagating step (Entry 6). On the other hand, the cyclization reaction of the disubstituted dienes (**1g**) with CCl_3Br and CBr_4 occurred without the monoadducts with higher *cis*-stereose-

Table 1. Samarium (II) Iodide Catalyzed Addition of Bromotrichloromethane and Tetrabromomethane to Olefinic Compounds[a]

Entry	Substrate	Haloalkanes	Product[b]	Reaction Temp (℃)	Conditions[6] Temp (℃)	Isolated Yield[c] (%)
1			X_3C, Br			
	1a	CCl_3Br	2a X=Cl	90	16	74
		CBr_4	3a X=Br	80	10	73
2	OAc		X_3C, Br, OAc			
	1b	CCl_3Br	2b X=Cl	90	24	71
		CBr_4	3b X=Br	80	24	60
3	OAc		Br, X_3C, OAc			
	1c	CCl_3Br	2c X=Cl	90	24	81
		CBr_4	3c X=Br	90	14	72
4	CO_2Et		X_3C, CO_2Et, Br			
	1d[5]	CCl_3Br	2d[5] X=Cl	90	12	65
		CBr_4	3d[5] X=Br	80	24	53