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Promotion of Radiation-Induced Formation of 8-Oxo-7,8-dihydro-2′-deoxyguanosine by Nitro 5-Deazaflavin Derivatives

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Abstract—6-Nitro- and 8-nitro-5-deazaflavin derivatives have been found to enhance prominently the radiation-induced formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) at the expense of formation of 2,6-diamino-4-hydroxy-5-formamidopyrimidine nucleosides (FapydGuo) both in deaerated and in N_2O saturated aqueous 2′-deoxyguanosine solutions. The radiosensitizing capacity of a 9-nitro-5-deazflavin derivative was observed only in the N_2O saturated aqueous solutions.

Radiosensitization of poorly oxygenated cells[1] in solid tumors in the treatment of human cancers is an important clinical application of the radiation chemistry of nitroheteroaromatic compounds.[2] Since radiation therapy requires oxygen to induce maximum damage in cellular radiation targets and to be maximally cytotoxic,[3] the hypoxic cells show radioresistance[4] and survive to allow tumors to regrow. Nitroheteroaromatic compounds utilized clinically as radiosensitizers[5] are considered to mimic oxygen to sensitize the cellular targets to the effects of radiation by enhancing oxidation of the potentially damaged reactive target radicals.[2]

Recently, we have developed nitro 5-deazaflavin derivatives[6a] as a novel class of nitroheteroaromatic compounds possessing an electrophilic redox coenzyme ring system and reported their selective cytotoxicities towards hypoxic cells[6b] as well as antitumor activities[6a,c] and their DNA damaging capacity[6d] under anaerobic conditions. We have also demonstrated that reductively activated 6-nitro- and 8-nitro-5-deazaflavin derivatives interact significantly with guanine base to give rise to the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo).[6e] Since nitro 5-deazaflavin derivatives are more electrophilic[6a,b] than other nitroheteroaromatic compounds, they would be anticipated to be more effective in sensitizing the cellular targets to the effects of radiation, which may result in the eradication of hypoxic cells in solid tumors.

DNA is considered to be one of the principal cellular targets for ionizing radiation-induced damage.[7] 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo)[8] is one of the major products of oxidative DNA damage and has attracted considerable attention as a consequence of its capacity to induce misparing and mutation.[9] Radiation-induced formations of 8-oxodGuo in DNA[10a,c] and in aqueous 2′-deoxyguanosine (dGuo) solutions[10b,d] have been studied well, however, promotion of 8-oxodGuo formation by radiosensitizers[11] has rarely been reported. In the present paper, we wish to report the promotion of radiation-induced formation of 8-oxodGuo by nitro 5-deazaflavin derivatives both in deaerated and in N_2O saturated aqueous dGuo solutions.

A series of nitro 5-deazaflavin-3-acetic acids **1**–**4** (Scheme 1) as novel radiosensitizers was prepared similarly as described in a previous paper.[6d] Aqueous dGuo solutions in the presence or in the absence of the radiosensitizers **1**–**4** were deaerated by flushing with N_2 or saturated with N_2O and were exposed to the gamma rays.[12] Metronidazole **5**[13] and etanidazole **6**[14] as radiosensitizers towards hypoxic cells were employed as

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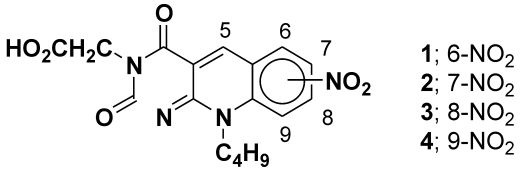


Scheme 1. Structure of compounds **1**–**4**.

reference compounds. As Cadet reported, 8-oxodGuo, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), and its nucleosides (FapydGuo isomers) were found as main products in the gamma-irradiated reaction solutions of dGuo.[10b] The dosage level of 8-oxodGuo was measured by an HPLC-ECD method[15] and the degradation of dGuo was monitored simultaneously by a UV detector. The reaction solutions were treated with aqueous 80% formic acid at room temperature under argon stream[10d,16] to convert a mixture of FapydGuo isomers[10b] into FapyGua. After complete removal of formic acid by freeze-drying, the level of FapyGua was measured by an HPLC-ECD method.[17] The G values[18] for the radiation-induced formation of 8-oxodGuo and FapyGua [G(8-oxodGuo) and G(FapyGua)] and for the radiation-induced degradation of dGuo [G(dGuo)] and reduction potentials of the compounds are shown in Tables 1 and 2.

