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NMR Study on Binding Interactions of Cationic Porphyrin Derivatives with double helical d(CGCGAATTCGCG)$_2$

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Abstract : Binding interactions of cationic porphyrins, T4MPyP and TMAP with DNA oligomer d(CGCGAATTCGCG)$_2$ were studied with NMR spectroscopy, UV and CD spectroscopic method. Two porphyrins showed significant differences in NMR, UV and CD data upon binding to DNA. T4MPyP was considered to position more closely to DNA bases through partial intercalation as well as ionic intercalation between the positive charges of porphyrin and phosphate group of DNA at 5'-GC-3' steps. Contrast to this, TMAP was thought to bind to phosphate of DNA more or less outside of the groove.

INTRODUCTION

Cationic *meso*-substituted porphyrins and their metallo-derivatives are considered to be very useful in probing nucleic acid structure and nucleic acid-ligand binding.[1-6] Recently, several studies for potential use of porphyrins as anticancer drugs have been reported because the molecules are able to selectively accumulate on the surface of cancer cells, and then to induce DNA strand cleavage.[7-10] Cationic porphyrins can bind to DNA in various ways depending on the type of substituent groups, presence of metal ions as well as the kind of metal complexed, the sequence of nucleic acids. Porphyrins which have no axial groups, such as *meso*-tetrakis(N-methylpyridinium-4-yl)porphine(T4MPyP), Cu(II)T4MPyP, and Ni(II)T4MPyP are regarded to intercalate at 5'-GC sites of DNA.[11,-13] In contrast, *meso*-tetrakis(2-N-methylpyridyl)porphine (TMPyP-2), *meso*-tetrakis(p-trimethylanilinium-4-yl)porphine(TMAP), Zn(II)T4MPyP, Mn(III)T4MPyP, Fe(III)T4MPyP and Co(III)T4MPyP are regarded to bind to the groove or to phosphate backbones of DNA.[13,14] Many researchers have proposed the models for various kinds of porphyrin-DNA complexes, based on X-ray, NMR or other spectroscopic data. Among them, Guliaev et al. suggested the most probable model for T4MPyP intercalation to d(GCACGTGC)$_2$, which two of substituents of

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porphyrin exist in the major groove and the other two are in the minor groove.[15] Bennet et al. proposed a model for T4MPyP-d(CCTAGG)$_2$ obtained from X-ray data, which porphyrins exist at the both terminals of DNA.[16] Here we report the effects of T4MPyP and TMAP on binding to double helical d(CGCGAATTCGCG)$_2$ with NMR, UV and CD spectroscopy.

MATERIAL AND METHODS

Sample Preparation

The model DNA oligomer d(CGCGAATTCGCG) was synthesized with a DNA synthesizer(ABI 391 PCR MATE) by β -cyanoethylphosphoramidite chemistry in solid phase. They were purified by using dialysis tubing with molecular weight cutoff range of 2,000 and passing through a Chelex100 column to remove heavy metal ions and then lyophilized. DNA concentration was calculated by measuring its absorbance at 260 nm with an extinction coefficient $\varepsilon_{260} = 1.15\times10^5\ M^{-1}cm^{-1}$.[17] All sample solutions were prepared in 20mM sodium phosphate buffer (pH 6.92) containing 100 mM NaCl.

The cationic porpyrins of *meso*-tetrakis(4-N-methylpyridyl)porphine tetra-*p*-tosylate salt (T4MPyP) and *meso*-tetrakis(para-N-trimethylamilinum)porphine tetra-*p*-tosylate salt(TMAP) was purchased from Sigma Aldrich Chemical Co. and were used without further purification. Concentrations of the T4MPyP and TMAP were calculated spectrophotometrically by measuring their absorbances at the Soret band with extinction coefficients $\varepsilon_{422} = 2.26\times10^5\ M^{-1}cm^{-1}$ (T4MPyP) and $\varepsilon_{412} = 4.16\times10^5\ M^{-1}cm^{-1}$(TMAP).[18] Porphyrin solutions were prepared in the same way as for DNA for all the experiments.

