CHANGES IN RNA SYNTHESIS DUE TO N-METHYL-N-NITROSOUREA

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Alkyl derivatives of nitrosourea are used to treat many tumors in man [7]. However, it is known that these substances also have a well-marked carcinogenic effect. For instance, a single injection into pregnant animals gives rise to a high percentage of tumors in the progeny [8]. Alkyl derivatives of nitrosourea inhibit nucleic acid synthesis and induce injuries incompatible with the normal activity of the cell [2, 6, 7].

The causes of the depression of RNA synthesis in isolated nuclei, in isolated chromatin, and in protein-free DNA after exposure to N-methyl-N-nitrosourea (NMU) are analyzed below.

EXPERIMENTAL METHOD

Wistar rats were used. Hepatic nuclei were purified through 2.2 M sucrose solution [4]. Chromatin preparations were obtained by homogenization of the nuclei in 0.02 M EDTA, 0.075 M NaCl, pH 8.0, followed by repeated washing in decreasing concentrations of Tris-HCl buffer, pH 8.0. DNA was isolated by centrifugation of chromatin dissolved in 2 M NaCl and 5 M urea for 36 h at 105,000g. Templates for RNA synthesis were treated with NMU at pH 7.4 for 1 h at 37°C, after which the preparations were dialyzed overnight at 4°C. RNA synthesis, sedimentation analysis of RNA, calculation of the molecular weights and the number of molecules synthesized, and reconstruction of chromatin (dialysis against decreasing NaCl concentrations) were done as described previously [4, 5]. The results were subjected to statistical analysis by the method described in [1].

EXPERIMENTAL RESULTS

It is well known that under physiological conditions NMU decomposes with the formation of methylcarbonium ions (which then react with nucleophilic centers of nucleic acids and proteins) and of isocyanates (carbamoyling mainly proteins and lipids and also, to a lesser degree, DNA and RNA) [7]. To determine whether RNA synthesis could be reduced through the carbamoyling action of NMU, the effect of increasing concentrations of NMU, the potassium salt of isocyanic acid (KNCO), and KCl (taken as the control of ionic strength created by the KNCO) on template activity of the chromatin was compared. It will be clear from Fig. 1 that the level of RNA synthesis on NMU-treated chromatin decreased with an increase in the NMU concentration and did not differ significantly from the control, in which chromatin was treated with KNCO and KCl.

These results are evidence that methylation processes and not carbamoylation were responsible for the decrease in RNA synthesis.

To determine the importance of DNA injuries in the depression of the template properties of chromatin, the level of RNA synthesis was measured on reconstituted templates. Experimental samples of DNP were reconstituted from DNA treated with NMU in a concentration of 35 mM, and normal chromatin proteins. In the control reconstituted DNP, neither DNA nor proteins were treated. Both experimental and control samples of DNA had protein spectra on SDS electrophoresis identical with the original chromatin. Nevertheless, the template activity

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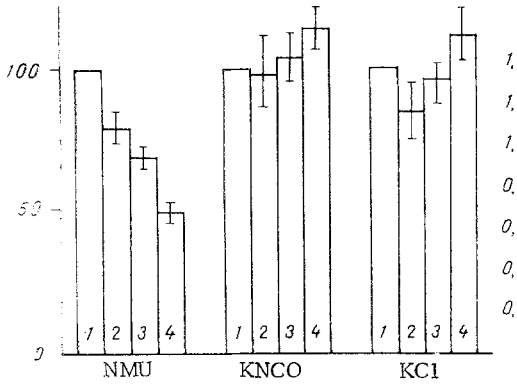

Fig. 1

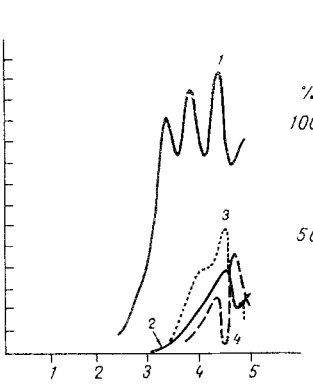

Fig. 2

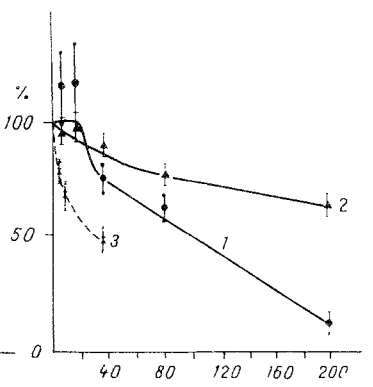

Fig. 3

Fig. 1. DNA synthesis on chromatin treated with NMU, KNCO, and KCl (in % of control, M ± σ). 1) Untreated samples of chromatin; 2) chromatin treated with agent in concentration of 1.5 mM, 3) 5 mM, 4) 35 mM.

