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Significant Formation of 8-Hydroxydeoxyguanosine in Photoirradiation of "Photo-Fenton Reagent" with Calf Thymus DNA and L 5178Y cells

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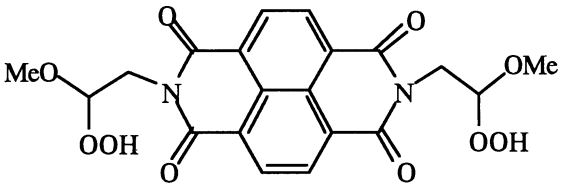
A significant formation of 8-hydroxydeoxyguanosine (8-OHdG) was observed in photoirradiation of "photo-Fenton reagent" (**1**) with calf thymus DNA in maximum yield of 1.1 %. The ten-fold enhancement of the formation of 8-OHdG compared to the control level was observed in photoirradiation of L5178Y cells with **1**. The 8-OHdG formation well corresponds to the site specific DNA-cleaving activity of **1** upon photoirradiation.

The importance of oxidative DNA damage has been widely recognized.[1] A special interest for the oxidative DNA damage is the correlation of the oxidative damage with cancer, aging,[2] or some other deseases.[3] Various compounds resulting from the oxidative DNA base damage by active oxygen species have been isolated.[4] Among such compounds, 8-OHdG has attracted much current attention,[5] because this compound can induce serious lesion such as point mutation *in vitro* [6] and *in vivo*.[7] The formation of 8-OHdG has been reported in the reactions with carcinogens,[8] anti-cancer drugs,[9] singlet oxygen,[10] and in γ-radiation.[11] We have recently designed a molecule referred to as "photo-Fenton reagent" that can generate hydroxyl radical (·OH) upon longer wavelength photoirradiation.[12] A sequence specific DNA strand scission was observed at -GG- site upon photoillumination of "photo-Fenton reagent" **1**. It is extremely important to clarify the actual role of ·OH in the formation of 8-OHdG by using this reagent , because precise concentration-dependent formation of 8-OHdG is available in this case. In other systems like metal-H_2O_2 or γ-radiation, the total amount of ·OH generated is not accessible and other active species such as superoxide anion or singlet oxygen may also be produced. Our reagent is particularly useful as a stoichiometric and pure hydroxyl radical source. In this paper we wish to describe the formation of 8-OHdG in photoirradiation of **1** in the presence of calf thymus DNA or L5178Y cells.

A reaction mixture containing **1** and L5178 Y cells in 5 mM (1 M = 1 mol dm^{-3}) tris buffer solution was irradiated from transilluminator (366 nm) at a distance of 10 cm for 1 h. After irradiation, DNA in the cell was extracted by using a Marmur's method,[13] and the DNA was digested by nuclease P_1 and alkaline phosphatase. 8-OHdG thus produced was analyzed by HPLC equipped with ECD developed by Floyd *et al*.[14] The formation of 8-OHdG increased with increasing concentration of **1**, but was saturated at *ca.* 50 μM concentration (Table 1). The tenfold enhancement observed in this study is surprisingly high compared to the case of γ-radiation of the same cell. Namely, 54 krad γ-radiation of L 5178Y cells resulted in a formation of 8-OHdG with a 2.5-2.6 8-OHdG / 10^5 dG ratio. This value is only 3 times larger than the control value.[15]



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This γ-radiation is so powerful to cause cell necrosis, whereas the formation of 8-OHdG was not so significant compared to the present "photo-Fenton" system. These results suggest the importance of the DNA binding ability[16] of **1** for the efficient formation of 8-OHdG.

Table 1. Formation of 8-OHdG in Photoirradiation of L5178Y cells with Photo-Fenton Reagent **1**[a]

Additive	Concentration / μM of **1**	8-OHdG/ 10^5 dG
none	----------	0.649
1	2	1.179
1	5	1.715
1	10	3.386
1	20	5.360
1	50	7.052
1	100	6.888

a) A reaction mixture containing L5178Y cells (2 x 10^7 cells / ml) and **1** dissolved in acetonitrile was irradiated from transilluminator (366 nm) at a distance of 10 cm for 1 h at room temperature.

