DNA Structural Integrity and Base Composition Affect Ultraviolet Light-Induced Oxidative DNA Damage†

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ABSTRACT: We previously demonstrated that ultraviolet (UV) light (254 nm) induced the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in DNA via a singlet oxygen mechanism. In the present paper, we provide novel findings that DNA structure and base composition significantly affect the yield of 8-OHdG by UV radiation. Unlike ionizing radiation that induces 8-OHdG both in free 2′-deoxyguanosine (dG) and in DNA, UV light induced 8-OHdG formation in intact DNA and polydG·dC, but not in dG. When thermally denatured DNA was irradiated with UV light, the yield of 8-OHdG was reduced by more than 80% compared to intact DNA. Oxygenation of the denatured DNA solution did not restore the yield of UV-induced 8-OHdG. Irradiation of DNA with different AT/GC ratios showed that the yield of UV-induced 8-OHdG varied in proportion to the AT content, suggesting that AT base pairs in DNA enhance generation of the oxidizing species and subsequent oxidation of dG. The natural antioxidants genistein, estradiol, protocatechuic acid (PCA), and oleanolic acid (OA) were investigated for their inhibition of UV-induced 8-OHdG. Genistein and estradiol, that intercalate into DNA as shown by a computer modeling, significantly quenched UV-induced 8-OHdG, whereas PCA and OA did not fit into DNA and exhibited weak or no effect. These results suggest that the intercalation of genistein and estradiol into DNA may alter the DNA structural integrity, interrupt the production of oxidizing species, and subsequently reduce the formation of 8-OHdG by UV radiation.

Ultraviolet (UV)[1] light has been known to be an etiological agent of photocarcinogenesis for a long time. One of the proposed mechanisms is that UV radiation induces various photoproducts in DNA, some of which may be responsible for gene mutations and carcinogenesis (*1*, *2*). Although pyrimidine dimers and [6−4] photoproducts are the predominant DNA modifications by UV radiation, particularly short-wave UV (<280 nm), other non-pyrimidine dimer DNA damage has also been reported (*1*−*3*). Reactive oxygen species (ROS) have been known to damage many biological macromolecules, including lipid, protein, RNA, and DNA (*4*−*7*). Increasing evidence has shown that UV radiation is capable of generating ROS, such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and molecular singlet oxygen ($^1O_2$) (*8*−*12*). These ROS, if not scavenged timely, may cause oxidative damage to macromolecules such as DNA (*4*−*7*).

Oxidative DNA damage has been known to be associated with gene mutations, carcinogenesis, and age related-disorders (*4*−*7*), and 8-hydroxy-2′-deoxyguanosine (8-OHdG), a frequently measured indicator or marker of oxidative DNA damage (*6*, *7*), has been implicated in several carcinogenic processes such as kidney (*13*, *14*), liver (*15*, *16*), and skin carcinogenesis (*17*, *18*). The levels of 8-OHdG are significantly increased in several types of cancerous tissues (*19*, *20*). The formation of 8-OHdG in DNA has been shown to be responsible for gene mutations (*21*, *22*) and activation of certain oncogenes such as H-*ras* and K-*ras* (*23*, *24*). Evidence has accumulated that UV and fluorescent light plus photosensitizers are capable of inducing the formation of 8-OHdG (*25*−*33*). Generally, the mechanism of action is thought to be through either the generation of $^1O_2$ from sensitized chromophores (*25*−*28*) or the production of hydroxyl radicals (HO•) from irradiation of coexisting oxidants such as H_2O_2 (*29*).

We have previously demonstrated that a germicidal UV source (254 nm) substantially induced the formation of 8-OHdG in a dose-dependent manner and that the pattern of UV-induced 8-OHdG was different from that induced by either Fenton reaction or ionizing radiation (*32*). We have proposed that $^1O_2$ plays a vital role in oxidation of DNA bases by the short-wavelength UV light. In the current paper, we demonstrate that DNA structure and base composition significantly affect the formation of 8-OHdG by UV radiation.

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[1] Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; dG, deoxyguanosine; UV, ultraviolet; ROS, reactive oxygen species; $^1O_2$, singlet oxygen; HO•, hydroxyl radicals; DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; ECD, electrochemical detection.

