THE INFLUENCE OF CAFFEINE ON DNA-POLYMERASE USING UV-IRRADIATED DNA AS TEMPLATE

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1. Introduction

Caffeine has been shown to act as an inhibitor of dark repair and co-mutagenic agent [1–3]. Despite its widespread use for inhibition of dark repair the mode of action is not well known. The inactivation of repair enzymes [1–3] by caffeine has been discussed as well as its interaction with DNA [4–6]. In the latter, caffeine is supposed to bind to locally-denatured regions in the DNA and to interfere with the repair system of the damaged cell. Because of the insertion of molecules of caffeine the repair enzymes may well be blocked.

Thus we attempted to investigate the influence of caffeine on DNA polymerase using UV-irradiated DNA as primer, especially since it could be demonstrated by Witte and Böhme [7] that the inhibitory effect of caffeine on dark repair is due to interaction with DNA.

If the above assumption is correct a decrease in the incorporation rate of [^{3}H]dTTP should be observed.

In addition our investigation should clarify the discrepancy between the results of Wragg and co-workers [8], who observed an inhibition of polymerase activity by caffeine, while Mouton and Fromageot [9] could not confirm this. Therefore we have compared the incorporation rate of [^{3}H]dTTP on unirradiated and irradiated primer DNA following addition of caffeine at concentrations of 8×10^{-7} to 8×10^{-3} M.

2. Materials and methods

We used DNA polymerase from *Proteus mirabilis* which shows exonucleolytic properties (comparable with polymerase I of *E. coli*). It was prepared by the method of Nüske et al. [10] and purified to a stage like step VII in the preparation of Richardson et al. [11] for DNA polymerase I from *E. coli*. Deoxyribonuclease was prepared by the method of Kunitz [12]. Its activity was 25% of that of crystallized deoxyribonuclease. DNA was a lyophilized preparation of highly polymerized DNA from calf thymus (Th. Schuchardt, München). Activation was obtained by incubation with DNAase I according to Richardson [13] for 60 min.

The assay of activity of DNA polymerase was done according to Richardson [13], but the volume of reaction mixture was reduced to 0.125 ml. It contained 0.005 ml (4 nmoles) each of dATP, dGTP, dCTP, and dTTP, 0.010 [^{3}H]dTTP (11.2 Ci/mmole, Amersham, diluted 25-fold), 0.030 ml DNA solution (nicked or unnicked), 0.030 ml glycine buffer, pH 8.2, 0.282 M containing 28.1 mM $MgCl_2$ and 4.2 mM 2-mercaptoethanol, 0.015 ml H_2O, 0.010 ml of the desired caffeine solutions, and 0.010 ml of the enzyme (0.8 units). The evaluation was done by the filter disk method of Bollum [14] in a liquid scintillation counter (Packard) with a toluene scintillation liquid.

The nicked or unnicked calf thymus DNA was irradiated in quartz cuvettes at 20°C with stirring by bubbling wet nitrogen through the solutions. Irradiation at 254 nm was with a low-pressure Hg-lamp (Hanovia, 75 W). The incident intensities amounted to 2.14×10^3 erg $\times$ mm$^{-2} \times$ min^{-1}.

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3. Results and discussion

At the beginning we studied the effect of caffeine on irradiated, nicked primer DNA. Fig. 1 indicates that caffeine in the concentration range 8×10^{-7} to 8×10^{-3} M does not show any effect on the polymerase activity. This confirms the results of Mouton and Fromageot [9], they also could not observe any effect of caffeine at concentrations of 1×10^{-3} to 5×10^{-3} M on DNA polymerase. In addition we can point out that caffeine 8×10^{-7} to 8×10^{-3} M does not affect DNA polymerase using unirradiated, nicked DNA as template.

Using UV-irradiated DNA as primer there was also no decrease by caffeine in the incorporation rate of [^{3}H]dTTP (figs. 2 and 3). On the contrary we can state that caffeine promoted the incorporation of thymidine nucleotide in some cases. So the template activity of slightly UV-irradiated DNA in fig. 2 is increased by caffeine at low ($< 8 \times 10^{-5}$ M) and at high concentrations.

Using very highly UV-irradiated DNA the effect of caffeine is different. Concentrations of 8×10^{-7} to 8×10^{-4} M are without any effect on DNA polymerase activity but high concentrations ($> 8 \times 10^{-4}$ M) bring about an increase of incorporation rate. The effect is the same whether nicked or unnicked DNA is used as template (fig. 3). In both cases the incorporation rate of [^{3}H]dTTP is not reduced by caffeine at concentrations below 8×10^{-4} M but it is increased by higher ones.

