

A common chromatin factor involved in the repair of *O*⁶-methylguanine and *O*⁶-ethylguanine lesions in DNA

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Received 13 April 1982; revision received 11 June 1982

1. INTRODUCTION

Repair of DNA containing *O*⁶-methylguanine or *O*⁶-ethylguanine by rat liver total proteins was observed in [1,2]. We have shown that the factor responsible for the disappearance of *O*⁶-ethylguanine is mostly located in chromatin [3] and that the ethyl group is transferred onto 2 cysteine residues of acceptor proteins [4]. Using a factor partially purified from mouse liver supernatant repair of DNA containing *O*⁶-methylguanine and transfer of the methyl group onto cysteine residues of proteins was shown in [5].

This work shows that chromatin proteins from rat liver contain factors acting on *O*⁶-methylguanine as well as *O*⁶-ethylguanine in DNA. Competition experiments indicate that a limiting component is common to both repair systems.

2. MATERIALS AND METHODS

2.1. Chromatin proteins

Rat liver nuclei and chromatin were isolated according to [6]; the non-histone proteins were extracted using heparin—Ultrogel as in [4]. The extract was dialyzed against buffer A (50 mM Tris—HCl, 1 mM EDTA, 2 mM dithiothreitol, pH 8.0).

2.2. The substrates

Calf thymus DNA was alkylated with [³H]ethylnitroso-urea (4.5 Ci/mmol; IRE Belgium) or with [³H]methylnitroso-urea (1.6 Ci/mmol; NEN). The alkylated DNA, partially depurinated according to [7] contained 8 residues of *O*⁶-ethylguanine (*O*⁶-etG) or 10 of *O*⁶-methylguanine (*O*⁶-meG)/

10⁶ guanines. For the ethylated DNA, ~15% of the radioactivity was in *O*⁶-etG, whereas, for the methylated DNA, ~50% was in *O*⁶-meG. The radioactive alkylated DNAs were kept at 4°C in 0.15 M NaCl, 15 mM Na-citrate, 1 mM EDTA (pH 7.0) buffer, 1% benzyl alcohol. Before use, the solutions were dialyzed against buffer B (10 mM Tris—HCl, 1 mM EDTA, pH 8.0).

2.3. Incubation of alkylated DNA with chromatin proteins followed by separation using isopycnic centrifugation

Proteins in buffer A (3 vol.) and alkylated [³H]DNA in buffer B (1 vol.) were incubated together for 2 h at 37°C.

CsCl gradient centrifugation and fractionation were carried out as in [4]; the radioactivity of each fraction was measured.

2.4. Incubation of alkylated DNA with chromatin proteins followed by purine analysis

The incubation was carried out as in section 2.3. To 200 µl incubation medium were added 33 µl 1 M HCl and 100 µl 10 mM HCl containing 25 µg unlabelled *O*⁶-meG and 25 µg unlabelled *O*⁶-etG; the mixture was heated at 70°C for 50 min. After addition of 36 µl 1 M K₂HPO₄ and 65 µl acetonitrile, the precipitate was discarded by centrifugation and 250 µl supernatant was chromatographed on a 250 × 10 mm Column of Ultrasphere ODS (5 µm) using an ALTEX 332 HPLC system. The eluent was a mixture of 15% acetonitrile and 85% 50 mM ammonium phosphate adjusted at pH 4.0 with 6 M H₃PO₄. The elution was performed at a flowrate of 2 ml/min and at a pressure of 70–90 bars.

The analysis takes 15 min; the retention times are $8.02 (\pm 0.09)$ min for O^6 -meG and $11.52 (\pm 0.09)$ min for O^6 -etG.

Peaks of O^6 -meG and O^6 -etG were automatically collected. Calculated by the integrator for absorbance at 260 nm, O^6 -meG and O^6 -etG recoveries were usually $\sim 95\%$. The total radioactivities of the fractions were corrected for the recoveries; taking account of the specific activities of the alkylating agents, the amounts (fmol) of O^6 -meG and O^6 -etG present in the alkylated DNAs before and after incubation with the chromatin proteins were calculated. The standard error on the values obtained for the substrate DNAs was 1–2%.