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MATERIALS AND METHODS

Chemicals and Reagents. Calf thymus DNA, *Clostridium perfringens* DNA, *E. coli* DNA, *Micrococcus lysodeikticus* DNA, genistein, estradiol, protocatechuic acid (PCA), oleanolic acid (OA), 2′-deoxyguanosine (dG), dimethyl sulfoxide (DMSO), polydT·dA, and polydG·dC were purchased from Sigma Chemical Co. (St. Louis, MO). Nuclease P1 and alkaline phosphatase were purchased from Boehringer Mannheim Co. (Indianapolis, IN). 8-OHdG standard was prepared as previously described (*17*).

Preparation and Pretreatment of DNA Samples. DNA was solubilized in 10 mM Tris-HCl (pH 7.0) to a concentration of approximately 200 μg/mL, and dG was prepared at a concentration of 0.2 μM in 10 mM Tris-HCl buffer. All reactions or treatments prior to UV radiation, e.g., oxygenation of the DNA solution with 100% oxygen for 10 min, thermal denaturation of DNA by boiling for 5 min, or incubation with different reagents, were performed in 2 mL of DNA solution in a 15 mL polyethylene tube. All tested chemicals were added to the DNA solution in 20 μL of DMSO to give a final concentration of 1%. The DNA solutions were then transferred to a 6-well plastic cell culture flask for UV radiation.

Irradiation of Samples. DNA and dG solutions in a 6-well plastic cell culture flask (3.4 cm diameter) were exposed to UV light from a GE 15T8 germicidal lamp with the predominant wavelength of 254 nm. The distance between the UV lamp and the surface of the DNA solution was 20 cm and the solution layer thickness was 0.4 mm. The radiation energy used for most experiments was approximately 5 kJ/m^2 measured with a Model IL 1700 research radiometer/photometer from International Light, Inc. (Newburyport, MA). The ionizing radiation was conducted for 5 min at 400 rad/min as previously described (*32*). After irradiation, DNA was precipitated using 1 M NaCl plus 2 volumes of 100% ethanol. DNA pellets were washed with 70% ethanol, dried in the air, and solubilized in 0.5 mL of 10 mM Tris-HCl (pH 7.0) for enzyme digestion using nuclease p1 and alkaline phosphatase as previously described (*31*).

HPLC−ECD Assay of 8-OHdG. The amount of 8-OHdG and dG in the DNA was measured using high-performance liquid chromatography−electrochemical detection (HPLC−ECD) as previously described (*31*). A Model 400 EC detector (EG&G, Princeton Applied Research, NJ) and a UV spectrophotometric detector were linked in-line with an HPLC (Waters, MA) and a computer using Millennium 2010 Chromatography Manager for data processing and quantitation. All experiments were repeated 2−3 times with each assay performed in duplicate or triplicate. The results were expressed as the number of 8-OHdG per 10^5 dG.

RESULTS

UV and Ionizing Radiation-Induced 8-OHdG Formation in dG and Calf Thymus DNA. UV and ionizing radiation exhibited a different effect on the formation of 8-OHdG in free dG and DNA. Figure 1 shows that UV radiation substantially increases the level of 8-OHdG in calf thymus DNA in a dose-dependent manner, but has little induction of 8-OHdG in free dG. For example, the level of 8-OHdG was increased by more than 400-fold at a UV dose of 12.5 kJ/m^2, whereas the same UV dose barely induced the formation of 8-OHdG (less than 2-fold) in dG. In contrast, exposure to ionizing radiation substantially increased the formation of 8-OHdG in both calf thymus DNA and dG (Figure 2). Further comparison of the yield of 8-OHdG induction by UV irradiation exhibited a decreasing order of DNA > polydG·dC > dG (Table 1).

