Enhanced DNA Cleavage by Mercury(II) Porphyrin at a Low Concentration of HaeIII Restriction Enzyme

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Mercury(II) porphryin enhanced DNA cleavage in the presence of 0.2 units μL^{-1} of HaeIII at which concentration the restriction enzyme could not cleave DNA in the absence of the porphyrin. This was ascribed to the synergistic effect of the bound Hg^{2+} ions to DNA and the intercalated free base porphyrin, released from the mercury(II) porphyrin complex upon binding to DNA, which was confirmed by UV–visible and CD spectroscopic measurements.

The type II restriction enzyme, *Haemophilus aegyptius* (HaeIII) recognizes specific DNA sequences and cleave it at precise positions within or close to the recognition sequence. In particular it cleaves DNA at the 5'GG–CC3' recognition sites on the DNA helix. It requires Mg^{2+} as a cofactor to catalyze the hydrolysis of the phosphodiester bonds leaving the free 5' and 3' hydroxy groups.[1,2]

Mercury(II) compounds are toxic and strongly interact with DNA thereby changing its conformation.[3–5] In addition, some cationic porphyrins are known to inhibit the action of telomerase, an enzyme which presents a potentially selective target for the design of new antitumor drugs.[6] It is therefore expected that metalloporphyrin derivatives of mercury(II) will significantly affect the activity of HaeIII. We report here about our findings that tetrakis(1-methylpyridinium-4-yl)porphyrinatomercury(II), Hg(II)TMPyP, enhanced the ability of 0.2 units μL^{-1} of HaeIII restriction enzyme to be able to cleave DNA at concentrations of 1.0×10^{-6} mol dm^{-3} and 1.0×10^{-7} mol dm^{-3} and at physiological pH and a temperature of 37 °C.

Since it has been shown by Tabata et al.[7] that at very low concentration (i.e. 0.2 units μL^{-1}) HaeIII cannot cut DNA in the presence of a high amount of magnesium ions, we first assessed the effect of increasing concentrations of the restriction enzyme (0.2–20 units μL^{-1}) on DNA (1.0×10^{-4} mol dm^{-3} in base pairs). This was later followed by the addition of Hg(II)TMPyP, H_2TMPyP and Hg^{2+} ions in separate experiments under the same conditions. The change in both intensities and conversion of supercoiled (F-1) and closed circular (F-II) pBluescript plasmid DNA to linear (F-III) and other small fragments was monitored by agarose gel electrophoresis. Figure 1 shows typical gel electrophoresis data for DNA scission by HaeIII in the presence of Hg(II)TMPyP and H_2TMPyP. In Figure 1 it can be observed that Hg(II)TMPyP enhanced the ability of HaeIII to cleave DNA thereby converting it to small fragments at low concentration (0.2 units μL^{-1}) at which condition DNA was not cleaved by HaeIII without it. Most especially, the DNA scission occurred in the presence of Hg(II)TMPyP at concentrations of 1.0×10^{-6} -10^{-7} mol dm^{-3} but this did not occur in the presence H_2TMPyP and Hg^{2+} alone. The higher concentration of the Hg(II)TMPyP ($> 10^{-6}$ mol dm^{-3}) did not enhance the DNA cleavage.

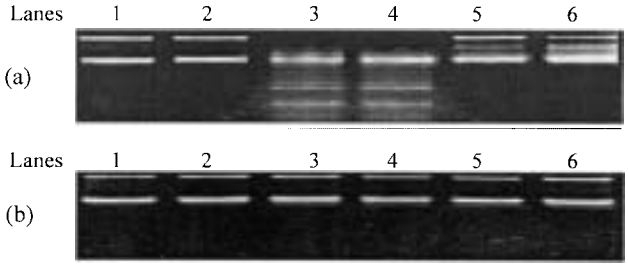

Figure 1. Plasmid DNA cleavage (1.0×10^{-4} mol dm^{-3} in base pairs) by HaeIII (0.2 units μL^{-1}) in the presence of (a) Hg(II)TMPyP and (b) H_2TMPyP of concentrations (1) 0, (2) 1.0×10^{-5}, (3) 1.0×10^{-6} (4) 1.0×10^{-7}, (5) 1.0×10^{-8} (6) 1.0×10^{-9} mol dm^{-3}. Lane 1 is DNA control. Fig. 1 (a) shows enhancement of DNA cleavage by Hg(II)TMPyP in lanes 3, 4, 5 and 6 and Fig. 1 (b) shows non-enhancement by H_2TMPyP.

