MULTIPLE FORMS OF DNA-METHYLASES FROM HEPATIC NUCLEI OF ANIMALS IN HEALTH AND DISEASE

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The level of enzymic methylation of cytosine in DNA of eukaryotes is considered to be one mechanism responsible for regulating gene activity [3]. It is now generally accepted that eukaryotic DNA-methylases (DNA-MTL) perform two types of methylation: de novo (replicative) and maintenance (postreplicative) [3, 9]. Meanwhile there is much evidence in the literature of the existence of multiple forms of DNA-MTL, differing in molecular mass [8, 14], and physicochemical properties [11, 14], in eukaryotic cells, and in some cases, depending on the physiological status of the cell, the ratio between the different forms of methylases may vary [2, 8]. Thus the study of the profile of DNA-MTL, of both replicative (I) and postreplicative (II) type, may serve as one approach to the study of the character of changes in the genome connected with expression of individual genes under normal conditions and in various types of pathology.

The aim of this investigation was to analyze the profile of DNA-MTL from rat and chicken liver nuclei from normal animals and animals with hyperthyroidism and virus transformation.

EXPERIMENTAL METHOD

The experimental material consisted of hepatic nuclei from adult rats, both intact and with mild hyperthyroidism [5], and hepatic nuclei from 7-day-old chicks, either healthy or infected with hepatoma virus MS-29 [5]. Nuclei were obtained by the method in [7] in the modification [1]. Free nuclear DNA was isolated [12] with additional treatment by pronase (100 μg/ml, "Serva," West Germany). Nonmethylated acceptor DNA of phage S_d was obtained by treatment of a concentrated purified suspension of phage S_d particles twice with phenol, saturated with 0.1 M Tris-HCl, pH 8.0 [6]. Pooled preparations of DNA-MTL obtained from nuclear extracts in accordance with [2] were analyzed by hydrophobic column chromatography on phenyl-sepharose. All solutions for chromatography were made up in buffer consisting of 20 mM Tris-HCl, 1 mM dithiothreitol (pH 7.5, buffer A). The ammonium sulfate (AS) fraction of DNA-MTL was dissolved in buffer A with 1 M $(NH_4)_2SO_4$ (buffer B) and applied at the rate of 5 ml/h to a column (0.8 × 15 cm) with phenyl-sepharose ("Pharmacia," Sweden), equilibrated with buffer B. The column was washed with buffer B, the yield of material absorbing at 280 nm being monitored down to the minimal quantity. Proteins were elated from the phenyl-sepharose by a linear or stepwise combined double descending gradient of $(NH_4)_2SO_4$ from 1 M to 0 and an increasing glycerol concentration from 0 to 60%. In some cases Triton X-100 was used instead of glycerol. At the end of the gradient additional elution was carried out with 5-6 volumes of 60% glycerol. The rate of elution was 5 ml/h and the volume of each fraction 5 ml. DNA-MTL activity was determined in the fractions thus obtained. The DNA methylation reaction was carried out as in [13]. The 5-methylcytosine concentration was determined in the standard way [4]. S-adenosine-L-methionine (^{3}H-SAM), with specific activity of 15 Ci/mmole ("Amersham," England) was used as donor of methyl groups.

