Enhanced DNA Photocleavage by a Subnanomolar Amount of Mercury(II) Porphyrin

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Mercury(II)-porphyrin complex as low as 3 x 10^{-7} mol dm^{-3} significantly accelerated single and double strand scissions of pBluescript plasmid DNA upon visible photoirradiation with the formation of singlet oxygen, hydroxyl radical, and other oxygen radicals which were confirmed by EPR measurement.

Mercury(II) is a toxic metal ion and leads to serious diseases in biological system. Thus, the interaction between mercury(II) and nucleic acid has received a great attention in many research fields to elucidate the mechanism of toxicity and binding pattern of mercury(II).[1] Since mercury(II) itself does not cleave DNA, active oxygen species are suspected to be damaging agents. Fluorescence dyes like porphyrins can produce active oxygen species in the presence of light, and porphyrins, especially cationic porphyrins, show high affinity for DNA.[2,3] Furthermore, mercury(II) can react with porphyrins to form stable mercury(II)-porphyrin complexes.[4,5] Thus, it is expected that DNA would be damaged in the presence of mercury(II) porphyrins under light irradiation. We describe here our finding that tetrakis(1-methylpyridinium-4-yl)porphyrinatomercury(II), $[Hg(tmpyp)]^{4+}$, accelerates DNA strand scission even at a concentration of 3 x 10^{-7} mol dm^{-3} upon visible photoirradiation at physiological pH and 25 °C.

The photoinduced DNA (1.27 x 10^{-4} mol dm^{-3} in base pairs) cleavage by the mercury(II) porphyrin, Hg^{2+} and free base porphyrin (H_2tmpyp^{4+}) at concentration of 3 x 10^{-7} mol dm^{-3} was examined by monitoring the change in the intensity and the conversion of the supercoiled closed circular (F-I) pBluescript plasmid DNA to open circular (F-II) DNA in 1% agarose gel electrophoresis as shown in Figure 1. The effect of visible light on DNA cleavage was examined under the wavelength of 365.0 ~ 366.3, 404.7 and 435.8 nm using a 500 W mercury lamp cut with a S-T1B Toshiba filter. A short irradiation (5 min) of the visible light formed F-II rapidly and then the F-II was gradually destroyed to small particle of DNA with conversion of F-I to F-II by a long irradiation time and completely disappeared in 25 min in the presence of mercury(II) porphyrin (Figure 1). On the other hand, the partial breaking of F-I DNA and producing F-II DNA were observed in 20 min irradiation for the free base porphyrin (Figure 1, lane 7) and lead(II) and cadmium(II) porphyrins. Slight production of F-II was observed when the supercoiled DNA alone or with Hg^{2+} was exposed to the light for 20 min. No significant DNA cleavage was observed by the mercury(II) porphyrin in the dark. These results indicate that the DNA photocleavage was synergystically accelerated in the presence of the mercury(II) porphyrin under visible light and that the supercoiled (F-I) DNA was converted to open circular (F-II). The formation of linear (F-III) DNA was not observed due to its fast cleavage.

In order to understand the mechanism of the above DNA photocleavage, we studied the binding of the mercury(II) porphyrin with pBluescript plasmid DNA by UV-Vis spectroscopy and CD spectral measurements. At neutral pH, H_2tmpyp^{4+} formed 1:1 product with mercury(II). Titrating a solution of mercury(II) porphyrin (3 x 10^{-6} mol dm^{-3}) with DNA, a shift of absorption maximum from 456 nm to 441 nm was observed. This shifted absorption maximum (441 nm) is the same as that obtained for free base porphyrin with this DNA. The results indicate displacement of Hg^{2+} from the mercury(II) porphyrin and binding of H_2tmpyp^{4+} to DNA. Since the ionic radius of mercury(II) is large, mercury(II) can not incorporate well into the porphyrin core and just sits on the porphyrin plane.[5] Therefore, mercury(II) porphyrin can not intercalate into DNA and Hg^{2+} is replaced with DNA. The released H_2tmpyp^{4+} interacted with DNA and displayed a negative CD spectrum at visible region (446 nm) (Figure 2). Thus, $[Hg(tmpyp)^{4+}]$ and H_2tmpyp^{4+} give the same CD spectra in visible region (446 nm) indicating intercalation of H_2tmpyp^{4+} into DNA.[6] A new strong negative CD band, however, was observed at 282 nm for the mercury(II) porphyrin. This band is indicative for conformation change of DNA from right handed B form to a left-handed non-B transition of DNA by binding of Hg^{2+} to DNA.[1,7] The intercalative binding of H_2tmpyp^{4+} to DNA seems to make it easy for Hg^{2+} to bind to DNA.

