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The Release of 5-Methylene-2-Furanone from Irradiated DNA Catalyzed by Cationic Polyamines and Divalent Metal Cations

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Abstract

Release of 5-methylene-2-furanone (5-MF), a characteristic marker of DNA deoxyribose oxidative damage at the C1' position, was observed in significant quantities from X-irradiated DNA. This observation, which held for DNA irradiated either in aqueous solution or as a film, requires postirradiation treatment at 90° C in the presence of polyamines and divalent metal cations at biological pH. The 5-MF product was quantified by using reverse-phase HPLC. The radiation chemical yield of 5-MF comprised more than 30% of the yield of total unaltered base release. Polylysine, spermine and Be(II) showed the strongest catalytic effect on 5-MF release, while Zn(II), Cu(II), Ni(II), putrescine and Mg(II) were substantially less efficient. We have hypothesized that the 5-MF release from irradiated DNA occurs through catalytic decomposition of the 2' - deoxyribonolactone (dL) precursor through two consecutive β - and δ -phosphate elimination reactions. A stepwise character of the process was indicated by the S-shaped time course of 5-MF accumulation. If dL proves to be the precursor to 5-MF formation, it would then follow that dL is a very important lesion generated in DNA by ionizing radiation.

INTRODUCTION

Oxidation of DNA deoxyribose at the C1' position plays an important role in the chemistry of DNA damage by a variety of agents, such as (1,10-phenanthroline) copper complex [(OP)₂Cu] (1–8), enediyne antibiotics (9–12), cationic manganese porphyrins (13,14), chromium (V) complexes (15,16), oxoruthenium complexes (17), UV light (18), and ionizing radiation (19–22). As shown in Scheme I, 2' -deoxyribonolactone (dL), an alkaline-labile lesion that is structurally related to an abasic site, is believed to be an intermediate of the C1' pathway. dL is relatively unstable and readily undergoes β - and δ -elimination reactions of the 3' - and 5' - phosphate fragments, respectively, to give rise to DNA strand cleavage and a low-molecular-weight product, 5-methylene-2-furanone (5-MF) (3,5,23).

So far, little is known about the role of the C1' chemistry in DNA damage by ionizing radiation. Oxidation of DNA sugar at the C1' position upon γ irradiation of aqueous solutions of DNA (20) or 2' -deoxythymidine (19) or of frozen aqueous solutions of 2' -deoxycytidine (22) has been reported. Formation of 2-deoxy-1,4-ribonolactone, a low-molecular-weight analog of dL, has been detected for irradiated 2' -deoxythymidine (19) and 2' -deoxycytidine (22). However, the significance of this pathway in the radiation chemistry of DNA is still poorly understood. In particular, the estimates based on the influence of isotope substitution on product formation suggest that the C1' chemistry plays no significant role in damage to DNA by hydroxyl radicals in aqueous solutions, which reflects the low accessibility of the C1' hydrogen to solvent (24).

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These data imply that the contribution of the C1' chemistry to radiation damage to DNA in aqueous solutions might be low. From theoretical calculations, hydrogen abstraction from C1', with formation of the 1' -deoxyribosyl radical, is considered the most thermodynamically favorable among the five deoxyribose carbons (25,26). This finding suggests that two factors may play a role in governing the relative contributions of each pathway initiated by hydrogen abstraction from the different deoxyribose carbons. One factor is the aforementioned accessibility of deoxyribose hydrogens to oxidizing species, and the other factor is the relative strength of the C-H bonds in deoxyribose.

In the present work, we investigated the release of 5-MF, a signature of the C1' chemistry, from irradiated DNA. We demonstrate that 5-MF was released in large quantities from DNA irradiated both in aqueous solutions and as dry films in the presence of an appropriate catalyst. The catalytic effect was displayed by a series of polyamines and divalent metal cations at neutral or slightly acidic pH. This indicates that the role of the C1' pathway in DNA radiation chemistry may be much greater than previously believed.