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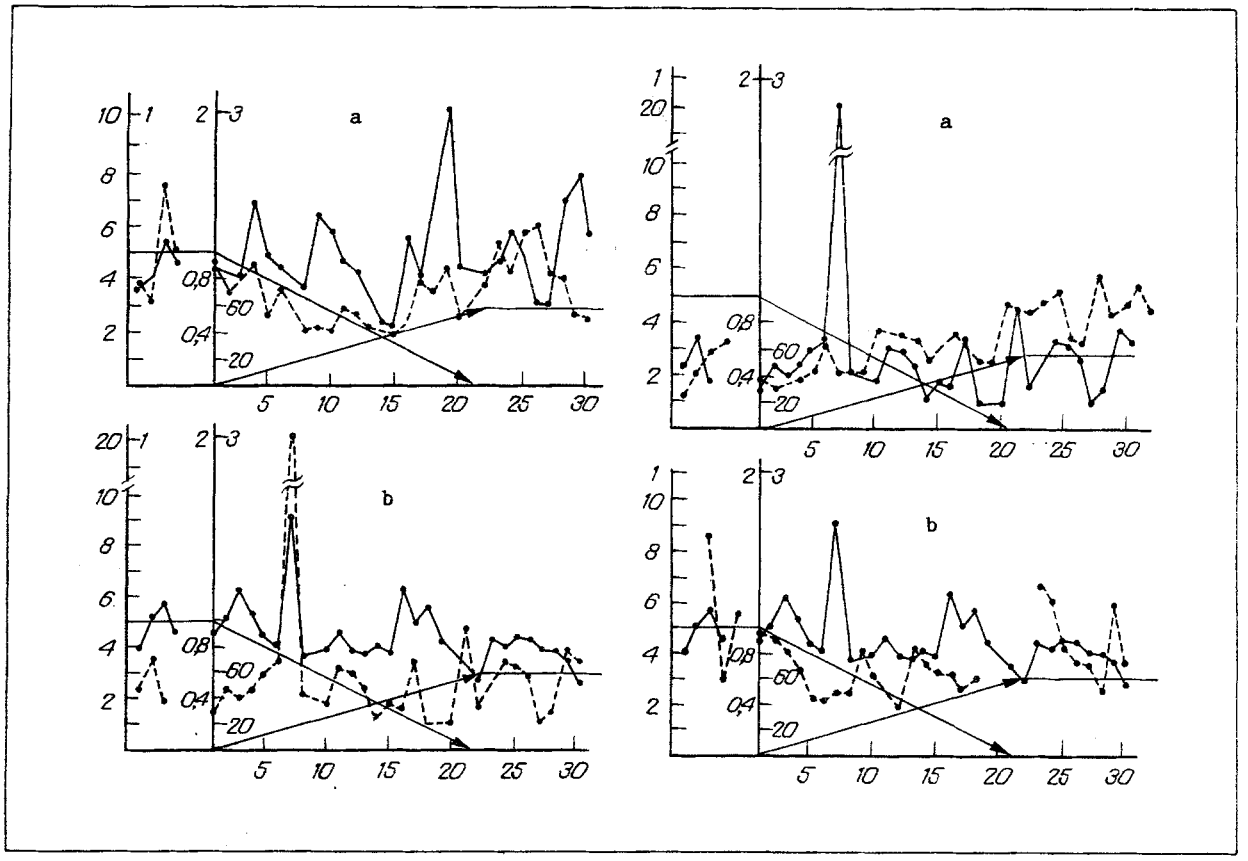

Fig. 1 Fig. 2

Fig. 1. Fractionation of DNA-MTL from normal rat and chicken liver nuclei on phenyl-sepharose: a) chromatographic profile of de novo methylases, substrate nonmethylated DNA from phage S_d; b) chromatographic profile of methylases of maintenance type, substrate homologous DNA. Continuous line — chicken liver enzymes, broken line — rat liver enzymes. Abscissa, serial nos. of fractions; ordinate: 1) ratioactivity of acceptor DNA (in cpm·10^{-2}), 2) concentration of $(NH_4)_2SO_4$ (in M), 3) glycerol concentration (in per cent).

Fig. 2. Fractionation of DNA-MTL of maintenance type from rat and chicken liver nuclei on phenyl-sepharose, under normal and pathological conditions: a) chromatographic profile of methylases from liver nuclei of normal and thyrotoxic rats; substrate, DNA from normal rat liver; b) chromatographic profile of methylases from liver nuclei of normal chickens and chickens with hepatoma; substrate, DNA from normal chicken liver. Continuous lines — enzymes from normal rat (a) and chicken (b) liver; broken lines — liver enzymes from thyrotoxic rats (a) and chickens with hepatoma (b). Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

