THE EFFECT OF IONISING RADIATION AND CHEMICAL METHYLATION UPON THE ACTIVITY AND ACCURACY OF E.COLI DNA POLYMERASE I

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Received October 7,1974

SUMMARY

The activity of E.coli DNA polymerase I decreases on treatment with γ-rays, methylnitrosourea or dimethyl sulphate. In the case of the first two agents the decrease in activity is accompanied by a decrease in the accuracy of the enzyme in an in vitro assay. There is no detectable change in the ratio of DNA polymerase activity to 3'->5' exonuclease activity on treatment.

INTRODUCTION

DNA synthesis in vivo should be a very accurate process in order that the integrity of the DNA base sequence of the genome be maintained. Even in in vitro systems DNA polymerases from both bacterial[1] and mammalian[2] sources have been found to incorporate only one wrong base in several hundred thousand. However, several recent reports have suggested that inaccuracies in DNA synthesis may be related to cellular ageing[3,4], carcinogenesis and tumour progression[5]. A mutant error-prone DNA polymerase has been found in human leukaemic cells[6].

The high accuracy of the DNA polymerases makes it possible to study small changes in the fidelity of the enzymes. We have been investigating the effect of carcinogens and other agents upon E.coli DNA polymerase I and now wish to report changes in the activity and accuracy of this enzyme following in vitro γ-irradiation or treatment with the methylating agents methylnitrosourea (MNU) or dimethyl sulphate (DMS).

Abbreviations: MNU - methylnitrosourea, DMS - dimethyl sulphate.

MATERIALS AND METHODS

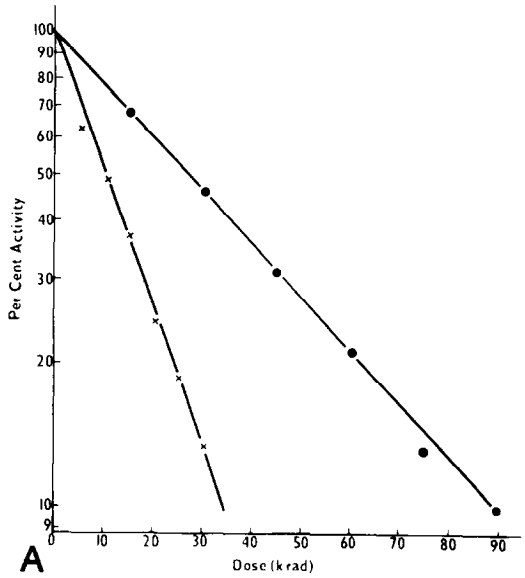
DNA polymerases and deoxyribonucleoside triphosphates were purchased from the Boehringer Corporation (London) Ltd., Poly(dA-dT).poly(dA-dT) was prepared by the method of Schachman et al[7]. Radioactive materials were purchased from the Radiochemical Centre, Amersham. The enzymes were irradiated in 0.02 M NaCl at 0°C in the presence of air using a ^{137}Cs source at a dose rate of 8.4 rad/sec. Treatment with MNU and DMS was in the presence of potassium phosphate buffer pH 7.5 (0.1 M for MNU and 0.5 M for DMS) at 4°C. The higher doses of DMS were given as a split dose with a dialysis against potassium phosphate buffer pH 7.5 in between.

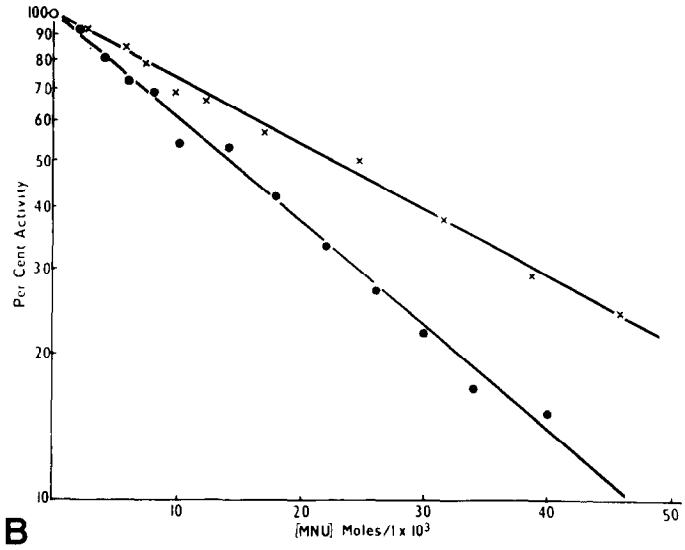
After treatment, inactivation of the enzyme was measured in a standard assay. Details have been given elsewhere[8]. Enzyme corresponding to 0.02 units of untreated enzyme was added before incubation at 37°C for 30 min. The incorporation of dG into newly synthesised material using a poly(dA-dT).poly(dA-dT) template was taken as a measure of the accuracy of the enzyme. This was measured by a method of concurrent assays using two assays which were identical in every respect apart from the deoxyribonucleoside triphosphate which was labelled with tritium. These assays (total volume 0.3ml) contained 50 nmoles each of dATP and dTTP, 5nmoles dGTP, 300 nmoles nucleotide phosphorus as poly(dA-dT).poly(dA-dT). One unit of enzyme activity (as calculated from the inactivation of the enzyme) was added before incubation. To measure total DNA synthesis 2 x 10^5 cpm/assay [^{3}H]-dTTP was used and when measuring wrong base incorporation 3 x 10^7 cpm/assay [^{3}H]-dGTP was used.