MATERIALS AND METHODS

Highly polymerized type I calf thymus DNA, poly-L-lysine hydrochloride with a weight-average molecular weight of 68,600, and DNA bases used as reference compounds in HPLC experiments were purchased from Sigma-Aldrich Chemical Co. 5-MF used as a reference compound was synthesized according to ref. (27). All other reagents and solvents were of the highest available grade from Sigma-Aldrich Chemical Co. or Fisher Scientific Co. and were used as received.

A DNA stock solution containing 12.6 mM DNA phosphates was prepared using DNase-free water and stored at 4° C. It was shown that buffering the stock with 20 mM phosphate (pH 6.8) and adding 0.1 mM sodium azide to prevent bacterial growth has no effect on the results obtained with polyamines. However, the use of phosphate was avoided in this study because of its metal-binding ability, which would obscure the catalytic effect of metals on 5-MF release.

In a typical experiment with DNA aqueous solutions, 200 μ l of the DNA stock solution was placed in a 2-ml flat-bottom ampoule and purged with oxygen or, if anoxic conditions were required, with argon. The ampoules were capped and irradiated at room temperature through the bottom with X rays from a Philips tube with a tungsten anode. The tube was run at a voltage of 40 kV and 20 mA that produced a dose rate of 10.8 kGy/h (measured by Fricke dosimetry). DNA aqueous solutions were irradiated at doses from 200 to 1500 Gy. After irradiation and addition of other reagents, the samples were diluted to the following final composition: 6.3 mM DNA phosphates, 10 μ M uracil (employed as an internal standard), 10 mM catalyst (if any), and 50 mM sodium acetate buffer (pH 5.2). The concentration of polylysine is expressed in amino acid residues. The samples were stored at -20° C until used.

DNA films containing 950 μ g of dry DNA were prepared from the stock solution in 9-well plates and dehydrated over saturated NaOH (5% relative humidity). Then the plates were sealed with aluminum foil either in air or in a glove box filled with argon. Films were irradiated in the plates with the same X-ray tube operated at 55 kV and 20 mA that produced a dose rate of 92 kGy/h determined by using radiochromic films (Far West Technology, Inc.). DNA films were irradiated at doses from 20 to 100 kGy. Immediately after irradiation the DNA samples were dissolved in 380 μ l of sodium acetate buffer containing the uracil standard and transferred into microcentrifuge tubes. Solutions in the tubes were treated with an aqueous solution of spermine tetrahydrochloride employed as a catalyst in this study to bring the mixture to essentially the same final composition as described above but with 70 mM sodium acetate buffer, pH 6.0. The samples thus prepared were stored at -20° C.

For further analysis, all the samples prepared as described above were heated at 90° C, then cooled. Aliquots of 100 μ l were treated with an additional 10 μ l of 0.2 M spermine to precipitate the remaining DNA and then centrifuged. The analysis of the supernatant solution was performed by reverse-phase HPLC on a Luna 250 X 4.6-mm 5 μ C18 analytical column (Phenomenex) operated at 30° C and equilibrated with 40 mM ammonium acetate. A linear acetonitrile gradient (1–9.6% over 20 min at a flow rate 1 ml/min) was applied to elute the products. The products were detected and quantified at 254 nm. Identification of the products was based on a comparison with authentic reference compounds by retention times and, in the case of 5-MF, by GC-MS.

Radiation yields of released DNA cleavage products were calculated from peak areas at 254 nm as described previously (28). Radiation damage to DNA was quantified as the total release of unaltered DNA bases, which has been shown to closely correlate with DNA strand breaks. Formation of a DNA strand break is most typically accompanied by release of a modified sugar fragment and an unaltered free base (21,29–31). Strand scission may be accompanied by the release of a base attached to a sugar fragment (e.g. a base propenal) (29,32), but this is relatively rare. Henle *et al.* (31) did not detect any altered DNA base release using HPLC in γ -irradiated aqueous solutions, and only traces of released altered bases were observed in irradiated solid-state DNA by Swarts *et al.* (33) (lyophilized DNA) and in the present work (results not shown).

The radiation yield of total base release, G_t , was obtained as a sum of radiation yields of individual base release.