UV and Circular Dichroism(CD) spectroscopy

UV experiments were carried out with a HP-8452A UV-VIS spectrophotometer equipped with a Peltier temperature controller. The melting experiments were performed by monitoring UV absorbance at 260nm from 10 ℃ to 80 ℃. Melting experiments with the complex between DNA and equimolar concentration of each porphyrin derivatives were also performed in the same method as described above.

Circular dichroism spectra were obtained on a JASCO J-600 circular dichroism spectropolarimeter. The spectropolarimeter was calibrated with aqueous 0.06 % NH_4-CSA solution. The regions of 220-320 and 400-500 nm were used to monitor the DNA region and the porphyrin Soret band, respectively.

NMR Spectroscopy

All NMR experiments were performed on a Unity Inova 400 spectrometer(Varian Associates, U.S.A.) with a 9.4 Tesla superconducting magnet in Central Research Facilities of Chungnam National University. Water signal suppression was achieved with the

(A) 5′→CGCGAATTCGCG→3′
3′←GCGCTTAAGCGC←5′

(B)

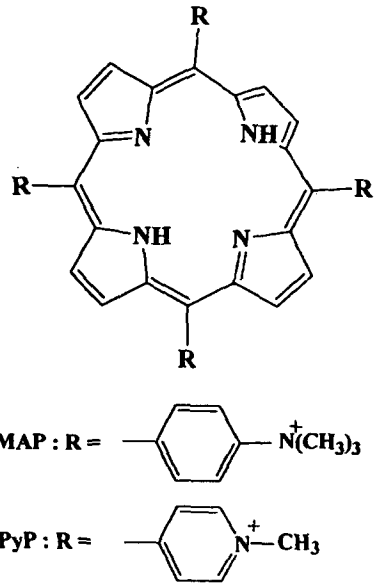


Fig. 1. (A) Self-complementary DNA duplex d(CGCGAATTCGCG)$_2$.
(B) Structure of meso-tetrakis(4-N-methylpyridyl)porphine(T4MPyP) and meso-tetrakis (para-N-trimethylamilinum)porphine(TMAP).

WATERGATE(Water suppression by gradient-tailored excitation) as well as presaturation pulse sequence. Chemical shifts of ^{1}H spectra were reported in ppm relative to the methyl resonance of internal 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) at 0.0 ppm. To observe labile proton signals, the sample was dissolved in aqueous 20 mM sodium phosphate buffer with 20% D_2O(pH6.92), containing 100 mM NaCl. The titrations of DNA oligomer with each of T4MPyP and TMAP were carried out at 20 ℃. To observe nonlabile proton resonances, DNA sample was dissolved in 20 mM sodium phosphate buffer (pH6.92) with 99.99 % D_2O, containing 100 mM NaCl. ^{1}H NMR spectra of porphyrin-DNA complexes were recorded as the same way described above. The ^{31}P-NMR spectra of DNA and DNA-porphyrin complexes were obtained with the sample dissolved in 20 mM phosphate buffer with 99.99 % D_2O solution, containing 100 mM NaCl and chemical shifts of ^{31}P spectra are recorded relative to external trimethyl phosphate(TMP).

RESULTS and DISCUSSION

UV absorbance and Induced CD Studies

Figs. 2 and 3 show UV absorption spectra of T4MPyP and TMAP at different mixing ratio between DNA and porphyrins. Binding with DNA caused observable red shift and significant hypochromicity in the Soret band for both cases. The Soret band initially appeared at 422 nm for free T4MPyP, but shifted upto 438 nm for DNA-bound T4MPyP. The initial Soret Band of TMAP was 412 nm, but shifted to 422 nm for the DNA-bound TMAP. Both of T4MPyP and TMAP showed almost same hypochromicity(around 50 %). In addition, there were clear isosbestic points in both spectra, indicating the single binding mode of porphyrins to DNA.