3' -> 5' exonuclease activity was measured by incubation with 3'-terminally labelled DNA[9] under conditions of the standard assay in the absence of deoxyribonucleoside triphosphates. The production of acid soluble radioactivity was used as a measure of the exonuclease activity.

RESULTS AND DISCUSSION

Effect of γ-irradiation or methylation on DNA polymerase I The specific activity of E.coli DNA polymerase I decreases exponentially with increasing dose following in vitro γ-irradiation or treatment with MNU or DMS (see fig.1). In order to reduce the enzyme activity 50% the doses required (D^{50}) are 27 krad, 14mM and 110mM respectively for γ-radiation, MNU and DMS treatment. The addition of MNU or DMS hydrolysis products to the enzyme had no





effect on its activity. Inactivation by DMS under the conditions of these experiments is apparently biphasic with the change in slope coinciding with the change over from single- to split-dose (see Methods). The D^{50} quoted above for DMS is obtained by extrapolation of the single-dose region.

Following γ-irradiation and MNU treatment there is also a decrease in the accuracy of the enzyme in the copying of a poly(dA-dT).poly(dA-dT) template. The amount of dG incorporated is taken as a measure of the accuracy of the polymerase (see fig 2). The assay used here is capable of detecting ca 1 wrong base in 200,000 (0.0005%), insufficient to detect the reported natural error of the enzyme of ca 1 wrong base in 500,000 (0.0002%)[1]. At high doses of radiation or of MNU the errors produced by the enzyme become detectable in the assays and increase with increasing dose (see fig 2). The addition of MNU hydrolysis products to the assay had no detectable effect on the fidelity of the enzyme. Following DMS treatment no significant wrong base incorporation was detected.

These results show that the fidelity of DNA polymerase can be lowered by γ-irradiation or methylation with MNU. It has been proposed[10] that the high fidelity of DNA polymerase I is due to the 3' -> 5' exonuclease activity

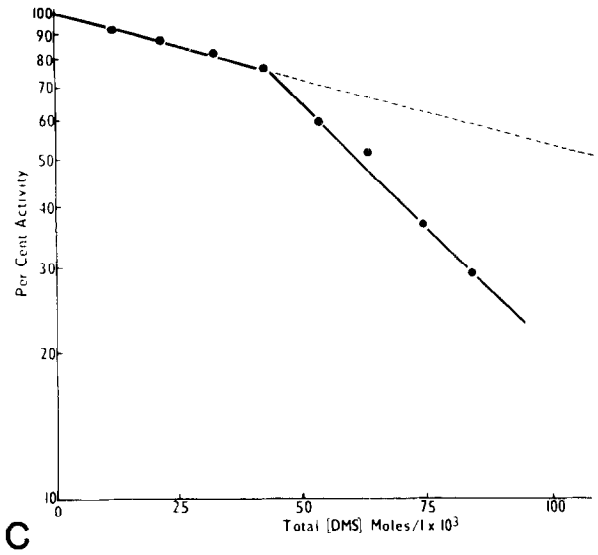


Figure 1. Inactivation of E.coli DNA polymerase by (a) γ-radiation, (b) MNU and (c) DMS. ●———● DNA polymerase I, X———X enzyme A of Klenow.

