Preferential binding of DNA methyltransferase and increased de novo methylation of deoxyinosine containing DNA

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Mammalian DNA-cytosine 5-methyltransferases methylate cytosines in deoxyinosine containing DNA polymers more rapidly than in other synthetic or naturally occurring DNAs. The initial methylation rate of poly(dI-dC)·poly(dI-dC) is about 10-times higher than that of poly-(dG-dC)·poly(dG-dC) or of the native *Micrococcus luteus* DNA. In competitive binding experiments, DNA methyltransferase has about 10-fold higher affinity for the dI-containing alternating DNA polymer than for poly(dG-dC)·poly(dG-dC). The observed high methyl accepting capacity of poly(dI-dC)·poly(dI-dC) may be a useful methodological advance to determine de novo DNA methyltransferase activity in extracts of mammalian cells.

DNA methyltransferase *Methylation* *Poly(dI-dC)·poly(dI-dC)*

1. INTRODUCTION

The methylation pattern of mammalian DNA appears to be cell and tissue specific, and clonally stable (review [1–4]). The majority of the more than 10^7 5-methylcytosines (5mC) in the genome is present in 5′-CpG-3′ dinucleotides and the hemimethylated duplexes arising shortly after replication are recognized and methylated by the maintenance activity of DNA methyltransferase. In many systems, an undermethylation of certain DNA sequences correlates with the expression of the gene [1–4].

Changes in the DNA methylation pattern can be introduced either by an impairment of the maintenance DNA methyltransferase activity or by the expression of de novo activity which methylates cytosine residues in unmethylated 5′-CpG-3′ or other dinucleotide sequences.

Interestingly, DNA methylating enzymes isolated from various mammalian tissues and cells are capable of methylating both hemimethylated and unmethylated DNA substrates, suggesting that one enzyme species is capable of performing both maintenance and de novo DNA methyltransferase activities [5,6]. Since the de novo methylation as compared to the maintenance methylation seems to be a rare event during the cell cycle, it is inevitable that mechanism(s) modulating these two types of activities operate within the cell. The nature of such mechanism(s) is unclear as yet.

Here, we report on the de novo methylation of the synthetic polymer poly(dI-dC)·poly(dI-dC), which is about 10-fold better at accepting methyl for mammalian DNA methyltransferases than any other known unmethylated substrate.

2. MATERIALS AND METHODS

2.1. *Materials*

Micrococcus luteus DNA was purchased from Sigma, all synthetic DNA polymers were obtained from PL-Biochemicals.

2.2. *Enzyme purification*

DNA methyltransferases were purified from human placenta and mouse mastocytoma P815 cells by chromatographies on DEAE-cellulose and

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heparin-agarose as described (Fractions IVA and IV, respectively, [7]).

2.3. *DNA methyltransferase assay*

The assay mixture contained in a final volume of 100 μl of 20 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.5 mM dithioerythritol, 6.7% (w/v) glycerol, 10 μM ^{3}H-labeled *S*-adenosyl-L-methionine (1 μCi), 1–8 μg DNA and 5–10 μg enzyme protein. After incubation at 37°C for various time periods, the DNA was isolated for radioactivity counting as described [5].

3. RESULTS

The DNA methylating enzymes used in the present study were isolated from terminal human placenta and from rapidly proliferating mouse mastocytoma P815 cells and have been extensively characterized elsewhere [5–8]. Both enzymes appear strikingly similar in their biochemical properties, i.e. molecular mass, mode of action, and immunological cross-reactivities with monoclonal anti-DNA methyltransferase antibodies. By studying methyl accepting activities of various synthetic DNA polymers, we have observed that both these enzymes methylate the deoxyinosine containing alternating polymer poly(dI-dC)·poly(dI-dC) with up to 10-times higher efficiency than the corresponding deoxyguanosine containing poly(dG-dC)·poly(dG-dC) polymer or the native 70% GC-rich *M. lutes* DNA (fig.1, table 1).