Table 1. Reduction potentials[a] of compounds and estimated G values[b] for radiation-induced formation of 8-oxodGuo and FapyGua and degradation of dGuo in N_2O saturated aqueous dGuo solutions[c]

Compd	Ep (V)	G(8-oxodGuo)	G(FapyGua)	G(dGuo)
—	—	0.12	0.34	−2.29
1	−0.560, −0.920	0.52	0.13	−1.10
2	−0.804	0.09	0.55	−1.01
3	−0.540, −0.850	0.52	0.12	−1.05
4	−0.682	0.46	0.11	−1.02
5	−1.100	0.06	0.47	−1.01
6	−0.980	0.05	0.53	−0.91

[a]All the reaction potentials were measured at 298 K in DMF, [compound] = 1.0×10^{-3} M, [Bu_4NClO_4] = 1.0×10^{-1} M versus an aqueous Ag/AgCl reference electrode under N_2.
[b]G values were estimated from the results of three independent experiments.
[c]In 1.0×10^{-2} M phosphate buffer, pH = 7.0, [dGuo] = 5.0×10^{-4} M, [compound] = 1.0×10^{-3} M.

Table 2. Reduction potentials[a] of compounds and estimated G values[b] for radiation-induced formation of 8-oxodGuo and FapyGua and degradation of dGuo in deaerated aqueous dGuo solutions[c]

Compd	Ep (V)	G(8-oxodGuo)	G(FapyGua)	G(dGuo)
-	—	0.02	0.64	−1.05
1	−0.560, −0.920	0.12	0.20	−0.51
2	−0.804	0.01	0.32	−0.48
3	−0.540, −0.850	0.23	0.08	−0.50
4	−0.682	0.02	0.31	−0.49
5	−1.100	0.01	0.35	−0.39
6	−0.980	0.01	0.35	−0.42

[a]All the reaction potentials were measured at 298 K in DMF, [compound] = 1.0×10^{-3} M, [Bu_4NClO_4] = 1.0×10^{-1} M versus an aqueous Ag/AgCl reference electrode under N_2.
[b]G values were estimated from the results of three independent experiments.
[c]In 1.0×10^{-2} M phosphate buffer, pH = 7.0, [dGuo] = 5.0×10^{-4} M, [compound] = 1.0×10^{-3} M.

In N_2O saturated aqueous dGuo solutions, where hydrated electrons (e_{aq}) could be efficiently converted into hydroxyl radicals,[19] 6-, 8-, and 9-nitro-5-deazaflavin derivatives **1**, **3**, and **4** have been found to enhance significantly radiation-induced formation of 8-oxodGuo in comparison with those without the radiosensitizers (Table 1), although the degradation of dGuo decreased in the presence of the compounds probably due to competitive reaction of hydroxyl free radical with the compounds.[20] In contrast to the results, 7-nitro-5-deazaflavin derivative **2** as well as metronidazole **5** and etanidazole **6**[21] appear to decrease the formation of 8-oxodGuo. Considering that one-electron reduction potentials of **1**, **3**, and **4** are higher than those of **2**, metronidazole **5**, and etanidazole **6**, the electron affinities of the compounds as radiosensitizers would affect significantly the promotion of radiation-induced formation of 8-oxodGuo under N_2O. These results suggest that **1**, **3**, and **4** are electrophilic enough to be capable of oxidizing 8-hydroxy-7,8-dihydro-2′-deoxyguanosine-7-yl radical (intermediate radical)[22] as a reaction intermediate for 8-oxodGuo.

In deaerated aqueous dGuo solutions, radiation-induced formation of 8-oxodGuo has also been found to be promoted prominently by **1** and **3** (Table 2). Although **4** enhances 8-oxodGuo formation in N_2O saturated aqueous dGuo solutions (Table 1), the radiosensitizing capacity has not been observed in deaerated aqueous solutions. These results suggest that **1** and **3** are capable of oxidizing the intermediate radical[22] into 8-oxodGuo faster than its reduction into FapydGuo by hydrated electrons (e_{aq}), on the other hand, the reduction of the intermediate radical would proceed much faster than its oxidation by **4**.

It is worthy of notice that **3** shows almost twice as large radiosensitizing capacity [G(8-oxodGuo) = 0.23] as **1** [G(8-oxodGuo) = 0.12] in deaerated solutions. The result could not be accounted for only by one-electron reduction potentials of the compounds. The stabilities of reduction products of **1** and **3** may account for the results; since **3** would be anticipated to give rise to more strongly stabilized reduction products than **1** by the conjugation with 5-deazaflavin ring system, electron transfer reaction between **3** and the intermediate radical[22] would proceed more smoothly than that with **1**.

