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TRANSFECTING ACTIVITY OF PHOTOIRRADIATED ϕx 174 DNA IN THE PRESENCE OF HYDROPEROXYNAPHTHALIMIDES

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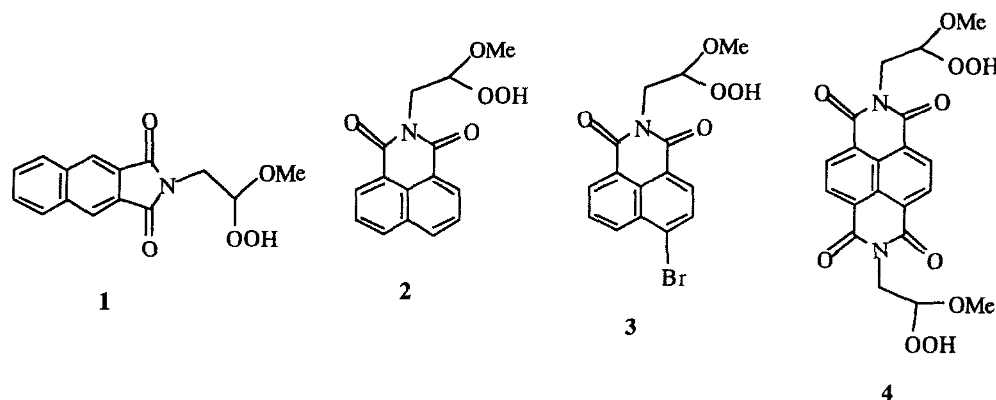
Abstract: Supercoiled double-stranded (form I) DNA of bacteriopharge ϕx 174 was photoirradiated with hydroperoxynaphthalimide derivatives (**1-3**) and "photo-Fenton reagent " **4** . The lesion caused by hydroxyl radical was deleterious enough to inactivate the ϕx 174 DNA. The plaque formation of ϕx 174 DNA was analyzed after 24 h incubation, indicating a fatal effect *in vivo* of hydroxyl radical generated from **4**.

The oxidative DNA damage caused by active oxygen radicals is a recent topic in medicinal and biological chemistry.[1] The analysis of oxidative DNA damage in a molecular level has been conducted, and many stable and unstable DNA oxidation products have been characterized.[2] Among such compounds 8-hydroxydeoxyguanosine[3] and thymine glycohol[4] have been paid much attention in connection with their biological interests. For the investigation to clarify the mechanism of the formation of these DNA oxidation products, a specific reagent that generates specifically a certain oxygen radical is highly desirable. We have been interested in the design of the compounds that generate specific oxygen radicals such as hydroxyl radical upon long wavelength photoirradiation (>350 nm).[5] Merits for the use of such compounds are followings: the detail stoichiometric analysis of DNA damage and the study on the initial oxidation step by hydroxyl radical are readily attainable, because the extent of DNA oxidation can be carefully controlled by photoirradiation time.

We have prepared a series of compounds (**1-4**) that generate hydroxyl radical upon long wavelength photoirradiation (>350 nm).[5,6] The assay of DNA-cleaving activities of these compounds showed that **4**, refered to "photo-Fenton reagent", has the strongest DNA-cleaving activity.[6] The formation of hydroxyl radical from these hydroperoxides upon photoirradiation was confirmed by ESR spin trapping technique using dimethylpyrroline N-oxide and the chemical trapping reactions using adamantane or benzene.[5,6] The sequence specific DNA cleavage by "photo-Fenton reagent" **4** upon photoillumination was also observed.[6] The -G-G- specific DNA strand scission induced by **4** suggested us to study the formation of 8-hydroxydeoxyguanosine (8-OHdG) from DNA. In fact, **4** afforded 8-OHdG in *ca.* 1.1% yield when **4** was photoirradiated with calf thymus DNA.[7] This value is almost the same as that for the methylene blue sensitized photooxygenation ($^1O_2$) of calf thymus DNA.[8] In an extention of these studies we have investigated whether the oxidative damage by these hydroxyl radical-generating molecules is serious enough for ϕx 174 DNA itself by examining the efficiency for the transfection of ϕx 174 DNA to *Escherichia coli* HF 4712 as a host cell.