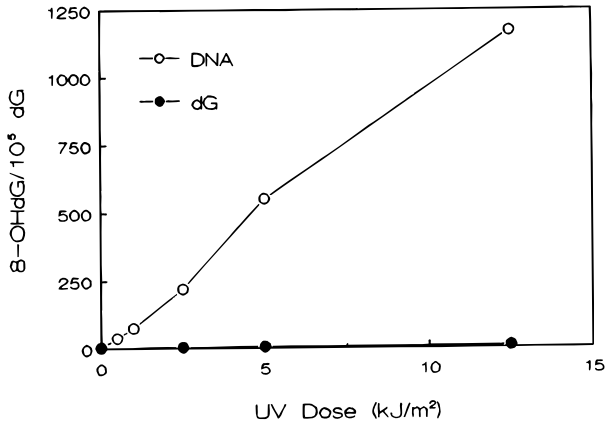

FIGURE 1: UV radiation-induced 8-OHdG in dG and DNA. DNA and dG were dissolved in 10 mM Tris-HCl and irradiated with the selected UV doses. The levels of 8-OHdG were quantitated as described under Materials and Methods. The results are expressed as mean from tow experiments with each assay point of at least four determinations. The standard deviations were less than 10% of the corresponding mean and are not shown in the figure. Open circles are DNA samples, and closed circles stand for dG.

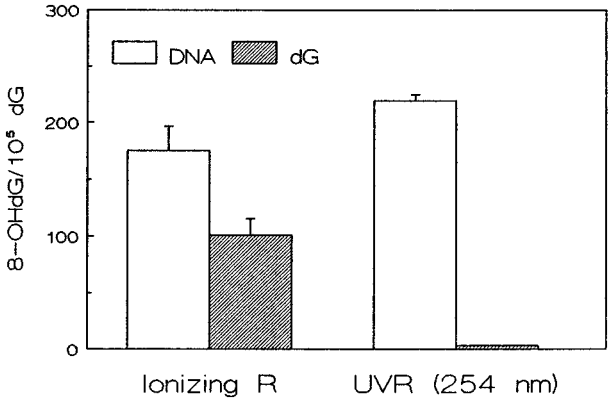

FIGURE 2: Formation of 8-OHdG in dG and DNA by UV and ionizing radiation. DNA and dG were irradiated with UVC (2.5 kJ/m^2) and ionizing radiation (2.5 krad). The levels of 8-OHdG were quantitated by HPLC−ECD. The results are expressed as the means from two to three experiments with standard deviations.

Formation of UV-Induced 8-OHdG in Denatured DNA. To examine the effect of DNA structural integrity on UV-induced 8-OHdG, calf thymus DNA was thermally denatured on a hot plate and then irradiated with UV light. As shown in Figure 3, the yield of UV-induced 8-OHdG was reduced by more than 80% in the denatured DNA as compared to the intact DNA. We previously demonstrated that oxygenation of intact DNA solution enhanced the production of UV-induced 8-OHdG in DNA (*32*). However, this oxygen enhancement could not be demonstrated in the denatured DNA since oxygenation of denatured DNA did not restore the yield of UV-induced 8-OHdG (Figure 3). Thus, this inhibition was not due to deoxygenation during boiling as oxygenation prior to irradiation had essentially no effect.

Table 1: Effect of UV Irradiation on the Formation of 8-OHdG in dG, PolydG·dC, and DNA[a]

treatment	8-OHdG/10^5 dG ($\bar{x} \pm$ SD)	change (%)
dG		
−UVC	1.71 ± 0.44	−
+UVC	2.41 ± 0.20	100
+UVC + dT	2.21 ± 0.32	92
+UVC + dT + dA	2.71 ± 0.1	113
polydG·dC		
−UVC	2.26 ± 0.06	−
+UVC	49.4 ± 5.80	100
+UVC + polydT·dA	37.4 ± 1.24[b]	76
DNA		
−UVC	2.51 ± 0.25	−
+UVC	173.0 ± 8.27	100
+UVC + dT	207.8 ± 5.37 [b]	120
+UVC + dT + dA	227.8 ± 24.2[b]	132

[a] The concentration of DNA is 200 μg/mL, and the concentrations of dG, dT, dA, polydT·dA, and polydT·dA are 0.2 μM. The fluence of UVC is 2.5 kJ/m^2. Results from two independent experiments with each assay performed in duplicate. [b] $p < 0.05$ versus UV irradiation.