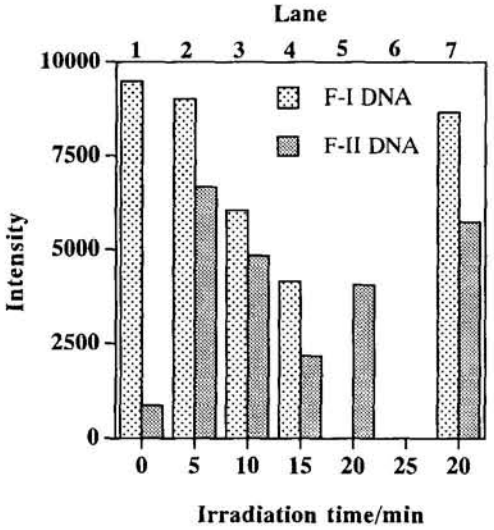

Figure 1. The change in intensities of F-I and F-II DNA as a function of irradiation time in the presence of $[Hg(tmpyp)]^{4+}$ (3 x 10^{-7} mol dm^{-3}) (lanes 1-6) and H_2tmpyp^{4+} (3 x 10^{-7} mol dm^{-3}) (lane 7).

EPR measurements were carried out to confirm radical formation under the present experimental conditions: microwave power, 4 mW; field, 330±5 mT (9.20394 GHz); modulation

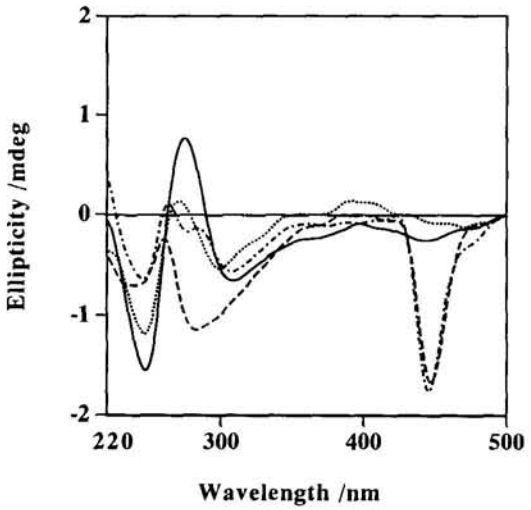

Figure 2. CD spectra of DNA in the absence (——) and the presence of $[Hg(tmpyp)]^{4+}$ (-----), Hg^{2+} (·····) and H_2tmpyp^{4+} (-·-·-·) at pH 7.5 (HEPES buffer, 0.02 mol dm^{-3}) and $[Hg(tmpyp)^{4+}$, Hg^{2+} or $H_2tmpyp^{4+}]$ / [DNA] = 0.188.

width, 0.1 mT; time constant, 0.1 s; sweep time, 0.50 s. Singlet oxygen and other radicals involving ·OH were trapped by 2,2,6,6-tetramethyl-4-piperidone (TMP) and phenyl-*tert*-butyl-nitrone (PBN), respectively. We detected the formation of TMPO radical[8] as shown in Figure 3(a) (1:1:1 spectrum, g = 2.0058 and a hyperfine coupling constant of 1.68 mT) by 5-20 min irradiation