A sodium cacodylate buffer solution (0.05 M, pH 7.0, 1M = 1 mol dm^{-3}) each containing a peroxide (**1**-**4**) and ϕx 174 (form I) DNA (10 ng) was photoirradiated from transilluminator (366 nm) at a distance of 10 cm for 90 min. *E. coli* cells of HF4712 *rec* A strain as well as *rec*+ strains grown in nutrient broth at 37 °C were treated according to the $CaCl_2$ method[9] and infected with the photoirradiated ϕx 174 DNA. Free phage was titrated by using *E. coli* HF 4712 as the indicator.[10]



The ability of ϕx 174 DNA for plaque formation on HF 4712 *rec* A cells was considerably decreased by photoirradiation in the presence of "photo-Fenton reagent" **4** at 10 μM concentration, whereas in the cases of other derivatives (**1**, **2**, and **3**) the plaque formation is only slightly inhibited at concentrations higher than 15 μM as shown in Fig. 1. The surviving fraction using the *rec* + cells was identical with that of the *rec* A cells, suggesting that the induced damages are not repaired by the host *rec* system.[11]

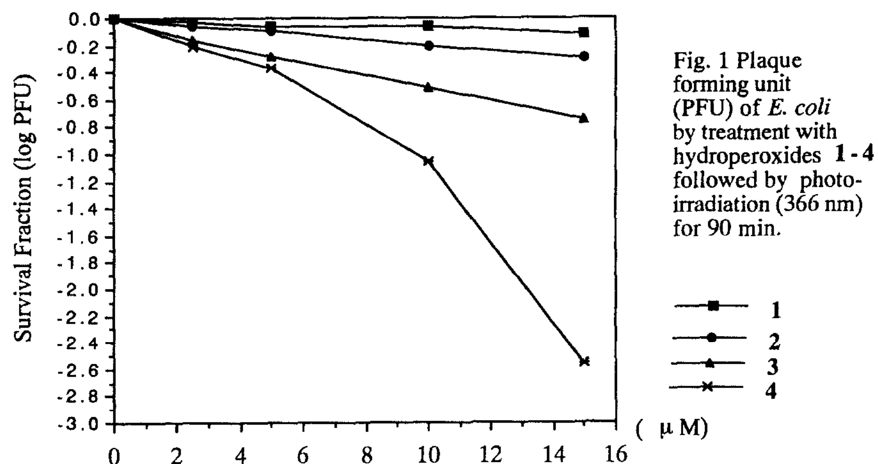


Fig. 1 Plaque forming unit (PFU) of *E. coli* by treatment with hydroperoxides **1**-**4** followed by photoirradiation (366 nm) for 90 min.

In order to clarify the relationship between the plaque formation and DNA-cleaving activity for these hydroperoxides, we have examined the DNA-cleaving activities of these compounds (**1** - **4**) under the same reaction conditions. The conversion of form I DNA to form II (open circular) DNA was monitored by the computer imaging

system .[12] Fig. 2 shows that ϕx 174 DNA is cleaved completely upon photoirradiation in the presence of **4** for 30 min., whereas in the case of **1 - 3**, form I DNA was still present even after photoirradiation for 90 min at 15 μM concentration. This is consistent with the results obtained from the amounts of the plaque formation *in vitro* studies using *E. coli*. Namely, upon photoillumination **4** cleaved supercoiled ϕx 174 DNA (form I) to give nicked (form II) and linear DNA (form III) even at 1 μM concentration, whereas for **1 - 3** only negligible DNA strand scission was observed at 1 μM even after prolonged photoirradiation. The form II DNA may be repaired in *E. coli* host cells, whereas the main effect on the plaque formation may probably be originated from the linear DNA (form III) formation.