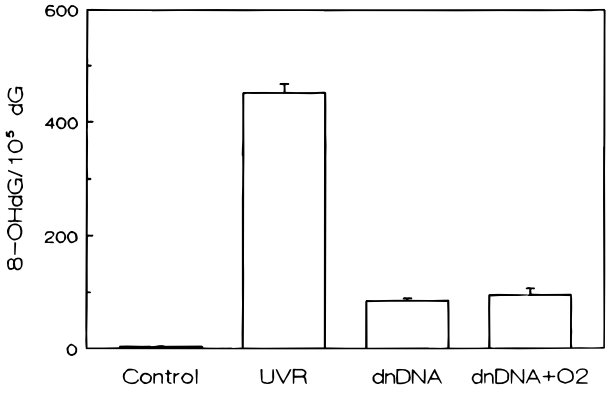

FIGURE 3: Formation of UV-induced 8-OHdG in denatured DNA. DNA was denatured by boiling on a hot plate for 5 min, cooled to room temperature, and then irradiated with UV light at a fluence of 5 kJ/m^2. Oxygenation of denatured DNA was performed by passing 100% oxygen through the solution for 10 min. The results are the means from three experiments with standard deviations. UVR, UV radiation at 254 nm; dnDNA, denatured DNA by boiling.

Effect of AT/GC Base Content of DNA on UV-Induced 8-OHdG. To examine the effect of DNA composition on UV-induced 8-OHdG formation, different types of DNA with various AT/GC ratios were investigated. These included calf thymus DNA, *Clostridium perfringens* DNA, *E. coli* DNA, *and Micrococcus lysodeikticus* DNA. As shown in Figure 4, when different types of DNA were exposed to UV radiation, the formation of 8-OHdG by UV radiation was more pronounced in AT base-rich DNA than in GC base-rich DNA. When dG, polydG·dC, and DNA were irradiated in the presence of dT, the mixture of dT and dA, or polydT·dA, the levels of UV-induced 8-OHdG were increased in DNA, but not in dG and polydG·dC (Table 1). These results indicate that the yield of 8-OHdG by UV radiation correlates proportionately to the AT content of DNA.

Structural Interaction between Quenchers and DNA Molecules. We previously demonstrated that the isoflavone biochanin A substantially reduced the formation of 8-OHdG by UV light, which was independent of the antioxidant activities (*31*). In the present study, we compared the inhibitory effect of natural antioxidants, genistein, estradiol, PCA, and OA (for structures, see Figure 5), on the formation

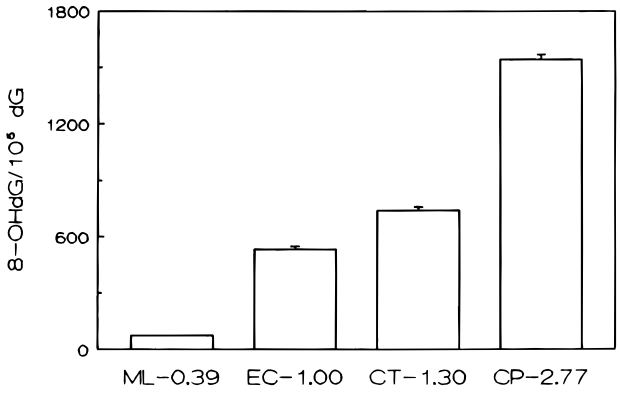

FIGURE 4: Formation of UV-induced 8-OHdG in different DNAs. DNA from different sources was dissolved in 10 mM Tris-HCl buffer (pH 7.0) and irradiated with UVC at a fluence of 5 kJ/m^2. The results are representative of two experiments with each assay performed in duplicate. Abbreviations of DNA: ML, *Micrococcus lysodeikticus*; EC, *E. coli*; CT, calf thymus; CP, *Cloistridium perfringens*. The numbers designate the ratio of AT/GC bases in DNA.

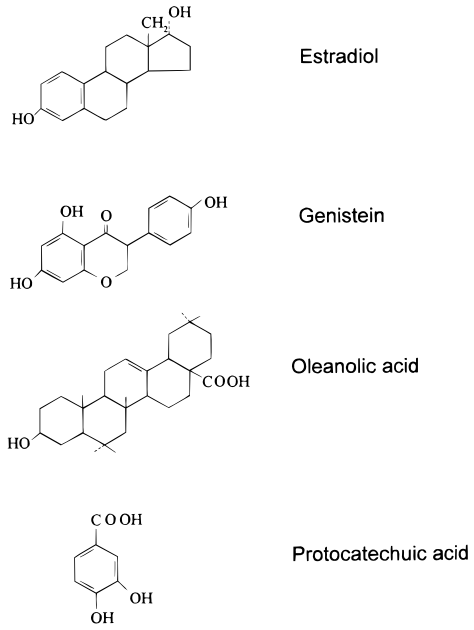

FIGURE 5: Chemical structures of estradiol, genistein, protocatechuic acid (PCA), and oleanolic acid (OA).

