BIOPHYSICS AND BIOCHEMISTRY

Participation of Methylcobalamin in *In Vitro* DNA Methylation

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Two DNA-methylases from nuclei of rat liver are compared for their ability to methylate thymic DNA in the presence of methyl group donors, radiolabeled ^{3}H-S-adenosyl-L-methionine and methylcobalamin. DNA-methylase with an isoelectric point of 5.2 is found, which uses both donors of methyl groups although its activity is more expressed with ^{3}H-S-adenosyl-L-methionine. ^{3}H-S-adenosyl-L-methionine and methylcobalamin presumably interact with different sites of the enzyme.

Key Words: *DNA; DNA-methylase; 5-methylcytosine; S-adenosyl-L-methionine; methylcobalamin; vitamin B_{12}; donors of methyl groups*

It is now thought that the level of cytosine methylation in eucaryotic DNA is a factor regulating gene activity [1]. Methylation of DNA is catalyzed by two DNA-methylases, or (DNA-cytosine-5)-methyltransferases, with the participation of S-adenosyl-L-methionine (SAM). SAM is considered to be a universal donor of methyl groups for DNA methylation [8]. The methyl residue in SAM originates *in vivo* from methylcobalamin (MC) [3], which is produced in the organism from vitamin B_{12} [12]. The participation of MC in *in vivo* DNA methylation, where MC acts as a precursor of methyl groups for SAM [3], may be conceived as follows:

$$\text{Homocysteine} \xrightarrow[\textit{homocysteine methyltransferase}]{+MC} \text{Methionine} \xrightarrow[\textit{methionin adenosyltransferase}]{+ATP}$$

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$$\text{Adenosylmethionine} \xrightarrow[\textit{DNA-methyltransferase}]{+DNA} \text{5-Methylcytosine-DNA} \rightarrow$$

At the same time, the possibility of direct methylation of DNA has been postulated [2,11]:

$$\text{DNA} \xrightarrow[\textit{DNA-methyltransferase}]{+MC} \text{5-Methylcytosine-DNA}$$

To verify the possibility of direct methylation of DNA by MC we compared preparations of DNA-methylases from nuclei of rat liver for their ability to methylate DNA *in vitro* in the presence of two ^{3}H-labeled donors of methyl groups, SAM and MC.

MATERIALS AND METHODS

Acceptor DNAs from calf thymus and *Micrococcus luteus* (Serva) were used in the experiments. Column isoelectric focusing (LKB) of proteins was performed with ampholytes with pH ranging from

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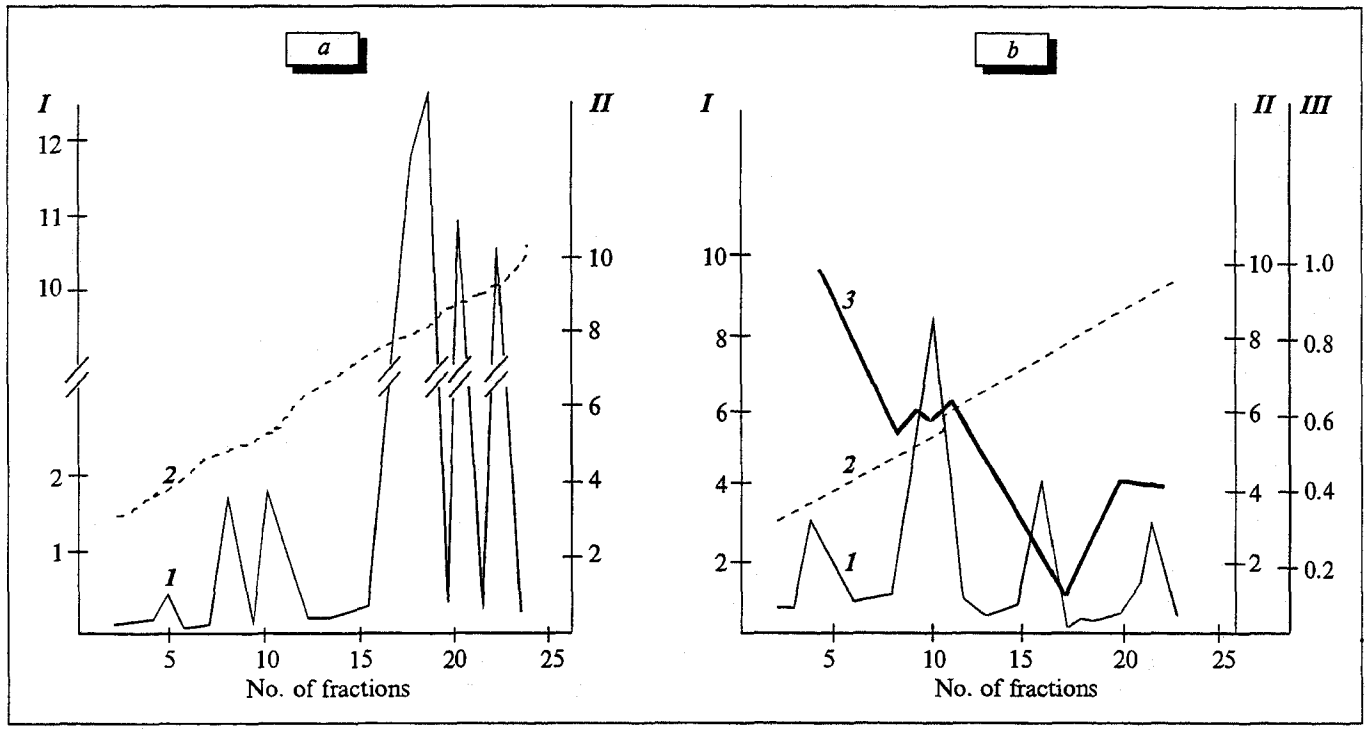

Fig. 1. Isoelectric focusing of a preparation of DNA–methylases from rat liver. Donor of methyl residue is ^{3}H–SAM (*a*) and ^{3}H–MC (*b*). Ordinate: *I*) radioactivity of substrate, cpm×10^3 (*a*) and cpm×10^2 (*b*); *II*) pH; *III*) protein concentration, mg/ml. *1*) methylase activity; *2*) pH gradient; *3*) protein concentration.