Theoretical fits to the time course of 5-MF release were obtained under the assumption that 5-MF is a product of two consecutive pseudo-first-order β - and δ -phosphate elimination reactions. This predicts S-shaped time dependences for 5-MF accumulation if 5-MF is stable under the reaction conditions. Thermal stability of an authentic 5-MF was tested in a reaction system containing 26.7 μ M 5-MF, 5 mM DNA phosphates, and 10 mM spermine in 50 mM sodium acetate buffer at pH 5.2. In this system we found less than 10% loss of 5-MF over 30 min at 90° C. If the reaction time is not much longer than 30 min, this justifies the use of the following classical expression for accumulation of 5-MF in our systems:

$$[5 - MF] = [dL]_0 \left[(k_\delta - k_\beta) [k_\delta (1 - e^{-k_\beta t}) - k_\beta (1 - e^{-k_\delta t})] \right], \quad (1)$$

where $[dL]_0$ is the initial concentration of dL; k_β is the rate constant of dL decomposition through β -elimination; k_δ is the rate constant of dL decomposition through δ -elimination.

RESULTS AND DISCUSSION

Catalytic Effects of 5-MF Release

Figure 1a–d illustrates the fact that 5-MF release is a postirradiation catalytic process since no effect was observed if nonirradiated or irradiated DNA was treated in the absence of a catalyst at pH 5.2 (spermine is shown as an example). The release of 5-MF was not accompanied by an additional release of free bases, as follows by comparison of traces 1a and 1b. This indicates that 5-MF was released from a radiation-induced oxidized abasic site, which is not heat-labile, at least at pH 5.2. However, we observed some 5-MF release even in the absence of a catalyst at slightly basic pH, indicating that the precursor to 5-MF is destabilized at higher pHs (results not shown). It has been shown in a model system that the stability of dL sites is greatly decreased with increasing pH (23). This agrees with our observations and makes dL a good candidate for the precursor to 5-MF in irradiated DNA.

The catalytic activity of various agents with respect to 5-MF release is compared in Fig. 2. All the studies of post-irradiation catalytic release of 5-MF presented in Fig. 2 were conducted in 50 mM sodium acetate buffer at pH 5.2 and 90° C. Under these conditions, 5-MF release was not observed when catalyst was absent. As described in the Materials and Methods, the curves shown in Fig. 2 are S-shaped. The most pronounced catalytic effect was observed for polylysine (PL), spermine and Be(II). The effect of other divalent metal cations ranged from moderate [Zn(II)] to weak [Cu(II), Ni(II)]. The effect of Mg(II) was detectable but was too weak to be quantified (results not shown). In contrast, methylviologen, a strong minor groove DNA binder, showed no catalytic effect under the same conditions (results not shown).

It has been reported that decomposition of dL is catalyzed by bases (5,23,34). In our system, however, the amino groups of polylysine and spermine are fully protonated at pH 5.2 and cannot act as base catalysts. But through hydrogen bonding with the phosphates, they can act as proton donors. Both polylysine and spermine are strong minor groove binders, which is facilitated by hydrogen bonding to the phosphates. Be(II), which displayed the strongest catalytic effect among the metals, and Zn(II) are also known for their tendency to form partially covalent bonds with electronegative oxygen. Based on that, we hypothesize that partial neutralization of negative charges on the phosphates through hydrogen or partially covalent bonding is critical for catalytic activity in 5-MF release. These types of bonds reduce negative charge on the phosphates and thereby make phosphate a better leaving group in the elimination reaction. Purely ionic interaction appears to be insufficient for catalytic activity as demonstrated with methylviologen dication, which is unable to form hydrogen bonds and shows no effect on 5-MF release.