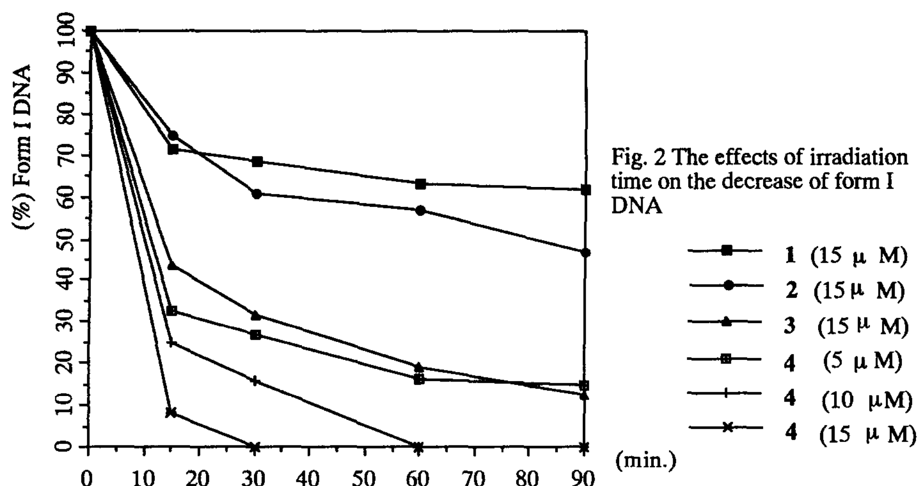


Fig. 2 The effects of irradiation time on the decrease of form I DNA

Next we examined the effects of the photoirradiation time on the plaque formation of ϕx 174 DNA on HF4712 *rec* A strain by using **4**. If hydroxyl radical generated during photoirradiation plays a significant role in the DNA strand scission as well as in the plaque formation of *E. coli*, then the time-dependent plaque formation should be expected. In fact, time-dependent decrease of the plaque formation was indeed observed as shown in Fig. 3. This clearly shows that the DNA lesion by hydroxyl radical produced from **4** is enhanced by longer photoirradiation time. Elongation of the photoirradiation time is also responsible for the propagation of the radical produced in DNA, and as a result, the initial lesion induced by hydroxyl radical became more serious by elongation of the photoirradiation time. The present result is also suggestive for a possible DNA lesion that may occur in a cell: if some radical species is generated in the vicinity of the nuclei or mitochodrial DNA, then the DNA in a cell should be damaged. When some other factors that can enhance the radical propagation reactions exist, the DNA lesion becomes more pronounced to result in a DNA strand scission or to induce point-mutation at the exact site (at -G-G domain as described). Further studies are now in progress in our laboratory.

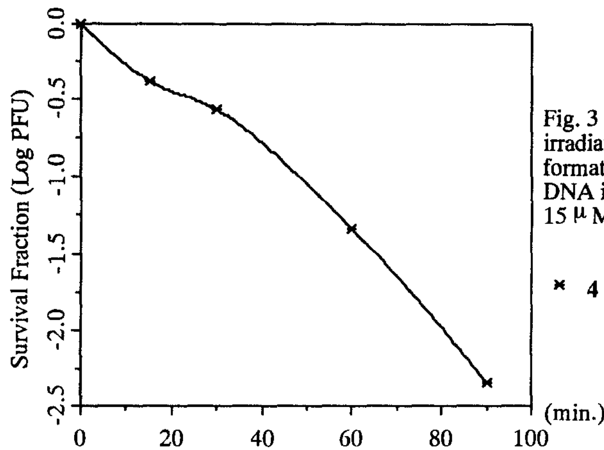


Fig. 3 The effects of photo-irradiation time upon plaque formation ability of Φ x 174 DNA in the presence of 15 μM of **4** .

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References and Notes

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12 The % yields of form I, form II, and form III DNA were determined by computer-imaging system. We thank Dr. Kensaku Ito (Toyama University) for the analysis of the data.