3. EXPERIMENTS AND RESULTS

3.1. Repair of O^6 -methylguanine and O^6 -ethylguanine lesions in DNA by chromatin proteins

Aliquots (100 μ l) of chromatin proteins (26, 35 or 60 μ g, depending on the preparation) in buffer A were mixed, in different tubes, with 100 μ l buffer B containing either (I) 35 μ g ethylated DNA (200 fmol O^6 -etG) and 31 μ g untreated DNA, or (II) 29 μ g

methylated DNA (205 fmol O^6 -meG) and 31 μ g untreated DNA, or (III) 35 μ g ethylated DNA and 29 μ g methylated DNA (200 fmol O^6 -etG and 205 fmol O^6 -meG). The incubation was carried out at 37°C for various times up to 120 min before measuring the remaining O^6 -alkylguanines.

Fig. 1 shows the result of 3 expt made with different preparations of chromatin proteins. In all cases, when there was a single substrate (methylated or ethylated DNA), the disappearance of O^6 -meG reached a plateau at 60 min (94, 102 or 145 fmol depending on the experiment) whereas the disappearance of O^6 -etG was still slowly progressing after 120 min. The curves for O^6 -etG are below those for O^6 -meG; but the relative positions of the experimental points (fmol) depend on the correctness of the specific radioactivities given for the labelled alkylating agents by 2 different suppliers.

When both substrates were simultaneously present, there was a decrease of the repair of either substrate indicating a competition. The methylated DNA always appeared to be better repaired than the ethylated DNA, but this also depends on the stated radioactivities of the alkylating agents. The

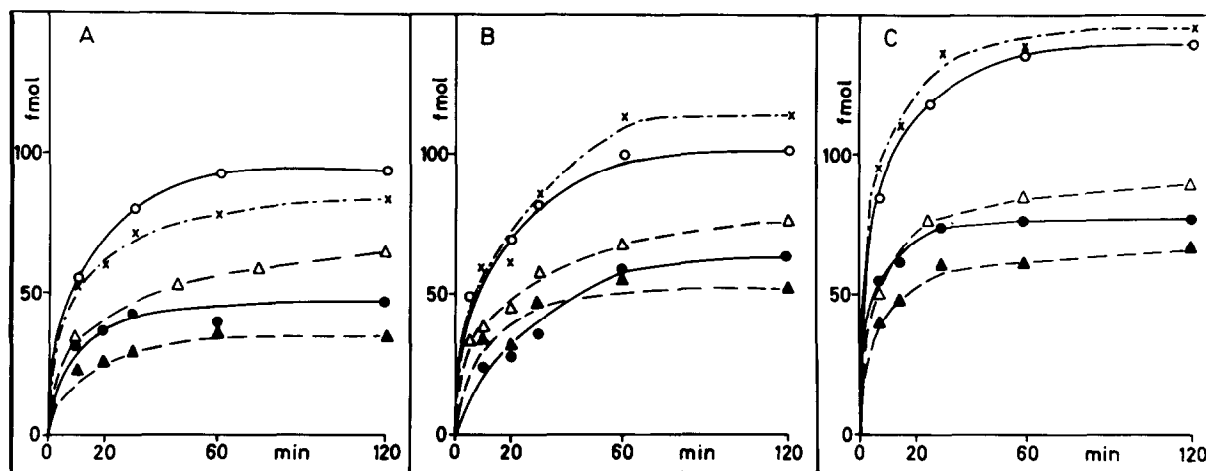


Fig. 1. Repair of O^6 -meG and O^6 -etG lesions in DNA by chromatin proteins. Buffer B containing methylated DNA (205 fmol O^6 -meG) and/or ethylated DNA (200 fmol O^6 -etG) is incubated at 37°C with buffer A containing 26 μ g (A), 35 μ g (B), or 60 μ g (C) of chromatin proteins from different preparations. The disappearance of O^6 -alkylguanines (fmol) from DNA is recorded as a function of time (min). Circles and continuous lines show the disappearance of O^6 -meG, while triangles and dashed lines follow the disappearance of O^6 -etG. Open symbols (\circ, Δ) are used when a single substrate (methylated or ethylated DNA) is present. Black symbols (\bullet, \blacktriangle) are used when both substrates are simultaneously present; ($\times \rightarrow \times$) is the sum of O^6 -meG and O^6 -etG disappearances.