3.5 to 10. Donors of methyl groups were ^{3}H-labeled SAM (specific activity 15 Ci/mmol, Amersham) and MC (specific activity 0.1 Ci/mmol) synthesized from oxycobalamin and ^{3}H-CH_3I as described previously [5] with a modification. DNA-methylases were isolated from the nuclei of rat liver isolated as described earlier [7]. Total preparations of DNA-methylases isolated from nuclear extracts by salting out with 0.8 N ammonium sulfate [6] were then fractionated by isoelectric focusing with ampholytes [10]. Methylase activity was measured as described elsewhere [4]. The sample contained either 40 μl ^{3}H-MC or 20 μl ^{3}H-SAM (specific activity of the working solutions was 0.1 Ci/mmol and 0.2 Ci/mmol, respectively). Since ^{3}H-MC is a photosensitive preparation, all manipulations were carried out in the dark or under red illumination [11]. The radioactivity of thymic DNA in cpm/g protein was taken as the unit of DNA-methylase activity. The concentration of protein was determined after Bradford [9].

RESULTS

In the first stage, we had to verify the presence of DNA-methylase activity in the total preparation from the nuclear extracts of rat liver using both ^{3}H-SAM and ^{3}H-MC. The data in Table 1 indicate that DNA-methylases from the total protein fraction are able to methylate thymic DNA *in vitro* in the presence of both labeled donors of methyl groups.

In the second stage, the total preparation of DNA-methylases was fractionated by isoelectric focusing with ampholytes allowing for separation of DNA-methylases with close isoelectric point values [4,10].

Using isoelectic focusing we hoped to identify DNA-methylase fractions differing in their ability to use SAM and MC. When SAM was used as the donor of methyl groups, the profile of DNA-methylase activity consisted of 6 peaks with different isoelectric points: 3.7, 4.8, 5.2, 7.6, 8.3, and 8.8. The maximal activity is concentrated in the alka-

TABLE 1. Level of *in Vitro* Methylation of Thymic DNA by ^{3}H–MC and ^{3}H–SAM, cpm/mg Protein

Source of DNA-methylases	Donors of Methyl Groups	
	^{3}H-MC	^{3}H-SAM
Total preparation from nuclei of rat liver	12,321	29,821
Enzyme after isoelectric focusing (pI 5.2)	25,938	56,250

TABLE 2. Effect of the Second Donor of Methyl Groups on Enzymatic *in Vitro* DNA Methylation

Added components, concentration	Level of DNA methylation, % of initial value			
	^{3}H-SAM		^{3}H-MC	
	thymic	*M. luteus*	thymic	*M. luteus*
MC				
0.005 μM	116	131	–	–
0.05 μM	138	129	–	–
SAM, 1 mM	–	–	104	218
DL–methionine, 0.02 μM	0	0	95	141

line range. The phenomenon of plurality of eucaryotic DNA-methylases has been described earlier when total preparations of methylases from human and animal tissue were fractionated by isoelectric focusing [4,6]. The profile of methylase activity in the presence of ^{3}H-MC is characterized by a lesser number of methylase peaks and a lower activity (Fig. 1, *b*). There was only one distinct peak with maximal activity in the neutral range (isoelectric point 5.2).

The experiments reveal that ^{3}H-MC may act as the donor of methyl groups in *in vitro* DNA methylation. Both a total preparation of DNA-methylases and the individual enzymes may use SAM and MC as the donors of methyl groups, although methylase activity is more expressed with SAM.

DNA-methylase is known to use donors of methyl groups differing in their chemical structure. Under standard conditions it exhibits different activity with SAM and with MC, and presumably the donors interact with different sites and possess unequal affinity to the enzyme. To confirm this assumption we carried out methylation of DNA with ^{3}H-SAM and ^{3}H-MC in the presence of another donor, unlabeled MC and SAM, respectively. Given the fact that two types of DNA-methylases exist in the eucaryotic cell, we identified *de novo* DNA-methylase and maintenance DNA-methylase using as the substrate unmethylated DNA from *Micrococcus luteus* and thymic DNA, respectively.

Table 2 shows that unlabeled MC in a concentration of 0.05 μM does not prevent methylation of DNA by ^{3}H-SAM and even activates methylation of DNA from *Micrococcus luteus* by 29% and thymic DNA by 38%. Methionine (0.02 μM), a constituent moiety of SAM, completely inhibits methylation of both unmethylated and methylated DNA (Table 2). Unlabeled SAM does not prevent methylation of thymic DNA with ^{3}H-MC and activates methylation of DNA from *Micrococcus luteus* (Table 2). Methionine has a similar effect (Table 2). These results indicate that MC and SAM interact with different sites of the enzyme. Moreover, both MC and SAM may act as agents stimulating the participation of another donor in the reaction.

Our results are in conformity with the data reported earlier [11]. However, in our experiments MC in lower concentrations (0.005 - 0.05 μM) activates both types of methylation (Table 2), whereas in the literature [11] MC in a concentration of 1 μM does not affect the maintenance type of methylation. Unlabeled SAM does not prevent methylation of DNA in the presence of ^{3}H-MC (Table 2). From our findings and the data reported earlier [11] we can deduce that there are two independent paths of *in vitro* DNA methylation governed by SAM and MC, respectively.

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