EFFECTS OF UV DAMAGE ON DNA AMPLIFICATION BY THE POLYMERASE CHAIN REACTION

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INTRODUCTION

Exposure of DNA to ultraviolet radiation can result in DNA damage; prominent forms of damage are pyrimidine dimers, DNA strand cross-links, and DNA-protein cross-links (Friedberg, 1985). Many studies have demonstrated that the extent of DNA damage increases as the level of UV exposure increases. DNA in evidence samples may suffer some degree of UV damage and thus it is important to know what effect UV damage might have on DNA analysis. We have investigated the effects of UV damage, specifically pyrimidine dimer formation, on the polymerase chain reaction (PCR).

METHODS AND MATERIALS

Both "naked" DNA and DNA in whole cells were exposed to measured doses of short (254nm) and long (365nm) wave UV irradiation. Purified genomic DNA was exposed in solution and whole blood and semen were exposed in both the liquid and dried stain forms. Exposures ranged from 0 to 30,000 J/M^2 as measured by an UV exposure meter. DNA from the UV exposed samples was isolated by standard procedures (Gill, et al, 1985) and amplified by PCR for 30 cycles using the thermostable Taq polymerase (Saiki, et al., 1988); the target sequences for amplification were a 110bp sequence from the ß-globin gene and the 242bp typing sequence from the HLA DQã gene (Saiki, et al., 1986). The quantity of PCR product produced for each sample was then assessed using agarose gel electrophoresis and ethidium bromide staining. DNA sequencing was performed by the Sanger di-deoxy method using the protocol and materials supplied with the Sequenase™ ver. 2.0 kit (U.S. Biochemical Corp.)

RESULTS

Exposure of "naked" DNA in solution to short wave UV radiation at doses exceeding $200J/M^2$ resulted in reduced PCR product; above $2000J/M^2$, PCR product was lost. In contrast, there was little loss of PCR product from extensively irradiated liquid whole blood, liquid semen, or blood stain material. These results suggest that the DNA in these materials is shielded from UV exposure. Irradiation of dry semen stains with short wave UV resulted in greatly reduced DNA recoveries with corresponding PCR product reductions. Irradiation of DNA in solution or of body fluids with long wave UV had no detectable effect on PCR, regardless of dosage.

Analysis of primer extension products gives evidence that Taq polymerase is blocked at pyrimidine dimer sites (Fig. 1). Samples of purified ß-globin PCR fragment DNA in solution were

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exposed to measured doses of short wave UV. The irradiated DNA was used as the template for a one cycle extension using a labeled primer and the reaction products were analyzed on a sequencing gel alongside sequence control lanes. The reaction products were fragments terminating at adjacent purines on the extending strand; this reflects termination at adjacent pyrimidines on the template strand. Doses up to $200J/M^2$ resulted in fragments which were fairly evenly distributed along the length of the template sequence. Higher doses resulted in a shift of the fragment distribution toward shorter fragments; doses above $7200J/M^2$ resulted in virtually no extension.

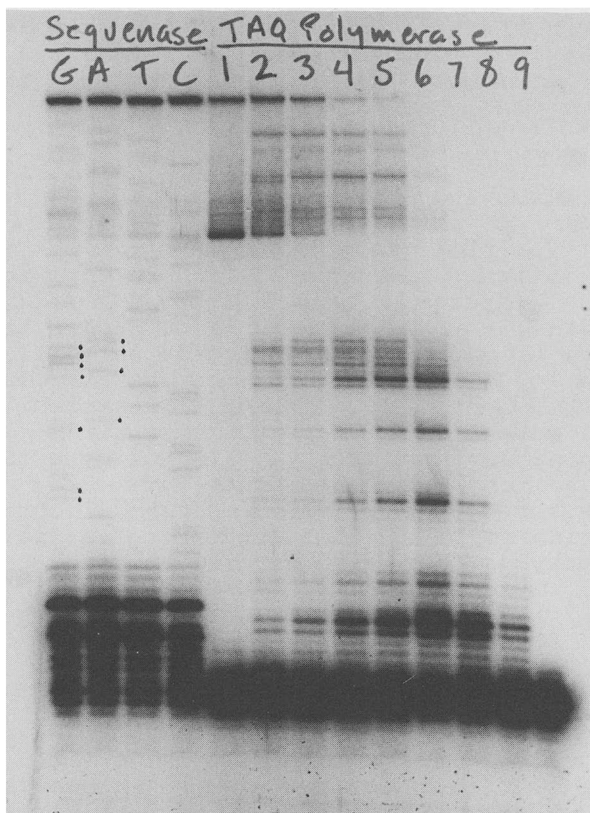

Fig. 1. Primer extensions by Taq polymerase on UV irradiated template DNA. Template strand exposures (in J/M^2) were 1, none; 2, 30; 3, 60; 4, 180; 5, 300; 6, 900; 7, 3600; 8, 7200; and 9, 14400. The sequencing reactions were run on un-irradiated DNA. Electrophoresis was on 7% acrylamide gels.

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CONCLUSION

The DNA in evidence materials appears to be shielded from direct exposure to damaging UV radiation. It is thus unlikely that evidence DNA will contain significant DNA damage unless it is exposed to direct, unfiltered sunlight for extended periods. This work indicates that if evidence DNA is UV damaged, its amplification by PCR will be blocked.

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