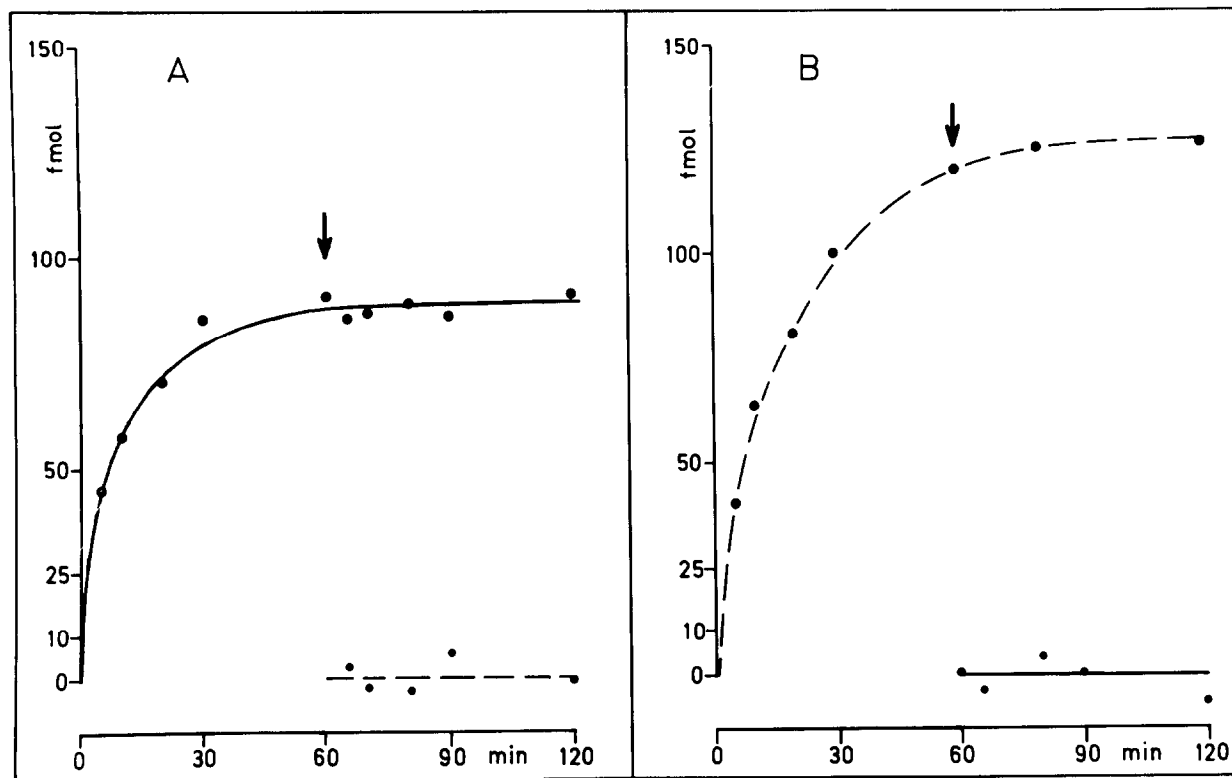


Fig.2. A common factor is consumed during the repair of O^6 -meG and O^6 -etG lesions in DNA. (A) Buffer A containing 35 μ g chromatin proteins is incubated at 37°C with buffer B containing methylated DNA (205 fmol O^6 -meG); after 60 min, ethylated DNA (200 fmol O^6 -etG) in buffer B is added. (B) Buffer A containing 58 μ g chromatin proteins is incubated at 37°C with buffer B containing ethylated DNA (200 fmol O^6 -etG); after 60 min, methylated DNA (205 fmol O^6 -meG) in buffer B is added. Disappearance (fmol) of O^6 -meG (—) and O^6 -etG (---) as a function of time (min).

sum of the repair of ethylated and methylated DNAs was in 2 cases above and in 1 case below that of the methylated DNA alone.