Results of subsequent experiments at a higher concentration of HaeIII (0.4 units μL^{-1}) indicated that H_2TMPyP and Hg(II)TMPyP did not inhibit DNA cleavage by HaeIII (0.4 units μL^{-1}) in all the concentrations studied. However, HaeIII (0.4 units μL^{-1}) could not cleave pBlusecript plasmid DNA at higher concentrations ($> 1.0 \times 10^{-6}$ mol dm^{-3}) of Hg^{2+} and the inhibitory effect of Hg^{2+} disappeared at the concentrations of 1.0×10^{-6} mol dm^{-3} and below. Although Hg(II)TMPyP did not inhibit DNA cleavage, the percentage of DNA fragments produced in the presence of this porphyrin (Hg(II)TMPyP) were higher (> 60%) compared to the DNA fragments produced in the absence of it at a higher concentration of HaeIII (0.4 units μL^{-1}).

In the UV spectroscopic studies, there was the development of a Soret band at about 430 nm when Hg(II)TMPyP was mixed with aliquots of restriction enzyme. In contrast to this, there was a marginal change in the UV spectra of H_2TMPyP upon the addition of HaeIII restriction enzyme. Similar results were obtained when Hg(II)TMPyP was mixed with aliquots of DNA in UV experiments. In particular, the Soret band of the Hg(II)TMPyP at about 455 nm decreased and the one at 439 nm developed with the appearance of isosbestic points between 380 nm and 650 nm (Figure 2). Since previous work has shown that the Soret band of H_2TMPyP-DNA complex is at about 439 nm[8] the results suggest that in the binding of Hg(II)TMPyP to DNA the mercury porphyrin was demetalated by the binding of the Hg(II) to DNA leading to the release of the free base porphyrin (H_2TMPyP) which intercalated into the DNA bases.

Results of the CD spectra of DNA upon the addition of the Hg(II)TMPyP, H_2TMPyP and Hg^{2+} showed that they caused a change in the DNA conformation based on their binding modes to DNA. The nucleic acid exists in the B-form conformation as evidenced by a pair of conservative CD bands, a positive band at about 273 nm and a negative band at about 248 nm.[9] In the case of Hg(II)TMPyP, it was observed that with increasing con-

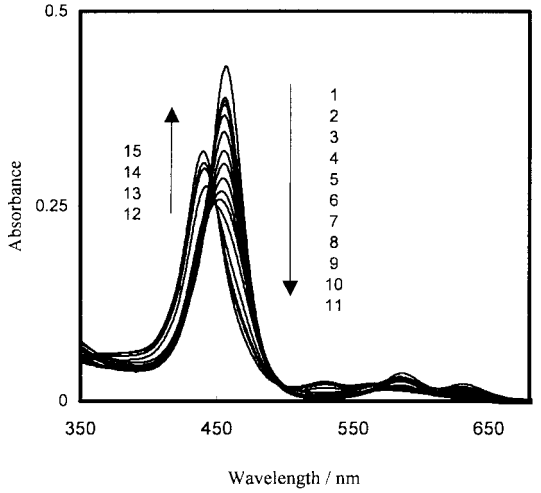

Figure 2. Change in absorption spectra of Hg(II)TMPyP in the presence of DNA at concentrations of (1) 0, (2) 0.16, (3) 0.32, (4) 0.40, (5) 0.60, (6) 0.79, (7) 0.95 (8)1.10 (9) 1.30, (10) 1.40, (11) 1.60, (12) 1.70, (13) 1.90, (14) 2.00, (15) 2.30×10^{-5} mol dm^{-3}. Total concentration of Hg(II)TMPyP is 3.0×10^{-6} mol dm^{-3}. Cell path length is 10 mm.

