

## Effects of Solar Ultraviolet Photons on Mammalian Cell DNA

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### Introduction

Solar ultraviolet and visible-light photons are strongly implicated in the etiology of human skin cancers (basal and squamous carcinoma, as well as malignant melanoma). The range of photons reaching the earth's surface extends from 290 nm, the boundary between UVC and UVB, to visible light; there is a measurable trace at 290 nm and the flux increases throughout the UVB (280-320 nm) and UVA (320-400 nm) regions (1). The carcinogenic effects of the UVC and UVB wavelengths have been known for decades (2), and recent evidence supports an important role for photoreactivable dimers in the process (3). This is consistent with the high probability that UVB photons will be absorbed by DNA, (4), resulting in the formation of photoproducts (especially pyrimidine photoproducts, which are produced in photochemical reactions involving the excited singlet or triplet state). The UVB region becomes even more important in terms of the possible environmental increase in UVB flux due to stratospheric ozone depletion (5).

UVA photons, long considered harmless, have recently been shown to be carcinogenic (6, 7). This is paradoxical, since the DNA absorption spectrum indicates a negligible probability that photons of wavelengths longer than 320 nm will be directly absorbed (8).

The observation of UVA carcinogenesis motivates the study of interactions between UVA photons and DNA. One strategy is to irradiate cultured cells with monochromatic, purified photons of UVA (available in the emission from a high-pressure mercury arc lamp). A practical advantage of using monochromatic rather than broad-band sources is

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that the latter comprise a mixture of photons which may have very different effects, and a minor component at the short-wavelength end of the broad band source may contribute overwhelmingly to the biological action (especially in the case of DNA effects, because UVB is much more efficient than UVA). Thus the contribution of the latter may be obscured (9).

A practical problem with working in the UVA region is the low fluences of radiation available with current sources, which results in long exposure times. For instance, at 365 nm, the  $F_{37}$  (fluence required to kill 37% of the population) of human P3 teratocarcinoma cells is more than  $1 \text{ MJ}^{-2}$  (10), which translates to about 30 minutes exposure at the fluence rate available from a 2500-W high-pressure mercury source with a monochromator. Further, because DNA repair may take only minutes, for instance,  $t_{1/2} = 2.5 \text{ min}$  for repair of single-strand breaks produced by x-rays in human epithelioid P3 cells (11), all breaks produced by 365-nm photons would be concomitantly repaired by the cells unless the repair were inhibited. Our practice is to keep the cells at  $0.5^\circ \text{C}$  during exposures, which slows the metabolic repair processes to beneath detectable levels.

#### Spectrum of DNA Changes Caused by Solar UV Photons

Both the type of DNA lesion and the chemical mechanisms (see below) whereby it is produced depend markedly on wavelength. Pyrimidine photoproducts, various isomers of cyclobutane-type (12), and (6-4) pyrimidine photoproducts (13) are produced in DNA by the direct DNA absorption of UVB photons. Evidence for this comes from the close correlation between the absorption characteristics of DNA between 254 and 334 nm and the action spectrum for the induction of these lesions (14). Although detectable, the yields of pyrimidine photoproducts induced by UVA photons are low by comparison, especially at longer wavelength photons, such as 365 nm. At 405 nm, pyrimidine dimers are not detectable (15).

UVA photons produce DNA lesions more characteristic of ionizing radiation: single-strand (15,16) and double-strand (17) DNA breaks and DNA-to-protein crosslinks (DPCs) (18). In the specific case of single-strand breaks induced by 405 nm photons in human P3 epithelioid cells, about half the breaks assayed by the alkaline elution technique are alkali-labile sites, as revealed by the convex shape of the elution profiles (19). Such profiles were not observed for other cell types such as Chinese hamster ovary cells (20) exposed to 405-nm photons; the nature of the alkali-labile sites produced in the human cells remains enigmatic.

Kleiman *et al.* (21) described the induction of DNA-DNA interstrand crosslinks in bovine lens cells exposed to a UVB broad-band source. There was no mention of the use of proteinase K in these experiments, therefore, they did not distinguish between DPCs and DNA-DNA crosslinks. We have only observed a barely detectable yield of DNA-DNA crosslinks after UVB exposures (i.e., crosslinks remaining after exposure to proteinase K).

Finally, no information is as yet available on the induction of such lesions as thymine glycols, urea residues, or apurinic sites by UVA photons in mammalian cells (22).

#### Initial Yields of Specific Lesions

A compilation of the initial yields (with cells meticulously kept cold throughout exposure and thereafter until lysis) of single-strand breaks and DPCs compared with pyrimidine dimers was presented by Peak and Peak (9; updated 23), and the yield for double-strand breaks was described by Peak and Peak (17).