Figs. 4 and 5 shows induced CD spectra of porphyrins absence and presence of DNA. No CD signal was observed in the absence of DNA, because porphyrins are optically inactive. Adding DNA to T4MPyP caused the negative induced CD signal, indicating intercalative binding interaction. In contrast, TMAP caused somewhat conservative induced CD bands which are showing positive and negative CD features. This clearly shows that TMAP binds to DNA in a different way from T4MPyP. Based on the previous reports TMAP might bind to DNA in the way of surface binding with TMAP self-stacking.

^{31}P NMR Study

Fig. 6 shows ^{31}P NMR spectra of DNA and porphyrin-DNA complexes. T4MPyP- and TMAP-bound DNA showed quite different ^{31}P NMR spectra compared to free DNA below 40 °C. And they also showed significantly different ^{31}P NMR spectra between them. Therefore we could say that binding porphyrin to DNA changed the environment near the phosphate group, but T4MPyP and TMAP induced the changes quite differently. Marzilli et al.(1986) reported that intercalation of T4MPyP to DNA caused a new, weak signal in significantly downfield region from phosphate resonance signal in ^{31}P NMR spectrum. But no new signal appeared in the downfield region upon binding T4MPyP to DNA in this study.

^{1}H NMR Study

Figs. 7, 8 and 9 show ^{1}H imino resonance signals of d(CGCGAATTCGCG)$_2$ in the absence and presence of porphyrins. In the absence of porphyrin, we could observe five imino resonsnces clearly, demonstrating that DNA duplex maintains a two-fold symmetry and both termini are fraying. Imino resonances of thymine and guanine bases could be observed until 60 °C, except that of the guanine base at position 2 which disappeared at 50 °C . Addition of T4MPyP caused a upfield shift and line broadening effect for imino resonances a little more than TMAP did. Guliaev et al.[15] demonstrated that the ^{1}H signal of a methyl group of thymine base was divided into two signals due to porphyrin-bound, and unbound state after adding porphyrin. According to our result, methyl ^{1}H signals were not splitted into bound- and unbound signals upon adding porphyrins, instead they became

broad TMAP did not cause a observable line broadening at the low [TMAP]/[DNA] ratio, but did at [TMAP]/[DNA] = 1. Contrast to this, T4MPyP caused a serious broadening effect at low [TMAP]/[DNA] ratio, 0.1 or 0.2. This might indicate that exchange between the bound- and unbound state was very fast for each of $d(CGCGAATTCGCG)_2$-T4MPyP and $d(CGCGAATTCGCG)_2$-TMAP. Fig. 10 shows the chemical shift changes of base protons caused by binding porphyrin to DNA. T4MPyP induced downfield shifts for most of base protons upon binding to DNA, but TMAP induced no observable shift for most base protons except for T7NH showing a downfield shift and for T9NH showing a upfield shift. This meant that binding pattern of T4MPyP and TMAP should be quite different and the preferring site of T4MpyP for DNA binding was thought to be 5'-GC- sequence. This is consistent with the result reported elsewhere.[12]