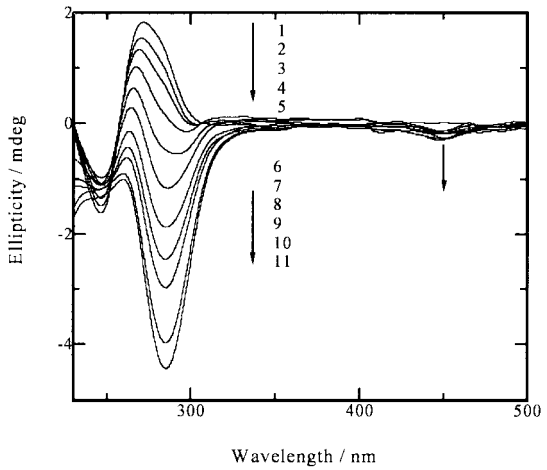

Figure 3. CD spectral changes of DNA (1.0×10^{-4} mol dm^{-3} in base pairs) upon the addition of Hg(II)TMPyP. Arrows indicate spectral changes upon the addition of Hg(II)TMPyP of (1) 0.0, (2) 0.2, (3) 0.6, (4) 0.8, (5) 1.0, (6) 1.2, (7) 1.4, (8) 1.6, (9) 1.8, (10) 2.0 and (11) 2.2×10^{-6} mol dm^{-3}. Cell pathlength is 1mm.

centrations there was the appearance of new negative and positive peaks at 282 nm and 257 nm (Figure 3) indicating that the DNA conformation was transformed from the original B-form to a Z-like conformation similar to observations made by Kuklenyik and Marzilli, and Gruenwedel and Cruikshank in the CD spectra of polynucleotides and calf thymus DNA respectively, by the addition of a large amount of Hg^{2+}.[4,5] Similar results were also obtained in the CD spectroscopic studies of the solutions of Hg^{2+} and DNA except that the change in DNA conformation in the UV region was smaller than Hg(II)TMPyP. These findings are comparable to results obtained in previous photocleavage studies on the interactions of Hg^{2+} and Hg(II)TMPyP with DNA.[8]

Moreover, the Hg(II)TMPyP-DNA complex showed a negatively induced CD spectrum between the wavelengths of 400–470 nm. The negatively induced CD spectrum in this case is similar to the binding of the free base porphyrin (H_2TMPyP) to DNA. This is because the ionic radius of Hg^{2+} is so large in Hg(II)TMPyP that mercury(II) just sits above the porphyrin plane and cannot incorporate well into the porphyrin core.[10] Therefore when Hg(II)TMPyP interacts with DNA, Hg^{2+} binds to DNA leading to the dissociation of the H_2TMPyP which intercalates into the DNA bases thereby displaying a negatively induced CD spectrum between the wavelength of 400–470 nm. The enhanced change in conformation of pBluescript plasmid DNA is therefore attributed to the synergistic effect of the bound Hg^{2+} and the intercalated H_2TMPyP in the DNA helix.

In the case of HaeIII, we observed that there was a marginal change in the CD spectra of the restriction enzyme upon the addition of the Hg(II)TMPyP, H_2TMPyP and Hg^{2+} compared to the results obtained in the DNA experiments. Therefore, the CD results of the mixed solutions of the Hg(II)TMPyP, H_2TMPyP and Hg^{2+} and the HaeIII restriction enzyme suggests that the effects caused by the binding of these porphyrins and the metal ion to the enzyme is insignificant in the presence of the high concentrations of DNA.

Since Hg(II)TMPyP induced the change in conformation in pBluescript plasmid DNA in CD experiments, the DNA cleavage by 0.2 units μL^{-1} of HaeIII can be attributed to the change in conformation of the DNA structure enhanced by the synergistic effect of the bound Hg^{2+} and the intercalated H_2TMPyP that were released from Hg(II)TMPyP upon binding to DNA. With the DNA deformed the low concentration of HaeIII is able to cleave the DNA.

Our findings suggest a new mechanism on the enhancement effect of metalloporphyrins on DNA cleavage and will contribute to further studies on the effects of metalloporphyrins in biological systems and in the medical field, especially in the research into the potential treatment of diseases such as the African trypanosomiasis (sleeping sickness), etc. Currently, this kind of research is being carried out in collaboration with a medical school.

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