Journal of Photoscience (2002), Vol. 9(2), pp. 488–490

Photodamage to Double-stranded DNA by Xanthone Analogues Increases Exponentially with Their HOMO Energies

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DNA photodamage mediated by photosensitizers are believed to play an important role in solar UVA carcinogenesis. We investigated the relationship between the DNA-damaging abilities of photoexcited xanthone analogues (as photosensitizers) and their highest occupied molecular orbital (HOMO) energies. DNA damage was examined using ^{32}P-labeled DNA fragments obtained from the *p53* tumor suppressor gene. These compounds induced DNA photodamage in a similar manner, and the extents of DNA damage were following order: xanthone > thioxanthone > acridone. Photoexcited xanthone caused nucleobase oxidation specifically at 5'-G of GG sequence in double-stranded DNA. An oxidative product of 2'-deoxyguanosine, 8-hydroxy-2'-deoxyguanosine (8-OHdG), was detected, and the amount was decreased by DNA denaturation. These findings suggest that photoexcited xanthone generates 8-OHdG at 5'-G of GG in double-stranded DNA through electron transfer. The calculated HOMO energies of these photosensitizers decreased in the following order: xanthone > thioxanthone > acridone. This study has demonstrated that DNA-damaging abilities of these photosensitizers increased exponentially with an increase in their HOMO energies.

Key words: UVA, DNA damage, HOMO energy, 8-OHdG, Electron transfer

INTRODUCTION

Solar UV light is a well-known carcinogen. Recent studies have demonstrated that solar UVA induces skin tumors in animals as well as UVB [1]. It has been reported that the mutagenic specificity of mutational spectrum of solar light in cells is not determined entirely by the UVB and that UVA greatly contributes to solar light-induced mutation [2]. Because only little UVA can be absorbed by the DNA, solar carcinogenesis would involve UVA-induced DNA damage mediated by activated photosensitizers. Photosensitizers from drugs and/or foods are believed to participate in UVA carcinogenesis. We have previously demonstrated that in the presence of

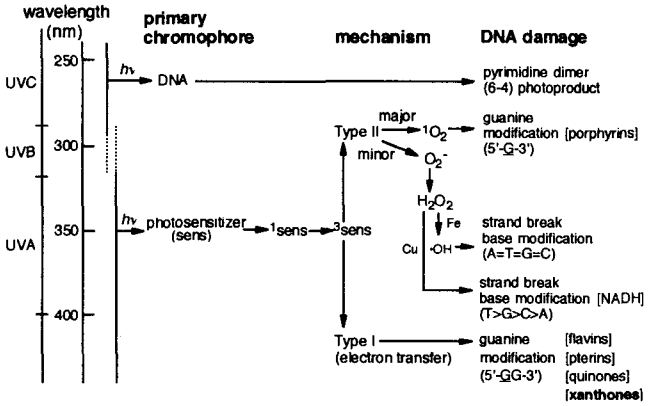

Figure 1. Mechanism of UV-induced DNA damage. Photosensitizers tested in our studies are listed in parentheses [ ].

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various photosensitizers, UVA causes DNA damage through electron transfer (Type I mechanism) and/or reactive oxygen formation (Type II mechanism) (Figure 1) [3-10].

In this study, we investigated the mechanism of DNA damage induced by exogenous photosensitizers, xanthone (XAN), thioxanthone (TXAN), and acridone (ACR). Derivatives of these XAN analogues are widely distributed in various plants and used as drugs. We also examined the relationship between DNA-damaging ability of these photosensitizers and their highest occupied molecular orbital (HOMO) energies.

MATERIALS AND METHODS

XAN and ACR were obtained from Wako Chemicals Co. (Osaka, Japan). TXAN was from Acros Organics (New Jersey, USA). The 5'-end-labeled DNA fragments from the *p53* tumor suppressor gene were prepared as described previously [11].

The standard reaction mixture in a microtube contained XAN analogues, ^{32}P-labeled DNA fragment, and 20 μM calf thymus DNA in 100 μl of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM diethylenetriamine-*N,N,N',N'',N''*-pentaacetic acid (DTPA) and 2.5 v% ethanol. Denatured DNA fragments were prepared by heating at 90 °C for 5 min followed by quick chilling before exposure to UVA light. The mixtures were exposed to 6 J/cm^2 UVA light using 10 W UV lamps (λ_{max}=365 nm). After the irradiation, the DNA fragments were treated with 1 M piperidine at 90 °C for 20 min. The DNA fragments were subjected to electrophoresis on an 8 M urea/8% polyacrylamide gel. The autoradiogram was obtained by exposing an X-ray film to the gel. The preferred cleavage sites were determined by direct comparison of the positions of the oligonucleotides with those produced by the chemical reactions of the Maxam-Gilbert procedure [12]. The amount of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured by the method as described previously [6,13].