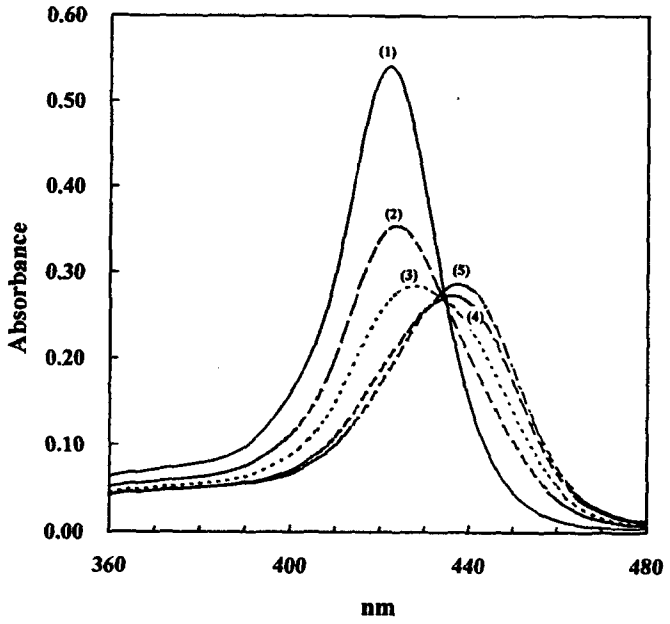


Fig. 2. Absorption spectra in the Soret region of T4MPyP at different mixing ratio of [T4MPyP]/[DNA], (1) T4MPyP (2) 0.3 (3) 0.5 (4) 1.0 (5) 2.0.

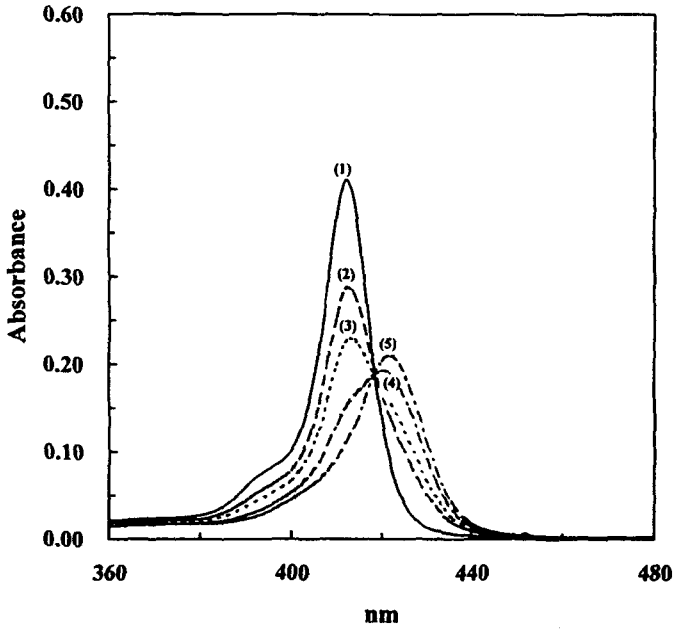
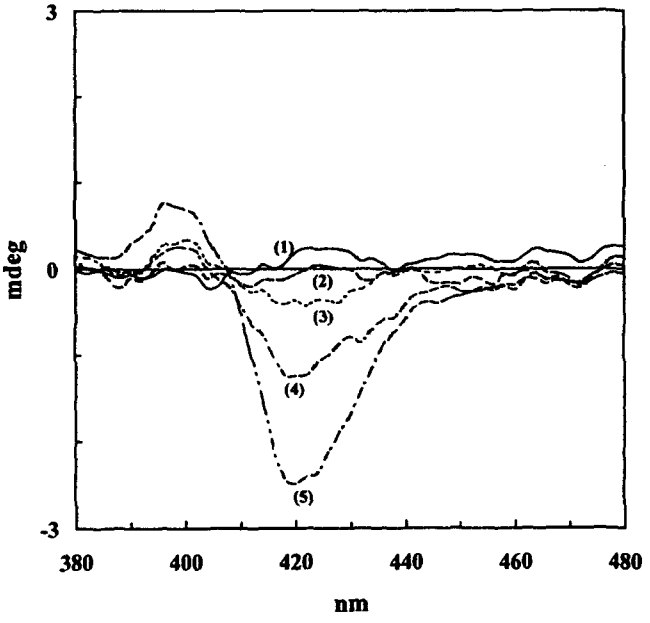


Fig. 3. Absorption spectra in the Soret region of TMAP at different mixing ratio of [TMAP]/[DNA], (1) TMAP (2) 0.3 (3) 0.5 (4) 1.0 (5)



(1) 2.0.