3.2. Exhausting the common pathway to search for a specific one

In a first experiment, several tubes received 35 μ g chromatin proteins in 100 μ l buffer A and 29 μ g methylated DNA (205 fmol O^6 -meG) in 50 μ l buffer B, and were incubated at 37°C. After various times, tubes were taken to determine the remaining O^6 -meG. After 60 min, 35 μ g ethylated DNA (200 fmol O^6 -etG) in 50 μ l buffer B were added to the remaining tubes; after various times up to another 60 min, tubes were taken to determine the remaining O^6 -meG and O^6 -etG. Fig.2(A) shows that the

elimination of O^6 -meG had reached a plateau (90 fmol) after 60 min; the O^6 -etG of the ethylated DNA added at that moment did not disappear at all.

In a second experiment, the tubes received 58 μ g chromatin proteins in 100 μ l buffer A and the order of addition of the alkylated DNAs in buffer B was inverted. Fig.2(B) shows that the elimination of O^6 -etG had nearly reached a plateau (125 fmol) after 60 min; the O^6 -meG of the methylated DNA added at that moment did not disappear at all.

3.3. Transfer of the alkyl group from O^6 -alkylguanine in DNA onto an acceptor protein

Chromatin proteins (630 μ g) in 1.8 ml buffer A were incubated with 0.6 ml buffer B containing