Quantitative Characteristics of 5-MF Formation

Figure 3 shows dose–response curves for 5-MF release from DNA irradiated in solution and from dry films under different gassing conditions. In this study the 5-MF release was quantified after 10 min of heat treatment at 90° C using 10 mM spermine as a catalyst. These conditions may not be optimal for complete 5-MF release, and the *G* values reported below should be treated as lower limits. The dose–response curves were found to be linear in all systems; the corresponding *G* values are reported in Table 1. As follows from Table 1, 5-MF yields are greater in the presence of oxygen for DNA aqueous solutions, with about a 1.4-fold decrease if oxygen is replaced with argon. This is not the case for the films since essentially the same *G* values were obtained for the DNA films irradiated under aerobic and anaerobic conditions. This is surprising since the formation of dL, a tentative precursor to 5-MF, requires one-electron oxidation of the intermediate C1' radical. As has been demonstrated previously (35–37), in the presence of oxygen this step proceeds through the formation and decomposition of an intermediate peroxy radical through elimination of superoxide $O_2^{\bullet-}$. In our experiments, the absence of oxygen only partially suppressed formation of 5-MF; therefore, another oxidant must play the role of oxygen in this anoxic system. Our working hypothesis is that this oxidant is the guanine radical cation; oxidation of the C1' radical would therefore be a reaction that terminates hole migration through DNA. Our observations also agree with the earlier finding that the dL lesions are generated by ionizing radiation in both oxygenated and anoxic systems (20).

For the reason stated above, the *G* values for 5-MF release reported in this study can be used as lower limit estimates for the yield of dL, the presumed precursor of 5-MF. It then follows from the values from Table 1 that dL is a highly important sugar lesion generated by radiation in DNA. A comparison of the *G* values for unaltered base release in oxygenated aqueous solutions [$G_t = 0.021 \pm 0.001 \mu\text{mol/J}$ from the present work, $G_t = 0.03 \mu\text{mol/J}$ from the literature (21)] with the *G* value for 5-MF release from the Table ($0.0067 \pm 0.0003 \mu\text{mol/J}$) shows that the dL lesions account for more than 30% of the total sugar damage. In a DNA-

polylysine system optimized to maximize release, the yield of 5-MF is 72% of the total yield of unaltered bases.

Our results may appear to contradict the data obtained by Balasubramanian *et al.* (24), but they do not. Balasubramanian *et al.* measured immediate strand breaks only, and for immediate strand breaks they found that in aqueous solutions the C1' pathway of DNA damage by hydroxyl radicals was the least significant. Based on our results, the release of 5-MF would not be expected under their conditions. Hydrogen abstraction at the C1' position, presumably leading to dL, would not result in an immediate strand break and, consequently, one should not expect 5-MF formation under conditions employed in the study (24).

CONCLUSIONS

In conclusion, the present results demonstrate that 5-MF, a marker of the C1' pathway, is produced in large quantities from irradiated DNA, which indicates a significant role of the C1' chemistry (up to 72% of the total sugar damage) in the radiation-induced DNA sugar damage by both indirect and direct effects. To the best of our knowledge, this is the first indication of such a significant role of the C1' pathway in the radiation chemistry of DNA.

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FIG. 1.

Release of 5-MF from irradiated aqueous DNA solutions analyzed by reverse-phase HPLC. X-irradiated solutions were heated in 50 mM sodium acetate, pH 5.2. (a) 1.5 kGy, 15 min at 90° C with 10 mM spermine. (b) Same as (a) but without spermine. (c) 1.5 kGy, no heating. (d) Same as (a) but no irradiation.

FIG. 2.

Catalysis of 5-MF release by DNA phosphate-binding compounds. DNA solutions, X-irradiated at 1.5 kGy, were heated at 90° C in 50 mM sodium acetate buffer, pH 5.2, with 10 mM of each catalyst.

FIG. 3.

Dose–response curves for 5-MF release from DNA X-irradiated in aqueous solution. Inset shows the dose–response curves for DNA films. Open symbols, anaerobic conditions; closed symbols, aerobic conditions. Irradiated samples were heated 10 min at 90° C with 10 mM spermine in 70 mM sodium acetate buffer, pH 6.0.

Scheme I.

TABLE 1
Radiation-Chemical Yields of 5-MF Release from X-Irradiated DNA

DNA state	Radiation-chemical yield, μ mol/J	
	Aerobic	Anaerobic
Solution	0.0067 ± 0.0003	0.0047 ± 0.0002
Film	0.032 ± 0.001	0.038 ± 0.006