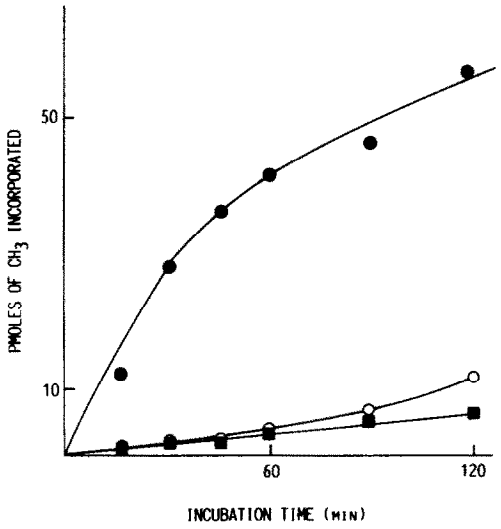

Fig.1. Time course of enzymatic methylation of poly(dG-dC)·poly(dG-dC) (○—○), poly(dI-dC)·poly(dI-dC) (●—●) and native *M. luteus* DNA (■—■). 1 μg of the DNA polymers was incubated with mouse DNA methyltransferase in the standard assay for various time periods.

The product of the enzymatic methylation of poly(dI-dC)·poly(dI-dC) and poly(dG-dC)·poly(dG-dC) was analyzed by thin-layer chromatography. After hydrolysis in formic acid, more than 99% of the radioactivity incorporated into both polymers during the methylation reaction co-chromatographed with 5-methylcytosine (not shown).

The deoxyinosine containing homopolymer poly(dI)·poly(dC), and also poly(dG)·poly(dC), do not serve as methyl-accepting polymers (table 1). This indicates a necessity of alternating cytosine-purine in polydeoxynucleotides for the transmethylation reaction. Deoxyadenosine however does not satisfy these conditions, as the capability of the alternating poly(dA-dC)·poly(dG-dT) polymer to accept methyl groups is less than 0.1% of that of poly(dI-dC)·poly(dI-dC) (table 1).

Mammalian DNA methyltransferases appear to have a high binding affinity for deoxyinosine containing polymers. This was shown in competitive binding experiments, in which the synthetic

Table 1

Enzymatic methylation of various DNA polymers by two different mammalian DNA methyltransferases

Substrate	pmol of methyl incorporated by DNA methyltransferase from	
	Mouse P815 cells	Human placenta
M. luteus DNA	5.39	2.22
Poly(dA-dC)·poly(dG-dT)	0.02	0.02
Poly(dG-dC)·poly(dG-dC)	6.27	1.38
Poly(dI-dC)·poly(dI-dC)	60.92	11.30
Poly(dG)·poly(dC)	0	0
Poly(dI)·poly(dC)	0	0

DNA methyltransferases from human placenta and mouse P815 cells were used to methylate 1 μg of the respective DNA polymer in the standard reaction mixture (see section 2). Incubations were performed at 37°C for 60 min

polymers poly(dI-dC)·poly(dI-dC) and poly(dG-dC)·poly(dG-dC) were mixed in different ratios and were then subjected to methylation by a limited amount of DNA methyltransferase. As demonstrated in fig.2, the half-maximal methylation rate of the polymer mixture was observed at a poly(dG-dC)·poly(dG-dC) to poly(dI-dC)·poly(dI-dC) ratio of 10:1. Because of the processive mode of action of mammalian DNA methyltransferases [5,6,9,10], this may indicate an approx. 10-fold higher initial binding affinity of the DNA methyltransferase for the poly(dI-dC)·poly(dI-dC) polymer. The observed differences are not due to a different length of the synthetic polymers used in these experiments, since both polymers exhibited similar size distribution ranging from 300 to 600 base pairs. The higher affinity for the poly(dI-dC)·poly(dI-dC) polymer is also not due to the appearance of single-stranded regions in this polymer at 37°C and low salt concentration. When the reactions were performed under conditions assuring the complete double-strandedness of the poly(dI-dC)·poly(dI-dC), i.e. 30°C and 100 mM NaCl [11], the DNA methyltransferase exhibited the same preference for the deoxyinosine containing polymer, even though the methylation of poly(dI-dC)·poly(dI-dC) was as strongly reduced at this salt concentration as the methylation of other unmethylated