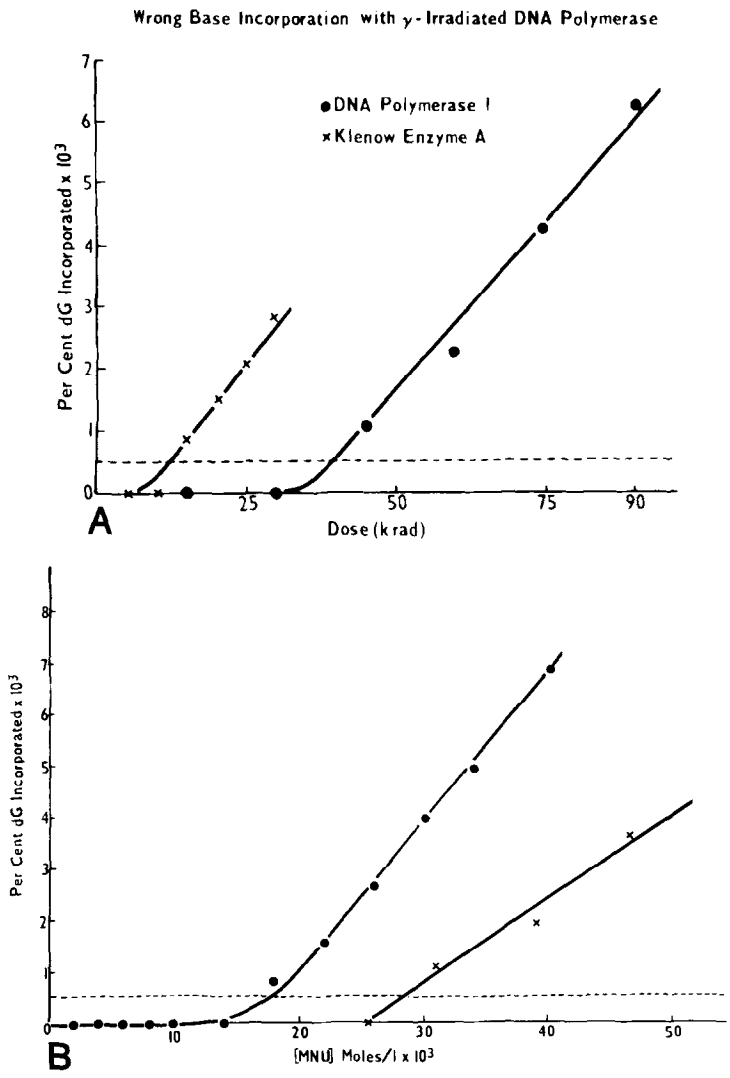


Figure 2. Wrong base incorporation by E.coli DNA polymerases following (a) γ-radiation and (b) MNU treatment. ●——● DNA polymerase I, X——X enzyme A of Klenow. The horizontal line indicates the limit of detection in the assays used.

associated with the enzyme; this activity acting as a "proof" reader and is available to excise any wrong bases that may be inserted. If this is so, the decrease in fidelity of the DNA polymerase on γ-irradiation or methylation could be due to a differential inactivation of the polymerase and 3' -> 5' exonuclease activities, the latter being the most sensitive.

Since DNA polymerase I also possesses a 5' -> 3' exonuclease activity that could interfere with the assay of the 3' -> 5' exonuclease activity it was decided to use the proteolytically cleaved DNA polymerase, the so called enzyme A of Klenow[11,12] for future work. This enzyme is obtained from DNA polymerase I by proteolytic cleavage and it only shows two enzyme activities, namely a DNA polymerase and a 3' -> 5' exonuclease.

Effect of γ-radiation or MNU on DNA polymerase enzyme A The specific activity of E.coli DNA polymerase enzyme A decreases exponentially with increasing doses of radiation or MNU with a D^{50} of 10 krad and 23 mM respectively (see fig 1a and 1b). Accompanying this decrease is a decrease in the accuracy of the enzyme (see fig 2) which is particularly noticeable at the higher doses. Like the intact DNA polymerase I the cleaved polymerase also shows a high fidelity which can be lowered by γ-irradiation or MNU treatment.

Effect of γ-radiation or MNU on the 3' -> 5' exonuclease activity The exonuclease activity associated with the cleaved polymerase was found to decrease at the same rate as the polymerase activity following γ-irradiation or MNU treatment with the ratio of DNA polymerase activity to 3' -> 5' exonuclease activity remaining constant over the dose range investigated. This lack of detectable selective inactivation of polymerase and exonuclease activities does not necessarily mean that the 3' -> 5' exonuclease activity is not responsible for the high fidelity of the polymerase. If an enzyme molecule which possesses a good polymerase activity but no exonuclease activity were to make a relatively large number of errors then the above results could be explained by the formation of relatively few error-prone polymerase molecules. In these circumstances the extra decrease in exonuclease activity would not be detectable in these assays.

This is the first report of a decrease in fidelity of a DNA polymerase following an in vitro treatment. The results reported here, coupled with the

presence of an error-prone DNA polymerase in human leukaemic cells[6] and the possibility of carcinogen modified DNA bases mis-coding during the copying of DNA[8,13], are indicative that there may be indeed a connection between inaccuracies in DNA synthesis and carcinogenesis.

Acknowledgements Miss A. Cogley is thanked for expert technical assistance. This work was supported by grants from the Medical Research Council and the Cancer Research Campaign.

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