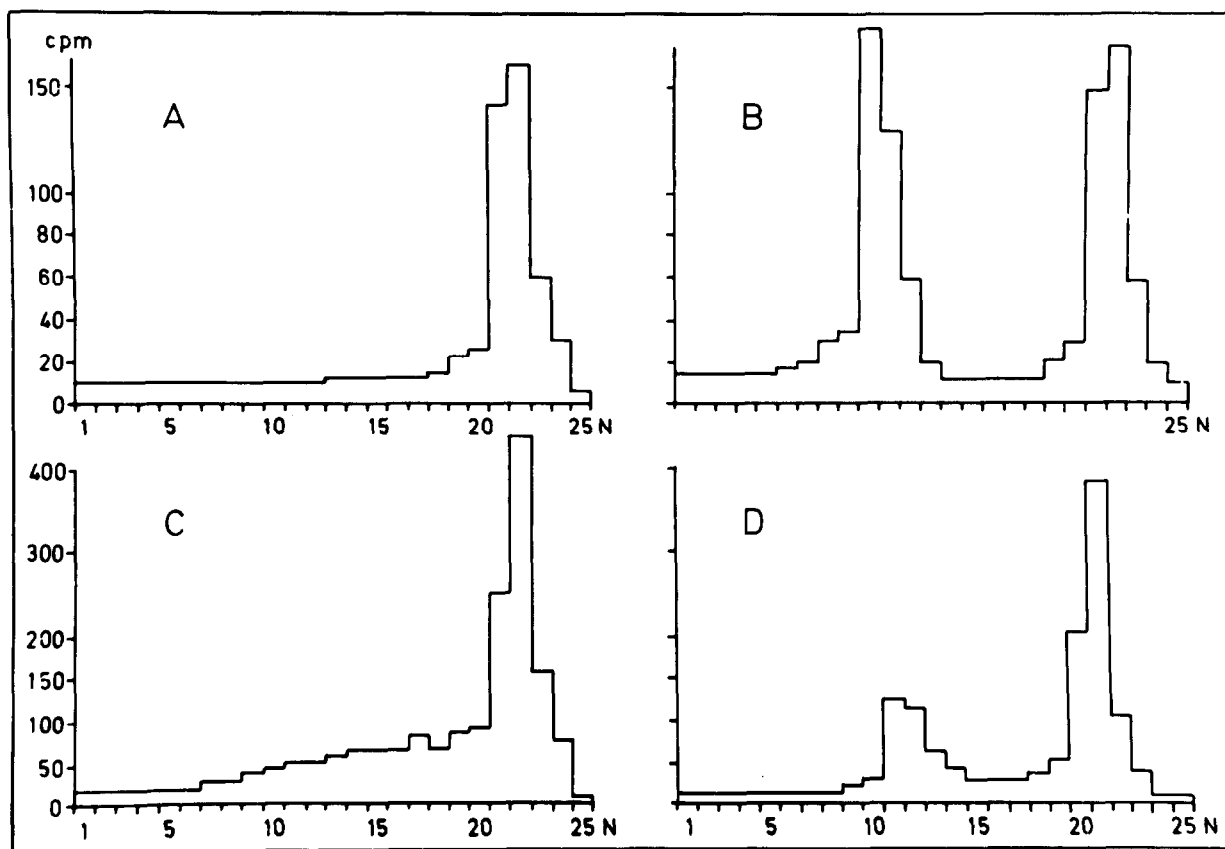


Fig.3. Transfer of the alkyl group onto an acceptor protein. Chromatin proteins in buffer A and methylated (A,B) or ethylated (C,D) DNA in buffer B are incubated for 0 min (A,C) or 120 min (B,D) at 37°C. The mixtures are submitted to CsCl isopycnic centrifugation; the gradients are fractionated (N = fraction number) for radioactivity determination (cpm).

either 345 μ g methylated DNA or 420 μ g ethylated DNA for 120 min at 37°C. Identical controls were not incubated. The mixtures were submitted to isopycnic centrifugation in CsCl gradients; fractionation was followed by determination of the radioactivity in each fraction.

Fig.3A,C shows that, without incubation, the radioactivity was only in the high density region with the DNA. After a 2 h incubation, part of the radioactivity was found in a lower density region (~ 1.4 g/ml) with the proteins (fig.3B,D). The result with ethylated DNA (fig.3D) was the same as that published in [4]. The result with methylated DNA (fig.3B) was qualitatively identical.

4. DISCUSSION

In *Escherichia coli*, 2 independent pathways repair O^6 -etG lesions in DNA; one, constitutive, is dependent on *uvrA,B,C* genes; the other, dependent on the *ada* gene, must be induced. The *ada* repair factor is common for O^6 -meG and O^6 -etG; the alkyl group is transferred onto a cysteine residue of an acceptor protein [8].

This work shows that the incubation of proteins prepared from rat liver chromatin leads to the disappearance of O^6 -meG and O^6 -etG in DNA; in both cases, the alkyl group is transferred in a molecule having the density of a protein. These repair

systems are constitutive.

The repair capacity toward methylated DNA is limited; the disappearance of O^6 -meG reaches a plateau after ~60 min under our experimental conditions. The repair of O^6 -etG lesions proceeds at a slower rate and a plateau is not yet reached after 120 min. Stopping or slowing down of the repair activities is not due to thermal inactivation of the enzymes since their half-lives were ~250 min in absence of substrates (not shown).

Competition experiments indicate that methylated DNA decreases the amount of repair of O^6 -etG lesions and, vice versa, ethylated DNA decreases the amount of repair of O^6 -meG lesions. It thus seems that the 2 repair pathways have at least 1 common component which is limiting the extent of the reaction. In 2 experiments, the sum of the amounts of O^6 -meG and O^6 -etG that had disappeared was higher than the amount of O^6 -meG when methylated DNA was the only substrate or the amount of O^6 -etG when ethylated DNA was the only substrate. Such a result suggests that in rat liver chromatin, as in *E. coli*, there might be a particular pathway for one of the substrate (or particular pathways for each of them) besides the common pathway. The result was, however, erratic so that another approach was tried to solve the question.

During a first incubation the limiting component of the common pathway was exhausted with an excess of one substrate (methylated or ethylated DNA), then the other substrate (ethylated or methylated DNA) was added. No disappearance of O^6 -etG or O^6 -meG was ever observed from the second substrate. A possible interpretation was that the specific repair systems were inactivated before

addition of their substrates; it is unlikely since, on incubation without substrate, the total repair capacity for either substrate decreased only very slowly. It is much more probable that these results indicate that no repair system acting on O^6 -etG or O^6 -meG other than those consuming the common limiting component is present in the proteins extracted from rat liver chromatin.

ACKNOWLEDGEMENTS

This work was supported by grants from the Fonds de la Recherche Scientifique Médicale and the Fonds Cancérologique de la Caisse d'Épargne et de Retraite; M.L. is a recipient of an IRSIA fellowship; A.R. is aspirant du FNRS.

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