Next we examined the formation of 8-OHdG from the reaction of calf thymus DNA with **1** under photoirradiation conditions. A solution of calf thymus DNA (0.1 mg / ml) and **1** was photoirradiated at a distance of 10 cm from transilluminator for 1 h at 0 °C. After irradiation, the solution was centrifuged to give rise to DNA precipitation, which was digested with nuclease P_1 and alkaline phosphatase. The yield of 8-OHdG was 1.1% at 10 μM concentration, whereas the yield of 8-OHdG was considerably decreased as the concentration of **1** being increased to more than 20 μM (Table 2). The reason for the decrease of 8-OHdG at higher concentrations may be rationalized in terms of double strand cleavage of calf thymus DNA induced by **1**.

In order to obtain further support, we examined the DNA cleavage by using supercoiled circular φx 174 DNA in the presence of **1** under photoillumination. A sodium cacodylate buffer solution containing φx 174 DNA and **1** was irradiated from transilluminator (366 nm) at 0 °C for 1 h. After irradiation, the solution was subjected to agarose gel electrophoresis, and the form II (nicked) and form III (linear) DNA were quantified by using computer imaging system.[17] The disappearance of form I DNA together with formation of form II and form III DNA's was observed as the concentration of **1** being increased. At concentrations higher than 50 μM, form I

DNA completely disappeared under the experimental conditions (Fig. 1). This is consistent with our proposal that the decrease of 8-OHdG at concentrations higher than 20 μM is due to the DNA double strand cleavage.

Table 2. Formation of 8-Hydroxydeoxyguanosine (8-OHdG) in Photoirradiation of Calf Thymus DNA with Photo-Fenton Reagent **1** a)

Additive	Concentration / μM of **1**	8-OHdG / 10^5dG
none	---	24.6
1	2	584
1	5	883
1	10	1149
1	20	822
1	50	807
1	100	492

a) Photoirradiation was carried out at a distance of 10 cm from transilluminator for 1 h at room temperature. Calf thymus DNA (0.1 mg / ml) was dissolved in tris-buffer and ten-fold excess of **1** in acetonitrile was added to the reaction system.

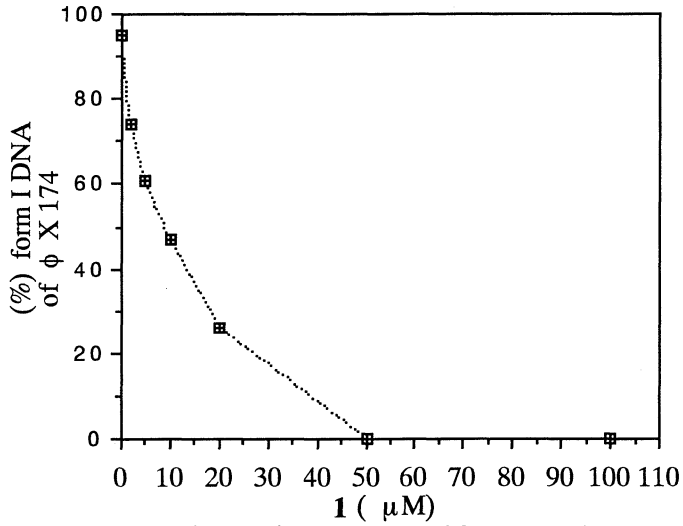

Fig. 1. Disappearance of form I DNA upon photoillumination of **1** at various concentrations.

The present result implies a smooth penetration of **1** to the inside cell and the drug strongly binds to DNA in the cell. Singlet oxygen is reported to produce 8-OHdG in yield 0.75% (750 of 8-OHdG / 10^5 of dG)[10a] from calf thymus DNA, where the photoirradiation was carried out at 20 μM of methylene blue for 30 min. In the reaction of iron-bleomycin with calf thymus DNA, only 50 of 8-OHdG / 10^5 of dG was produced.[9] The yield of 8-OHdG from the reaction of horseradish peroxidase-H_2O_2 system with calf thymus DNA was reported to be *ca.* 1%.[18] According to the reports on the formation of 8-OHdG from the reaction of various carcinogens with

cell DNA or some organisms *in vitro*,[19] the enhancement of the 8-OHdG formation compared to the control value was at most three times. In contrast, we were able to confirm an extraordinary high level of 8-OHdG formation in calf thymus DNA and L5178Y cells upon photoillumination of "photo-Fenton" reagent **1**.

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