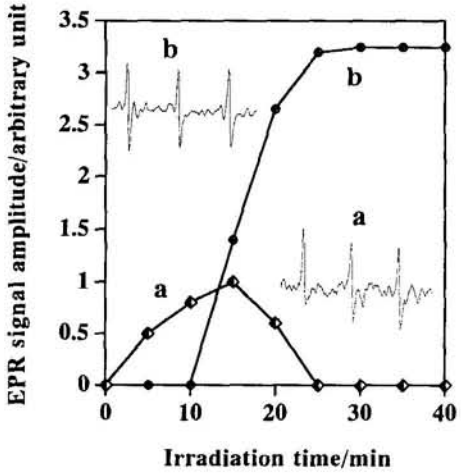

Figure 3. EPR signals and the change in intensities of trapped radicals by TMP (a) and PBN (b) as a function of irradiation time using a mercury lamp (365 - 366.3, 404.7 and 435.8 nm) in the EPR cavaty at 298 K. $[Hg(tmpyp)^{4+}] = 3 \times 10^{-7}$ mol dm^{-3}, $[DNA] = 1.27 \times 10^{-4}$ mol dm^{-3} in base pairs, [TMP] and [PBN] = 3.33×10^{-2} mol dm^{-3}, pH 7.5. The samples were irradiated in the EPR cell.

of DNA containing the mercury(II) porphyrin. The addition of NaN_3 to the above solution inhibited the formation of $^1O_2$ and did not show any EPR signals. In addition, the formation of free radicals like ·OH was confirmed from EPR signals in the presence of PBN under the visible light irradiation longer than 10 min. The EPR signals, however, did not show a clear doublet form. Thus, these signals are mixtures of ·OH and other oxygen radials bound to PBN.[9] With increasing the irradiation time, the intensity of the signal increased as shown in Figure 3(b). The g value and hyperfine coupling constant for these spectra were 2.0059 and 1.68 mT respectively. These values were identical to the values obtained from Fenton reaction that produced ·OH radicals (g = 2.0061; a^N = 1.68 mT, a^H = 3.08 mT) of PBN(OH)·. The EPR signal was suppressed by addition of ethanol, indicating that the trapped radical was hydroxyl radical. From these results, singlet oxygen was formed first and then, hydroxyl radical and other oxygen radicals were formed at a longer photoirradiation with decrease of $^1O_2$ in the presence of mercury(II) porphyrin (Figure 3). Taking into account of the decrease in $^1O_2$ at longer irradiation, we can assume that $^1O_2$ participates in the generation of ·OH and other oxygen radicals.[8]

Comparison of the photocleavage of DNA with the results of UV-Vis, CD, and EPR measurements indicates that the mercury(II) porphyrin interacts with DNA and deforms of DNA structure by the intercalation of H_2tmpyp^{4+} and the binding of Hg^{2+}. The photocleavage of DNA occurs first by singlet oxygen ($^1O_2$) formed at short irradiation time (5-15 min) and then by free radicals involving ·OH formed significantly at a longer irradiation (15-40 min) in the presence of mercury(II) porphyrin as low as 3 x 10^{-7} mol dm^{-3}. Total concentration of the oxygen radicals reaches maximum at 20 min photoirradiation. Thus F-I completely converted to F-II at this irradiation time and the concentration of F-II increased due to the slower decomposition rate of F-II than F-I. Mercury(II) assists the formation of the triplet states of H_2tmpyp^{4+} and DNA bases by its heavy metal effect under photoirradiation. Consequently, these triplet states enhance the production of $^1O_2$ that causes the rapid formation of ·OH, and other oxygen radicals in the presence of mercury(II) porphyrin, and then DNA is cleaved effectively by these active oxygen species.

References and Notes

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