of UV-induced 8-OHdG. Figure 6 shows that genistein and estradiol substantially quench UV-induced 8-OHdG, whereas PCA and OA have almost no effect at the comparable concentrations. Computer-simulated modeling indicates that genistein and estradiol intercalate into the supercoiled structure of DNA (Figure 7) whereas the structure of PAC or OA is either too large or too small to fit into DNA in a comparable manner. Further study showed that acridine, a DNA intercalator, also substantially decreased the formation of 8-OHdG by UV irradiation despite structure difference with genistein and estradiol (data not shown). This structural interaction between DNA and tested compounds may play an important role in the inhibition of UV-induced 8-OHdG.

DISCUSSION

Reactive oxygen species (ROS) have been known to cause the oxidative modification of DNA bases (*4*−*7*). It has been shown that oxidation of DNA bases can be induced by

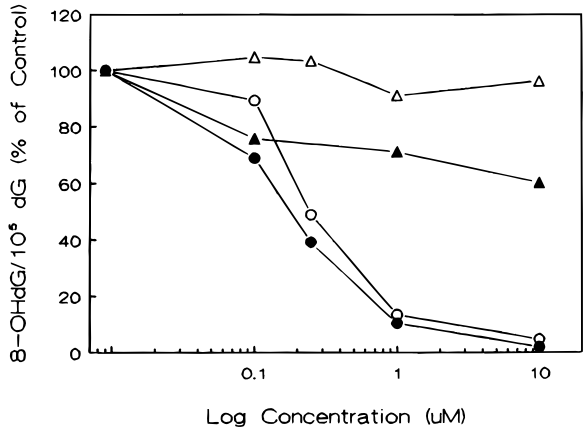

FIGURE 6: Inhibitory effect of tested compounds on UV-induced 8-OHdG. DNA was coincubated with different agents and irradiated with UV at a fluence of 5 kJ/m^2. The results are the means of at least three determinations and normalized to the percentage versus UV radiation only. The standard deviations are less than 10% of the mean and are not shown in the figure. Legend: genistein, closed circles; estradiol, open circles; PCA, closed triangles; and OA, open triangles.

exposure to redox cycling chemicals, Fenton reaction, respiratory burst in phagocytosis, ionizing radiation, and UV light (*4*−*7*). Generally, HO$^\bullet$ is considered to be the most common and deleterious ROS which nonselectively oxidize DNA bases (*33*). In the Fenton reaction system and ionizing irradiation, 8-OHdG is produced through generation of HO$^\bullet$ (*4*−*6*). In systems containing photosensitizers such as methylene blue and riboflavin plus long-wavelength light irradiation, 8-OHdG is predominantly formed via production of $^1O_2$ (*25*, *26*). Fisher-Nielsen et al. (*27*, *28*) reported that exposure of calf thymus DNA and V79 Chinese hamster cells to UV light (consisting of the unfiltered output of a mercury lamp or sun lamp) significantly increased the formation of 8-OHdG in DNA. More recently, we have provided evidence that $^1O_2$ is exclusively involved in the short-wave UV-induced 8-OHdG (*32*). We also found that inhibition of UV-induced 8-OHdG by certain isoflavones was independent of the antioxidant activities (*31*), suggesting that other mechanisms may be involved in inhibition of UV-induced 8-OHdG.

In the present study, we have demonstrated that UV radiation induced the formation of 8-OHdG in DNA and polydG·dC in a dose-dependent manner, but not in free dG. In contrast, ionizing radiation induced the formation of 8-OHdG in both DNA and free dG bases in amounts roughly proportional to the amount of dG in solution (Figure 2). This result corroborated our previous observation that UV radiation induced the formation of 8-OHdG through a mechanism different from that of the ionizing radiation (*32*). UV radiation most likely induces the formation of 8-OHdG in DNA through the generation of $^1O_2$, whereas ionizing radiation acts primarily via the radiolysis of water into HO$^\bullet$ (*33*). The current study confirms that UV radiation and ionizing radiation act differently on the induction of 8-OHdG in DNA and dG. For ionizing radiation, the major oxidizing species is HO$^\bullet$ that nonselectively oxidizes all four types of DNA bases, whereas UV radiation primarily generates $^1O_2$ that selectively oxidizes guanine (*33*). The oxidizing species generated by ionizing radiation occurs in solution and is

