Effect of DNA photosensitization mediated by promazine derivatives on transcription in vitro.

Promazine derivative (PZD) photosensitization is known to induce loss of bacteriophage infectivity (Merville et al., 1983). Since one of the primary events in replication of DNA viruses is DNA transcription, studies were undertaken in order to investigate the effects of DNA-PZD photosensitization on this transcription. SV40-DNA F1 was chosen because under definite temperature conditions (18 °C) and under a definite RNA polymerase (E. coli) DNA molar ratio (1:3) one specific initiation site of transcription is used on the SV40 genome (Reisig & Hearst, 1981). SV40 DNA F1 was isolated from infected Vero (African green monkey) kidney cells. SV40 DNA (36 μg/ml) complexes with the various PZD (0.5 mM) was irradiated with near-UV light (λ > 290 nm, Osram XBO150, 67 W/m²) in Tris pH 7.4 buffer. Aliquots (10 μl, 360 ng of DNA) were removed after increasing periods of irradiation. DNA was ethanol precipitated, washed with 70% ethanol in order to extract the free sensitizer. Transcription experiments were carried out according to Reisig & Hearst (1981). DNA pellets were resuspended in the initiation mixtures containing α32P-ATP, GTP, UTP and ApA dissolved in-HEPES pH 8.0 buffer. RNA polymerase (70 ng, Boehringer) was added and initiation of transcription was carried out at 18 °C for 5 min. The elongation step was allowed by addition of the four nucleotides and reinitiation was prevented by addition of heparin. Before stopping the reaction, aliquots were removed in order to determine the 32P incorporation by TCA precipitation. The nucleic acid precipitates were denatured and analysed by 7 M urea - 5% PAGE. In order to determine the termination sites of the RNA synthesis on PZD-photosensitized DNA, sequencing channels were run in parallel on the gel. RNA sequencing was performed by the inhibitor method as described by Reisig & Hearst (1981).

The amount of transcript was determined by measurement of 32P incorporation. The PZD-induced DNA photodamages led to a decrease in the amount of transcript. The efficiencies of PZD to promote these damages varied according to the scale: chlorpromazine - methoxy promazine - trifluoromazine - promazine. Acepromazine had no measurable effect. However, no termination site was detected on the gel.

We conclude that the PZD photoactdus on DNA (Merville et al., 1984) were unable to stop transcription and transcription inhibition was caused by DNA single-strand breaks known to be induced by PZD phototreatment (J. Decuyper et al., in preparation).

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References


J. Piette, J. De biologie gen Université de
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