Fig. 4. Induced CD spectra in the Soret region of T4MPyP at various mixing ratios of [T4MPyP]/[DNA] ratios of 0.1, 0.3, 0.5, 1.0, and 2.0.

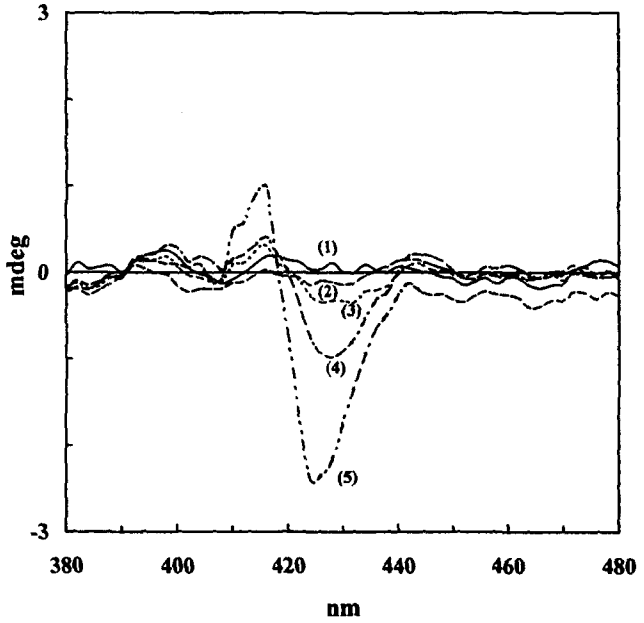


Fig. 5. Induced CD spectra in the Soret region of TMAP at various mixing ratios of [TMAP]/[DNA] ratios of 0.1, 0.3, 0.5, 1.0, and 2.0.

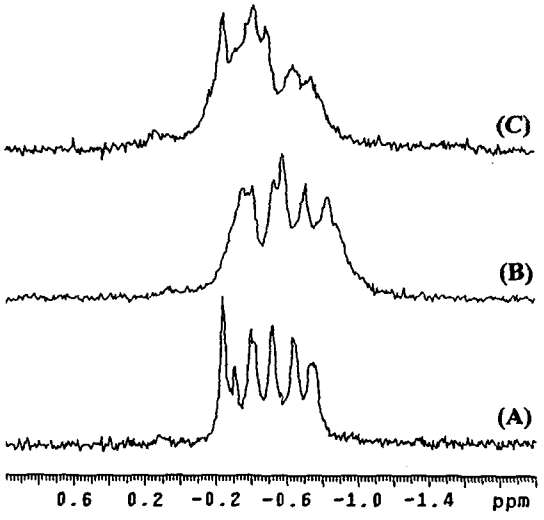


Fig. 6. ^{31}P NMR spectra of d(CGCGAATTCGCG)$_2$ and porphyrin-DNA complexes. (A) d(CGCGAATTCGCG)$_2$ duplex; (B) [DNA] : [T4MPyP]=1 : 0.5; (C) [DNA] : [TMAP]=1 : 1.