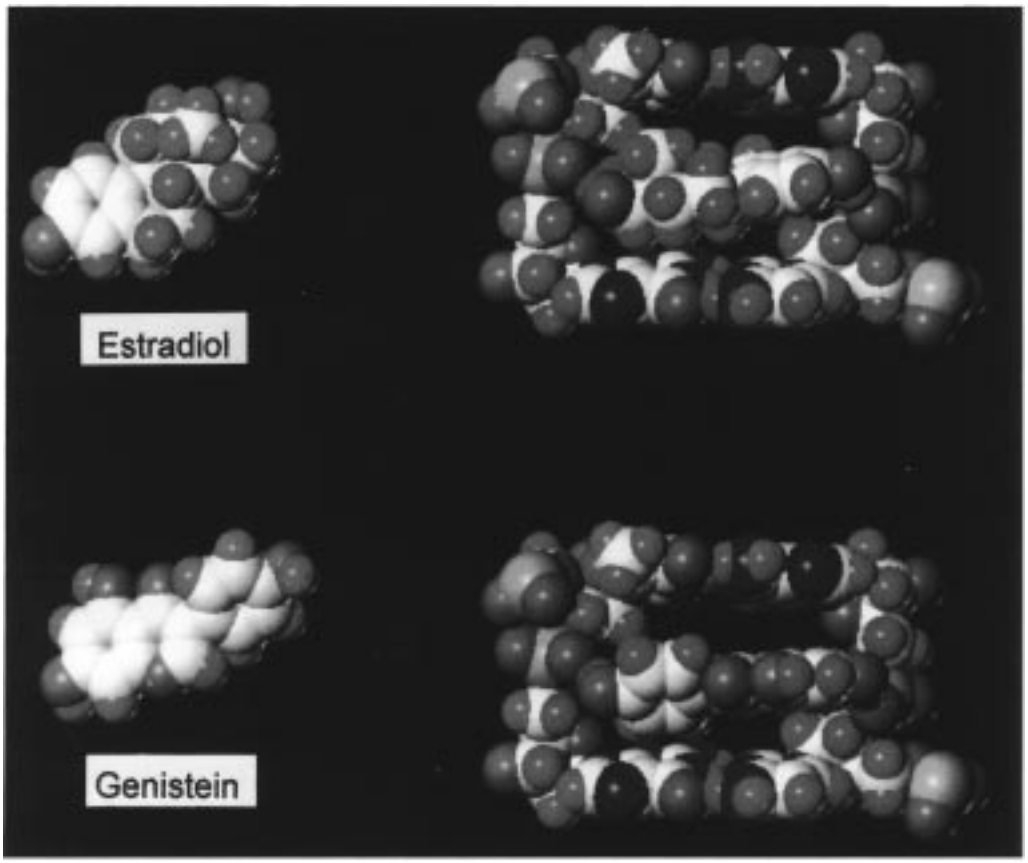

FIGURE 7: Fitting of estradiol and genistein into DNA by computer modeling. The computer-generated graphic was kindly provided by Dr. Lawrence B. Hendry from the Medical College of Georgia, Augusta, GA.

primarily a biophysical reaction independent of the DNA structure. Thus, oxidation of DNA bases may occur in both free dG and intact DNA. In contrast, UV radiation requires the absorption of energy by base chromophores in DNA to ultimately generate certain types of oxidizing species, such as singlet oxygen. This process is apparently far more dependent on the DNA structure. It appears the DNA structural integrity plays a crucial role in the generation of oxidizing species and subsequent induction of 8-OHdG upon UV irradiation.

Study on free dG, polydG·dC, and denatured DNA provides evidence for the importance of structural integrity. Free dG is a substrate for the induction of 8-OHdG by the Fenton reaction and ionizing radiation. However, UV light is unable to induce the formation of 8-OHdG in free dG unless there is an intact DNA structure (Figures 1 and 2). In addition, UV irradiation is able to induce the formation of 8-OHdG in polydG·dC, but far below the level induced in intact DNA. Our results have showed the levels of UV-induced 8-OHdG are significantly different with the order of DNA > polydG·dC > dG (Table 1). The experiment with the thermally denatured DNA indicates that the yield of UV-induced 8-OHdG is substantially reduced when the structural integrity of DNA is disrupted. In addition, oxygenation of DNA solution does not enhance the yield of UV-induced 8-OHdG in the denatured DNA (Figure 3) as it does in the intact DNA (*32*), suggesting the importance of structural integrity.