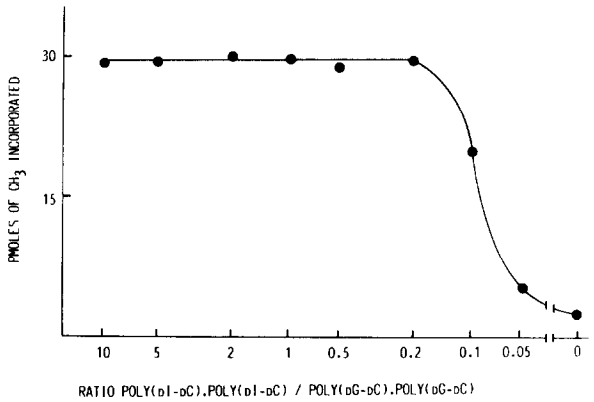

Fig.2. Competition between poly(dI-dC)·poly(dI-dC) and poly(dG-dC)·poly(dG-dC) for mouse DNA methyltransferase. The enzyme was added to preformed mixtures of the two polymers and the methylation reaction was carried out for 30 min. The total amount of polymers was kept constant (8 μg).

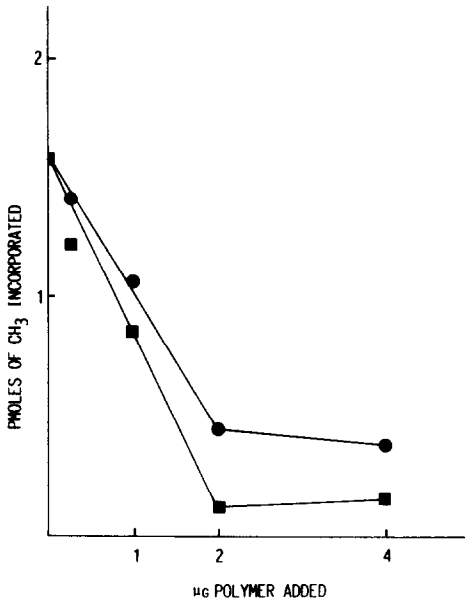

Fig.3. Inhibition of enzymatic methylation of poly(dG-dC)·poly(dG-dC) by poly(dG)·poly(dC) (●—●) and poly(dI)·poly(dC) (■—■). Various amounts of the homopolymers were mixed with 2 μg of poly(dG-dC)·poly(dG-dC). DNA methyltransferase from mouse P815 cells was added and the reaction continued for 60 min.

[6,9,10,12,13] and hemimethylated (unpublished) double-stranded DNA substrates.

The homopolymers poly(dI)·poly(dC) and poly(dG)·poly(dC), which do not serve as methyl accepting polymers (table 1), compete with the methyl accepting polymer poly(dG-dC)·poly(dG-dC) in binding to DNA methyltransferase. As the data in fig.3 show, poly(dI)·poly(dC) is in this respect more effective than poly(dG)·poly(dC). Similar results were obtained, when poly(dI-dC)·poly(dI-dC) was used as the methylatable polymer in competition with these two homopolymers, although the inhibitory effect of poly(dG)·poly(dC) on the methylation of poly(dI-dC)·poly(dI-dC) was weaker than its inhibitory effect on the methylation of poly(dG-dC)·poly(dG-dC) (not shown). The reactions were performed at 30°C in 100 mM NaCl in order to exclude melting of the poly(dI)·poly(dC) polymer [14]. The binding affinities of the DNA methyltransferase for the alternating deoxyinosine containing polymer as compared to the analogous homo-

polymer are quantitatively different (cf. figs 2 and 3). Nevertheless, DNA methyltransferase also appears to interact preferentially with homopolymers containing deoxyinosine.

4. DISCUSSION

In a series of experiments with DNA methylating enzymes from human and mouse cells, we have observed a dramatic increase of de novo methylation of synthetic polymers containing deoxyinosine. The efficiency of mouse DNA methyltransferase to methylate DNA polymers containing cytosine-purine alternating sequences increases according to a ratio of about 1:300:3000 for the purine bases adenine:guanine:hypoxanthine (table 1).