The most striking observation is the initial yields of single-strand breaks and DPCs produced by UVA radiation, more than an order of magnitude greater than the yields produced by ionizing radiation when analyzed on the basis of the lethal dose. This implies that if DNA breaks and DPCs are critical lesions, those inflicted by ionizations may be of a more deleterious type than those produced by UVA photons. One way to

study the severity of DNA lesions induced by radiation is to follow their repair within the cell.

#### Cellular Repair of DNA Lesions Induced by UVA Photons.

Many studies have reported on the repair of the pyrimidine dimers (24) and (6-4) adducts (25,26) caused by UVB radiation. More efficient repair of (6-4) photoproducts than dimers was reported (27). Repair of pyrimidine dimers, (6-4) photoproducts, Dewar isomers, and DNA breaks in human fibroblasts exposed to simulated radiations was described (25). The repair of pyrimidine dimers in mammalian skin exposed to artificial solar radiation was described by Strickland and Creasey (12), using hamsters. In the marsupial *Monodelphis domestica*, which possesses photolysase enzyme activity, both excision repair and concomitant photoreactivation of these two types of lesions were reported by Mitchell *et al.* (28).

Far fewer studies have been carried out on the repair of the types of lesions characteristically produced by UVA radiation, such as DNA breaks and DPCs. The consensus about single-strand breaks, as is the case with the breaks produced by ionizing radiation, is that normal mammalian cells have efficient enzyme systems that take care of these lesions (29,30; both studies using human lymphocytes). More recently, Peak *et al.* (31) described complete repair of the breaks induced by 365-nm monochromatic UVA in human epithelioid cells was seen, but in xeroderma pigmentosum cells, a fraction of the breaks were unrepairable. Churchill *et al.* (20,32) reported complete repair of breaks by wild-type CHO cells, AA8, whereas the repair-deficient mutant, EM9, showed inhibition of DNA repair after exposure to 365-nm photons, and to a lesser extent, 405-nm near-violet, and 434-nm blue-light photons. These studies demonstrate that break repair is important for cells exposed to UVA and visible light photons and that unrepaired DNA strand breaks might account for observed cellular sensitivity to these radiations.

Only one published study analyzed the cellular repair of DPCs induced by solar photons. Rosenstein *et al.* (33) followed crosslink processing in various frog cell lines exposed to an artificial solar source. The lesions increased during repair incubation, then were completely removed by 24 hours. Clearly, repair responses for this lesion are different from the response to DNA breakage. In unpublished experiments, we have confirmed an increase in DPCs in epithelioid cells exposed to a potent crosslinking regimen -- 405-nm photons in the presence of protoporphyrin IX -- during repair incubation, followed by partial elimination (25% remaining) of the lesions after 24 hours of repair incubation. More work is necessary to further elucidate this pathway.

At each wavelength studied, double-strand breaks were only detected at fluences greatly exceeding the  $F_{37}$  value (17). Any repair studies would thus involve a population of thoroughly dead cells. Analysis of the biological significance of these particular events will have to await the development of more sensitive assays.

#### Mechanisms of DNA Damage by Solar Photons

The mechanisms whereby photons in the UVB region exert their effects on DNA (direct triplet excitation and system crossing over) are well known. In the UVA region, the mechanisms are completely different and operate by photodynamic mechanisms of photosensitization. For instance, a role for oxygen in DPC formation and strand breakage was demonstrated by Rosenstein and Ducore (34) and Peak *et al.* (11,18). The pathways whereby ground state molecular oxygen becomes excited must involve nonDNA endogenous sensitizers, because action spectroscopy reveals that more events occur than are justified by the absorption of DNA. Riboflavin, porphyrins, quinones, and bilirubin, amongst others, are suspected to be sensitizers in mammalian cells. (34,23). The nature of the reactive oxygen species and the actual sensitizers involved is a complex issue that has yet to be resolved, as described by Kochevar and Dunn (35). Recent evidence using an isolated DNA model strongly supports a role for the hydroxyl radical in strand cleavage caused by 365-nm photons (36), and studies in which putative sensitizers were

irradiated showed that various UVA photons can produce a superoxide anion (37,38). However, there is little experimental evidence to prove that these species are of importance in mammalian cells. Sensitization of DNA damage in mammalian cells by an aqueous environment of deuterated water compared with light water is taken as evidence for a role for singlet molecular oxygen (11,18), although the decay behavior of other oxygen radicals such as superoxide anion in deuterated water is not known. Finally, although long suspected as an important molecule in UVA effects,  $H_2O_2$  has recently been shown to be insignificant in DNA breakage by 365-nm photons (31).

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