As Tables 1 and 2 show, radiation-induced formation of FapyGua appears to be decreasing as the formation of 8-oxodGuo increases. To have a clue for the reaction mechanism for the radiosensitization by 5-deazaflavin derivatives, the formation of 8-oxodGuo and FapyGua was investigated in deaerated aqueous dGuo solutions irradiated with a constant dose level of gamma ray (800 Gy) in the presence of increasing concentrations of **1** and **3**. As shown in Figure 1, the increase in the formation of 8-oxodGuo has been observed concomitant with

the decrease in the formation of FapyGua as the concentrations of **1** and **3** increase. These results indicate that **1** and **3** oxidize effectively the intermediate radical[22] to promote the formation of 8-oxodGuo at the expense of formation of FapyGua (Scheme 2).

In conclusion, we have first demonstrated prominent promotion of radiation-induced formation of 8-oxodGuo both in deaerated and N_2O saturated aqueous dGuo solutions by nitro 5-deazaflavin derivatives.

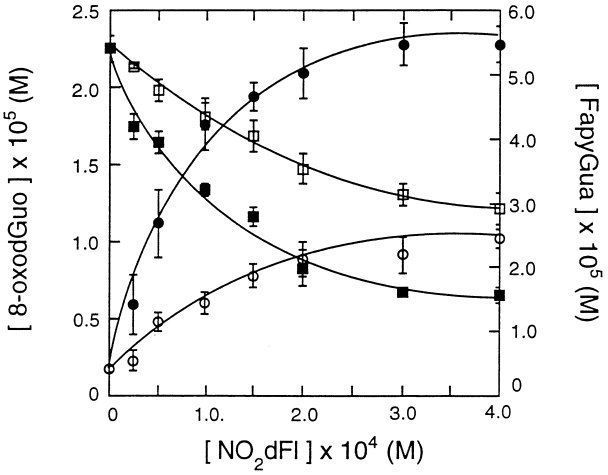
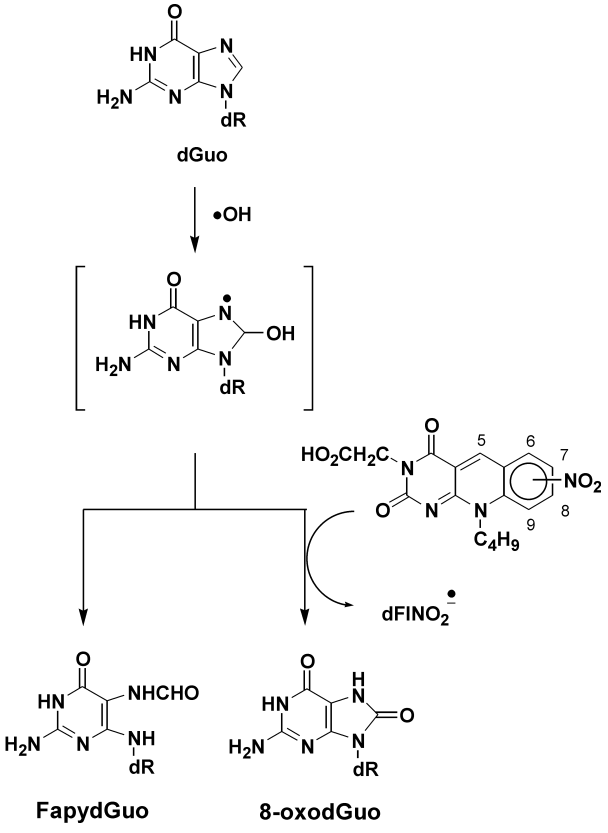


Figure 1. Radiation-induced formation of 8-oxodGuo (circle) and FapyGua (square) (800 Gy) in the presence of varying concentrations of **1** (open symbol) and **3** (closed symbol) in deaerated aqueous dGuo solution ([dGuo] = 5.0×10^{-4} M).



Scheme 2. Plausible reaction mechanism for promotion of radiation-induced formation of 8-oxodGuo by nitro 5-deazaflavin derivatives.

Detailed sensitizing effects of nitro 5-deazaflavin derivatives on radiation-induced DNA damage and radiosensitization of hypoxic cells by these unique radiosensitizers are under investigation.

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18. The number of chemical species formed by the absorption of 100 eV of energy in reaction solutions. Also see ref 2a
19. $e_{aq} + N_2O + H_2O \rightarrow N_2 + OH^- + {}^{\bullet}OH$. Also see ref 2a
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