The poly(dI-dC)·poly(dI-dC) polymer may be a useful substrate to determine de novo DNA methyltransferase activities in crude cellular extracts and in the course of enzyme purification.

It is unknown, whether the observed unusual high methyl accepting activity of poly(dI-dC)·poly(dI-dC) has a biological significance. In the DNA, deoxyinosine residues may arise due to deamination of deoxyadenosine; most of them, however, are recognized and removed by specific *N*-glycosidases [15]. Prior to elimination by the repair enzyme, deoxyinosines per se may lead to the enzymatic methylation of neighbouring deoxycytosines and a change of preexisting methylation patterns may be introduced.

A possible explanation for the high methyl accepting activity of poly(dI-dC)·poly(dI-dC) may be the particular helical structure of this polymer, which has been a subject of controversy. It was proposed to be a left-handed helix [16,17]; however, analysis of far UV circular dichroism spectra [18] and two-dimensional nuclear Overhauser effects [19] indicated a right-handed helical structure, resembling that of other B-DNAs. If the conformation of poly(dI-dC)·poly(dI-dC) is indeed different from that of normal B-DNA, it is possible that in the cell certain DNA segments transiently adopt a three-dimensional structure similar to that of poly(dI-dC)·poly(dI-dC) and that this leads to a de novo methylation event.

Similar results have been recently reported by Pedrali-Noy and Weissbach [20].

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REFERENCES

[1] Bird, A.P. (1984) Nature 307, 503–504.
[2] Doerfler, W. (1983) Annu. Rev. Biochem. 52, 93–124.
[3] Jaenisch, R. and Jähner, D. (1984) Biochim. Biophys. Acta 782, 1–9.
[4] Razin, A. and Szyf, M. (1984) Biochim. Biophys. Acta 782, 331–342.
[5] Pfeiffer, G.P., Grünwald, S., Boehm, T.L.J. and Drahovsky, D. (1983) Biochim. Biophys. Acta 740, 323–330.
[6] Grünwald, S. and Drahovsky, D. (1984) Int. J. Biochem. 16, 883–888.
[7] Pfeifer, G.P., Grünwald, S., Palitti, F., Kaul, S., Boehm, T.L.J., Hirth, H.P. and Drahovsky, D. (1985) J. Biol. Chem. 260, 13787–13793.
[8] Pfeifer, G.P., Spies, E., Grünwald, S., Boehm, T.L.J. and Drahovsky, D. (1985) EMBO J. 4, 2879–2884.
[9] Drahovsky, D. and Morris, N.R. (1971) J. Mol. Biol. 57, 475–489.
[10] Simon, D., Grunert, F., Von Acken, U., Doering, H.P. and Kröger, H. (1978) Nucleic Acids Res. 5, 2154–2167.
[11] Wells, R.D., Larson, J.E. and Grant, R.C. (1970) J. Mol. Biol. 54, 465–497.
[12] Drahovsky, D. and Morris, N.R. (1971) J. Mol. Biol. 61, 343–356.
[13] Turnbull, J.F. and Adams, R.L.P. (1976) Nucleic Acids Res. 3, 677–695.
[14] Inman, R.B. and Baldwin, R.L. (1964) J. Mol. Biol. 8, 452–469.
[15] Karran, P. and Lindahl, T. (1980) Biochemistry 19, 6005–6011.
[16] Mitsui, Y., Langridge, R., Shortle, B.E., Cantor, C., Grant, R.C., Kodama, M. and Wells, R.D. (1970) Nature 228, 1166–1169.
[17] Drew, H. and Dickerson, R. (1982) EMBO J. 1, 663–667.
[18] Sutherland, J. and Griffin, K. (1983) Biopolymers 22, 1445–1448.
[19] Mirau, P.A. and Kearns, D.R. (1984) Biochemistry 23, 5439–5446.
[20] Pedrali-Noy, G. and Weissbach, A. (1986) J. Biol. Chem. 261, 7600–7602.