Ionization potentials (IP), which indicate the HOMO energies, of XAN analogues were estimated by *ab initio* molecular orbital (MO) calculation at Hartree-Fock 6-31G* level.

RESULTS AND DISCUSSION

DNA damage was observed by UVA irradiation in the presence of 0.5 μM of XAN, and the extent of DNA damage increased depending on the concentration of XAN. XAN did not induce DNA damage without UVA irradiation. DNA photodamage induced by XAN was observed only when the DNA fragments were treated with piperidine, suggesting that DNA damage is due to base modification with little or no strand breakage. Photoexcited XAN caused DNA damage specifically at 5'-G of GG sequence in double-stranded DNA, as well as a type I photosensitizer, riboflavin. DNA denaturation decreased the extent of DNA damage and the damage occurred at most guanine residues. Similar DNA cleavage patterns were observed with photoexcited TXAN, and ACR, suggesting that DNA damage is induced in a similar manner by each molecule. As an oxidative product of 2'-deoxyguanosine, 8-OHdG was detected, and the amount was increased depending on the concentration of XAN analogues. DNA denaturation significantly decreased the amounts of 8-OHdG formation.

The scavengers of reactive oxygen species, such as catalase, SOD, and typical $^{\cdot}$OH scavengers (ethanol, mannitol, and sodium formate) showed no or little inhibitory effects on the DNA damage. The DNA damage did not enhanced in D_2O, in which DNA damage by singlet-oxygen ($^1O_2$) could be enhanced due to the elongation of lifetime of $^1O_2$ [9]. These results suggest that reactive oxygen species, such as H_2O_2, $O_2^{\cdot-}$, $^{\cdot}$OH, and $^1O_2$ were not involved in the DNA damage.

These results can be reasonably explained by assuming that nucleobase oxidation is induced by photoexcited XAN analogues through electron transfer (Figure 2). Guanine is most easily oxidized among the four DNA bases, because

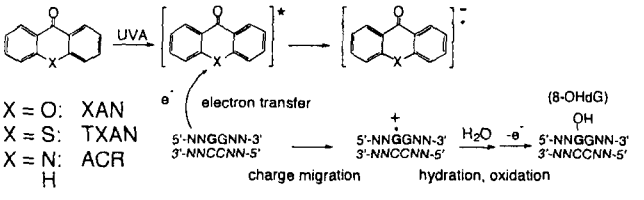

Figure 2. Proposed mechanism of guanine oxidation in double-stranded DNA induced by photoexcited XAN analogues.

the oxidation potential of guanine is lower than the other DNA bases [14,15]. MO calculations have revealed that stacking of two guanine bases in double-stranded DNA significantly lowers the HOMO energy, and electron-loss centers are localized on the 5'-G in GG sequence [16]. Thus, photoexcited XAN analogues oxidize DNA to form the cation radicals of nucleobase. The formed cation radicals are finally localized on the 5'-G in GG sequence through charge migration, and react with water molecule to form the C-8 OH adduct radical, followed by oxidation, leading to the formation of 8-OHdG [14,17]. The formation of 8-OHdG causes DNA misreplication that may lead to mutation such as G•C→T•A transversion [18,19]. Formed 8-OHdG can be converted into further piperidine labile oxidative product such as imidazolone and/or oxazolone [20,21]. Imidazolone forms stable base pair with G comparable with the Watson-Crick G•C base pair [20,21], and may cause G•C→C•G transversion [22,23]. These transversions can partly explain the mutation induced by UVA irradiation as previously reported [2].

The extent of DNA photodamage by XAN analogues increased in the following order: XAN > TXAN > ACR. The amounts of 8-OHdG formations by photoexcited XAN analogues have increased exponentially with an increase in their calculated HOMO energies, which are 8.64 eV (XAN), 8.22 eV (TXAN), and 7.78 eV (ACR). Strictly, DNA-damaging ability of photosensitizer should be determined by not only the oxidation-reduction potential estimated from the HOMO energies but also other factors such as the free energy of the reaction taking into account the solvation, the lifetime of photoexcited state, and non-covalent bonding to DNA. However, the DNA-damaging abilities of these photosensitizers are practically predicted by their HOMO energies.

In summary, this study has shown that photoexcited XAN analogues mediate poly-G-specific DNA damage through electron transfer. The DNA-damaging abilities of XAN analogues increase exponentially with an increase in their HOMO energies. Photo-carcinogenicity of drugs composed of XAN analogues might be speculated by their HOMO energies.

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