Assuming the existence of two types of DNA-MTL in the eukaryotic cell, these were identified in rat and chicken liver nuclei as follows: de novo DNA-MTL (I), using nonmethylated DNA of phage S_d as the substrate, and DNA-MTL of maintenance type (II), using homologous substrate as acceptor DNA. Methylase activity was tested in fractions obtained during hydrophobic chromatography on phenyl-sepharose. Work with the partially purified enzyme preparation containing nonspecific endonucleases as impurities, required an incubation period of 2 h for incubation of the enzyme with the substrate. According to data in Fig. 1a, two circumstances are common for rat liver MTL I (continuous line) and chicken liver MTL I (broken line). First, in both cases a heterogeneous set of DNA-MTL, differing in their hydrophobic properties, was found, and second, in both cases a fraction with strong hydrophobic properties and elated from the exchange resin only by 60% glycerol was present; this fraction, moreover, judging by its chromatographic profile, also was heterogeneous. According to data in Fig. 1a, chicken liver

TABLE 1. Changes During Pathology in Activity (per cent of activity of liver nuclear methylases from normal animals) of Different Fractions of DNA-MTL Obtained by Chromatography on Phenyl-Sepharose

Experimental conditions	Level of methylating activity of undermentioned type								
	I			II			II′		
	A	B	C	A	B	C	A	B	C
Chicken hepatoma MS 29	59	79	33	36	68	292	36	18	181
Rats with thyrotoxicosis	97	158	30	49	226	121	16	255	326

Legend. Normal conditions, substrate homologous DNA, II′) maintaining methylation, substrate homologous DNA isolated from liver nuclei of healthy animals (or thymus DNA). A, B, C) Stepwise elution from phenyl-sepharose by solutions of 0.65 M $(NH_4)_2SO_4$ + 20% glycerol (A); 0.2 M $(NH_4)_2SO_4$ + 60% glycerol (B); 60% glycerol (C).

MTL I demonstrated higher activity as a whole than the corresponding rat liver enzyme. The analogous data for MTL II are given in Fig. 1b.

The general pattern of multiplicity of DNA-MTL differing in their hydrophobic properties was preserved for both objects in this case also. Replacement of glycerol in the eluting system by Triton X-100, as an agent preventing protein aggregation, did not change the character of the methylase profile (data not given). It is an extremely interesting fact that the most active fraction of MTL II for both objects — both rat liver and chicken liver — was completely identical in its hydrophobic properties (as was found in both cases in fraction 7). A similar peak of enzyme activity was not found in the I methylase pool. The presence of multiple forms of MTL evidently reflects the diversity and polyfunctionality of these enzymes in the eukaryotic cell. In this connection it was interesting to undertake a comparative analysis of the heterogeneity of the methylase profile under normal and pathological conditions, and to discover as far as possible those enzyme fractions whose activity correlates with gene activity. A total MTL preparation from the liver of healthy animals, and from chicken hepatoma MS-29 and from the liver nuclei of rats with thyrotoxicosis (a disease known to be accompanied by activation of several hepatic genes [10]), was subjected to chromatographic analysis. The substrate in these experiments also was either DNA of phage S_d or homologous DNA, as reflecting the degree of methylation (or of under methylation) of DNA in vivo, and which in turn can be regarded as a preliminary test of gene activity, also was used as the substrate in these experiments. In some cases, thymus DNA was used as neutral substrate.

To obtain DNA-MTL preparations in this part of the work we used a system of stepwise elution and compared activity of total enzyme fractions under normal conditions and in the above types of pathology. According to data in Table 1, activity of de novo MTL in both types of pathology was lower than normally, as a rule, and only one enzyme preparation (IB), and only in thyrotoxicosis, demonstrated higher than normal enzyme activity. MTL II behaved differently on the whole. Both virus transformation and thyrotoxicosis of the experimental animals were accompanied by the appearance of individual enzyme fractions that were much more active in pathology than in the normal state. It must also be noted that the increase in the level of substrate methylation in vitro was manifested to the greatest degree, under the influence of the different enzyme fractions, in the case of thyrotoxicosis.

The level of substrate methylation in vitro also reflects activity of the enzyme used, and the state of the substrate reflects it conformational features and the degree of methylation in vivo, which under normal and pathological conditions may differ significantly. To differentiate strictly between activation of methylating enzymes in the types of pathology studied, independently of the properties of the substrate, activity of MTL from liver nuclei of normal and diseased animals was determined in a special series of experiments, using DNA from the liver of healthy animals as the acceptor of methyl groups. In this case, preexisting methylation in vivo affected the possibility of methylation in vitro equally.