Fig. 2. Sedimentation profiles of RNA synthesized on chromatin and protein-free DNA: 1) control DNA; 2) DNA treated with NMU in concentration of 53 mM; 3) control chromatin; 4) chromatin treated with NMU in concentration of 35 mM. Abscissa, volume of separating gradient (in cm^3), starting from bottom; ordinate, radioactivity in fractions (in cpm × 10^{-3}).

Fig. 3. Dependence of RNA synthesis on NMU concentration (in % of control, M ± σ). 1) RNA synthesis in nuclei with endogenous RNA-polymerase in presence of 0.2 M $(NH_4)_2SO_4$ and 1 μM $MnSO_4$; 2) RNA synthesis in nuclei by RNA-polymerase of *E. coli*; RNA synthesis on chromatin by RNA-polymerase from *E. coli*. Abscissa, NMU concentration (in mM) used to treat templates.

of DNP reconstituted from NMU-treated DNA was only 32 ± 5% of that of the reconstituted control specimens.

This observation indicates the important role of injuries to the DNA itself in the changes of chromatin template activity. The decrease in chromatin template activity after treatment with NMU was thus probably due to methylation of the DNA.

Inhibition of RNA synthesis on templates injured by NMU could be the result of a decrease both in the mean molecular weight of the transcripts and in the number of RNA copies. To test these hypotheses experiments were carried out to fractionate the newly synthesized RNA in a sucrose density gradient. The sedimentation profiles are shown in Fig. 2 and the results of calculations of the mean molecular weight of the RNA and the number of molecules (in % of the corresponding control) are given in Table 1. The results show that with an accuracy determined by the error of measurement, the number of molecules synthesized in NMU-treated DNA and chromatin templates did not differ from the control values. Meanwhile the relative molecular weight of synthesized RNA was reduced both on the chromatin template and also, particularly significantly, on DNA, on which it was only 25 ± 6% of the control.

The decrease in RNA synthesis was thus due mainly to a decrease in length of the transcripts.

The inhibitory action of NMU (depending on its concentration) on isolated nuclei and chromatin is illustrated in Fig. 3. RNA synthesis with RNA-polymerase from *E. coli* was inhibited much faster on chromatin than on nuclei. Considering that on protein-free DNA synthesis was inhibited by an even greater degree than on chromatin (Fig. 2; Table 1), it can be tentatively suggested that both chromatin proteins and other nuclear proteins play a protective role in the inhibition of the RNA-synthesizing ability of templates by NMU.

An inhibitory effect on NMU on the activity of certain enzymes is known [7]. For example, NMU significantly inhibits DNA polymerase 1 activity [2]. Accordingly it was interesting to compare RNA synthesis in nuclei with the endogenous polymerase (which could have its properties changed during treatment of the nuclei with NMU) and with polymerase from *E. coli*.

With an increase in the concentration of NMU used to treat the nuclei, RNA synthesis was found to decrease more rapidly with the endogenous RNA polymerase than with the enzyme from

TABLE 1. Characteristics of RNA Synthesized on Chromatin and DNA, Treated with NMU in a Concentration of 35 mM (in % of control)

Template for RNA synthesis	Rel. mol. wt. of RNA	Rel. no. of RNA molecules
Control chromatin	100	100
Chromatin treated with NMU	71±9	92±14
Control DNA	100	100
DNA treated with NMU	25±6	84±32

E. coli (Fig. 3). Consequently, the decrease in RNA synthesis after treatment with NMU could be due not only to methylation of DNA, but also to injury to the enzyme system responsible for transcription.

Inhibition of RNA synthesis by the action of NMU on the genome may be one of the main causes leading to death of the cells. NMU acts mainly on those regions of DNA where the double helix is most easily denatured [9]. In the cell genome the chief candidates for the role of these regions are the active genes which, as we know, are most accessible to DNase I and which are evidently closely bound with the nuclear template [4]. It has been shown by the use of direct methods that benz(a)pyrene metabolites bind mainly with these regions of chromatin [3]. The possibility cannot be ruled out that the spatial conformation of these regions makes them most accessible also for certain other chemical agents and, in particular, for NMU. This hypothesis is supported, on the one hand, by the fact that spermine, on binding with DNA, inhibits both the intercalation of benz(a)pyrene and the methylation of DNA by NMU [9], and on the other hand, by the fact that *in vivo* it is those regions of chromatin that are the most accessible to DNase I that are methylated before the rest [10].

As a result of the action of NMU on nuclear chromatin the lengths of the RNA transcript are significantly reduced (Fig. 2; Table 1) and the inhibitory action on RNA synthesis increases with an increase in the NMU concentration (Fig. 3). Active concentrations of NMU, acting *in vivo*, will evidently be much lower than the maximal concentrations which can be obtained in experiments *in vitro*. The number of injured genes must be reduced correspondingly. However, even if an incomplete RNA copy is transcribed on only one of the genes, this is sufficient to give rise to disturbances of protein synthesis and, ultimately to disturbances of normal activity of the cell.

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