In addition to the DNA structural integrity, we also investigated the effect of DNA base composition on UV-induced 8-OHdG using various DNAs with different AT/GC ratios. Our results show that the yield of UV-induced 8-OHdG is in proportion to the AT content of the DNA (Figure 4). The exact mechanism whereby the formation of 8-OHdG is substantially enhanced by UV light in AT-rich DNA remains unknown. However, we propose that thymidine in DNA may serve as an intrinsic photosensitizer that mediates the generation of ROS upon UV radiation. It is hypothesized that excitation of DNA with UV radiation activates thymidine to a triplet from a singlet ground state and that triplet thymidine transfers its energy to molecular oxygen to generate a reactive singlet state, e.g., $^1O_2$, which is responsible for the formation of 8-OHdG. In support of this mechanism, we note: (A) thymine has the lowest lying triple state of the four bases in DNA (*34*); (B) excitation of thymine but not guanine leads to singlet oxygen (*35*); and (C) the intersystem crossing for guanine at room temperature is weak, leading to low levels of triplet being formed (*36*). Further experiments demonstrate that the presence of exogenous dT or mixed dT and dA increases the level of UV-induced 8-OHdG by more than 20−30% in DNA but not in dG or polydG·dC. This study provides the direct evidence that dT plays an important role in production of 8-OHdG by UVC irradiation.

We previously demonstrated that the isoflavone biochanin A potently inhibited UV-induced 8-OHdG, which was independent of its antioxidant activities. We have proposed that the interaction between this quencher and DNA either alters DNA structure or inserts into the DNA for site-specific protection (*31*). This hypothesis was further tested by studying the interaction between other quenchers and DNA. In the current study, we compared several compounds, genistein, estradiol, PCA, and OA, for their inhibitory effects on UV-induced 8-OHdG (for structures, see Figure 5). The results show that genistein and estradiol potently quench UV-induced 8-OHdG, whereas PCA and OA have almost no effects at the selected concentrations (Figure 6). However, the inhibition of UV-induced 8-OHdG was independent of antioxidant activity since PCA was demonstrated to be equally potent in scavenging of hydrogen peroxide in comparison with genistein and estradiol (data not shown).

Hendry et al. (*37*−*39*) have proposed an "insertion hypothesis" to describe the interactions between DNA and certain biological molecules. In their hypothesis, intercalation of a molecule into the supercoiled structure of DNA through the best fit, but not covalent binding may exert a significant biological effect. In the current situation, the isoflavone or flavone core structure may account for this "favorable structural fitting interaction". With the help of Dr. L. Hendry, a computer modeling graphic has been generated which shows that genistein and estradiol intercalate into the DNA, whereas the molecules PCA and OA are either too small or too large for a proper fit. This "fitting or insertion into DNA" model correlates well with the inhibitory capacity of UV-induced 8-OHdG. We have shown that acridine, a DNA intercalator, also substantially reduced the level of UV-induced 8-OHdG in DNA. Thus, intercalation into DNA may alter the DNA structural integrity and inhibit the formation of 8-OHdG by UV radiation. This may explain, at least in part, the mechanism of inhibiting UV-induced 8-OHdG by isoflavone and related compounds.

In summary, we corroborated the previous observation that UV radiation induced the formation of 8-OHdG through a different mechanism than that of ionizing radiation and Fenton reaction. UV radiation induced 8-OHdG in DNA and polydG·dC, but not in free dG. The yield of 8-OHdG by UV radiation was substantially reduced in the thermally denatured DNA. The yield of UV-induced 8-OHdG was proportionally correlated with the AT content of the DNA. Intercalation of genistein and estradiol into the DNA may alter DNA structure and subsequently quench the formation of UV-induced 8-OHdG. Therefore, we conclude that DNA structural integrity and thymidine content significantly affect the formation 8-OHdG by UV radiation.

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