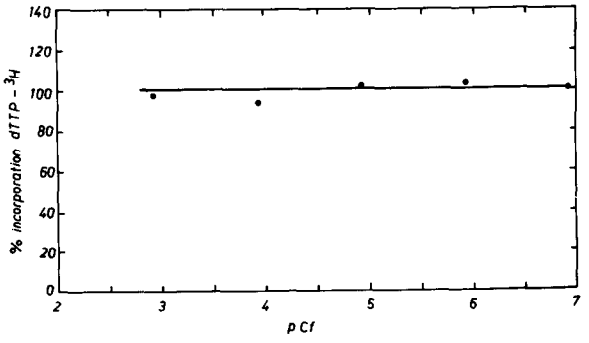

Fig. 1. The incorporation of [^{3}H]dTTP as a function of caffeine concentration in the *in vitro* polymerase system using nicked, unirradiated calf thymus DNA as primer. pCf = negative logarithm of caffeine concentration.

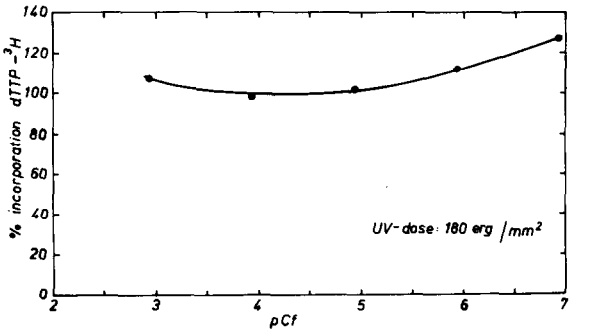

Fig. 2. The effect of caffeine on the incorporation rate of the *in vitro* polymerase system using slightly irradiated, nicked calf thymus DNA as template.

Figs. 2 and 3 indicate that two facts must be taken into account in considering the interaction of caffeine with this *in vitro* polymerase system: The time of UV-irradiation on the one hand and the level of caffeine concentration on the other hand. We first discuss the effect of UV-dosage.

In another paper [15] the influence of UV-light on the conformation of DNA has been described. Low doses of UV-irradiation cause conformational changes of the DNA. The DNA molecules are partially twisted and the bases become tilted. From this intermediate state with tilted bases the DNA molecules are converted to a C-like conformation at higher doses. In the intermediate state the conformational changes of DNA can be reversed by caffeine, but the changes of the secondary structure of DNA at high UV-doses can only be reversed to a small extent by caffeine (H. Lang, in pre-

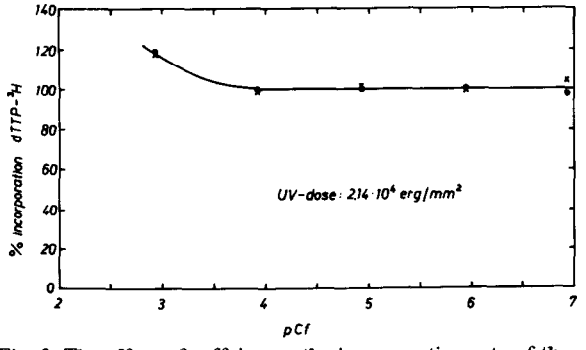

Fig. 3. The effect of caffeine on the incorporation rate of the *in vitro* polymerase system using highly irradiated, nicked (•) or unnicked (×) calf thymus DNA as template.

paration). Thus the effect of caffeine at low concentrations on the template activity of UV-irradiated DNA can be explained as follows. Caffeine molecules bind to DNA near the region of UV-induced conformational changes, reverse these changes and produce the increase of incorporation relative to the UV-irradiated DNA template without caffeine. At high UV-doses caffeine is not able to reverse the conformational changes and the rate of synthesis is uninfluenced. The increase of [^{3}H]dTTP incorporation observed at high concentrations of caffeine ($> 8 \times 10^{-4}$ M) must be considered in connection with the indirect binding processes of caffeine [16]. At this high concentration caffeine causes a destabilization of the secondary structure of DNA. Due to this destabilization the UV-damaged and unstable DNA molecules will be further destabilized and broken. As a consequence the incorporation rate is increased. On the basis of these results we can conclude that currently held ideas about the mode of action of caffeine on DNA polymerase are not correct and other possibilities must be taken into consideration.

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