The results are given in first part of Table 1 and in Fig. 2. The data in Table 1 indicate that viral transformation induces an almost twofold increase of activity only of MTL with marked hydrophobic properties. Meanwhile, in the case of thyrotoxicosis, a much sharper increase of enzyme activity was discovered. All these observations relate to fractions bound firmly with the hydrophobic sorbent. The opposite picture was observed in the case of MTL distinguished by low affinity for phenyl-sepharose, as Fig. 2 clearly demonstrates. The chromatographic profile was obtained during gradient elution of the material. According to data in Fig. 2a and b, enzyme activity of the weakly hydrophobic MTL fractions, in the case both of hepatoma (Fig. 2b, broken line) and of thyrotoxicosis (Fig. 2c, broken line) was appreciably lower than normally. In this case, a very clear illustration of the

change in methylase profile in pathology is disappearance of the most active methylase peak (fractions 6 and 7), which is invariably present in the liver nuclei of normal animals.

We must again concentrate our attention on the fact that the difference between the normal and pathological states in this series of experiments was determined entirely by a difference in activity of the enzyme and was in no way dependent on pre-existing methylation in vivo.

If the data in Fig. 2, obtained by gradient elution of MTL, are compared with the results in Table 1, obtained during stepwise elution, it will be evident that the change in activity of the total preparation in pathology reliably reflects only a general tendency, but it cancels out the marked differences observed between the normal and pathological states.

In a special series of experiments we determined the content of 5-methylcytosine (5-MC) in rat and chicken liver DNA from normal animals and animals with the corresponding pathology. The 5-MC concentration in the case of thyrotoxicosis was 60.8% and in the case of viral transformation 47.8% of normal. It is natural to suppose that the observed decrease in the total 5-MC concentration is an averaged value, but it must be assumed that the main decrease in the 5-MC level in DNA during pathology takes place on account of the regulatory regions of the genome. So far as DNA of the methylase peak block in both types of pathology is concerned, this can be regarded as a likely participant in the general mechanism of regulation of gene activity. On the basis of these same data relating to 5-MC concentration it can also be concluded that the enzyme capacities of fractions B and C (Table 1), which exhibit increased activity in vitro, remain unrealized in the in vivo situation during pathology for various reasons.

LITERATURE CITED

1. N. I. Bubnov, A. G. Basnak'yan, and I. I. Votrin, Byull. Éksp. Biol. Med., No. 2, 154 (1985).
2. N. N. Burtseva, O. A. Gimadutdinov, and B. F. Vanyushin, Biokhimiya, **52**, No. 2, 290 (1987).
3. Ya. I. Bur'yanov and G. I. Kir'yanov, Structural and Functional Bases of Enzymic Methylation of DNA [in Russian], All-Union Institute of Scientific and Technical Information, Moscow (1987), pp. 123-191.
4. V. K. Vasil'ev, Biol. Nauki, No. 9, 118 (1971).
5. I. I. Nikol'skaya, E. V. Sharkova, and E. G. Lopatina, Biokhimiya, **54**, No. 4, 564 (1989).
6. T. I. Tikhonenko and N. Ya. Solov'eva, Biokhimiya, **26**, No. 5, 795 (1961).
7. R. Axel, Biochemistry, **14**, 2921 (1975).
8. T. H. Bester and V. M. Ingram, Proc. Nat. Acad. Sci. USA, **82**, 2764 (1985).
9. A. H. Bolden et at., Prog. Nucleic Acids Res., **33**, 232 (1986).
10. D. B. Camp and J. H. Oppenheimer, Molec. Cell. Biochem., **55**, 159 (1983).
11. M. Qureshi, K. L. P. Adams, and R. H. Burdon, Biochem. Soc. Trans., **10**, No. 6, 455 (1982).
12. J. J. Marmur, J. Mol. Biol., **3**, 208 (1961).
13. I. I. Nikol'skaya, E. V. Sharkova, S. V. Suchkov, et al., Biochem. Internal., **7**, 605 (1983).
14. G. P. Pfeifer et at., J. Biol. Chem., **260**, 13787 (1985).