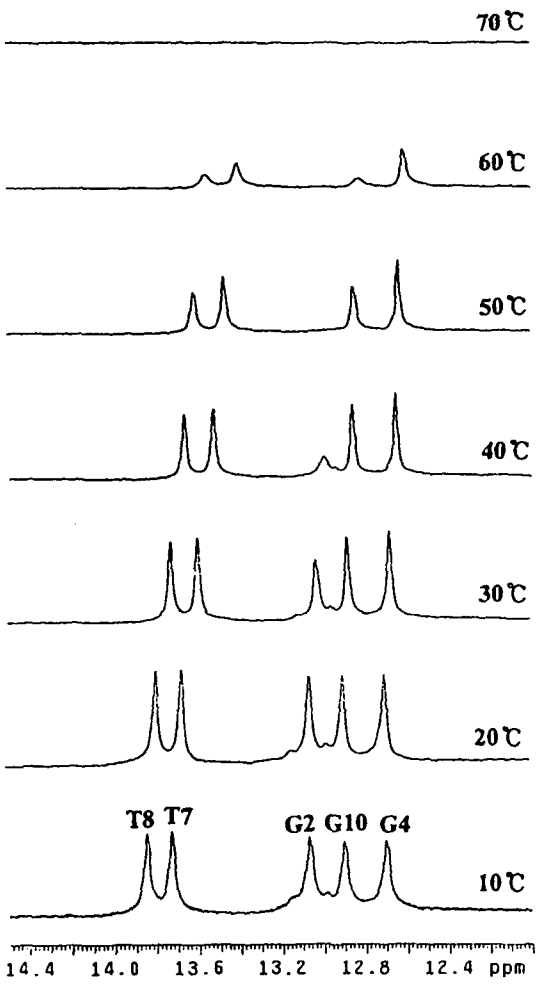


Fig. 7. ^{1}H imino resonance signals of $d(CGCGAATTCGCG)_2$ in 20mM sodium phosphate buffer (pH 6.92) with 100mM NaCl from 10°C to 70°C .

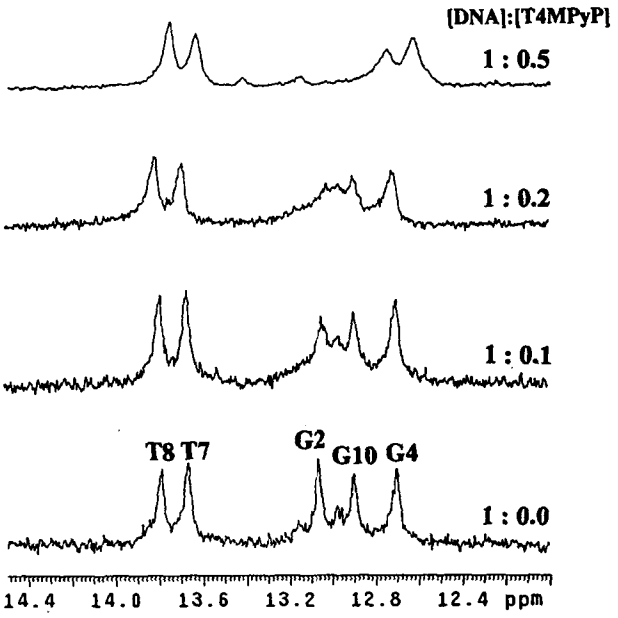


Fig. 8. ^{1}H imino resonance signals of T4MPyP-DNA complex at various mixing ratios

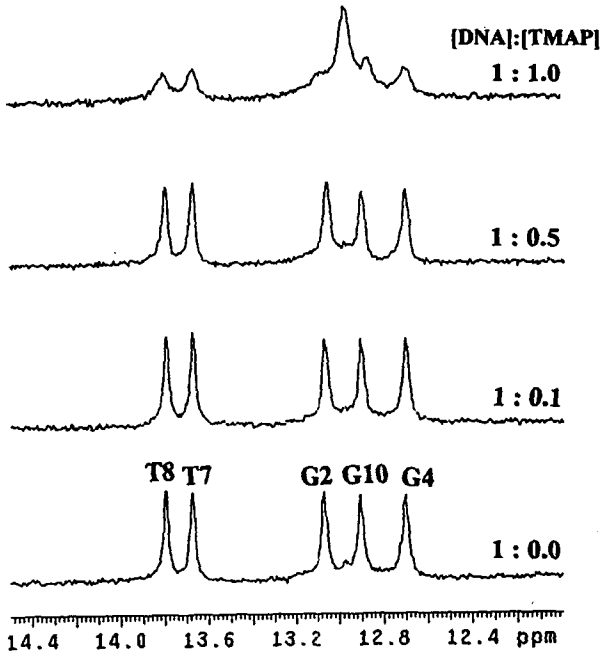


Fig. 9. ^{1}H imino resonance signals of TMAP-DNA complex at various mixing ratios.

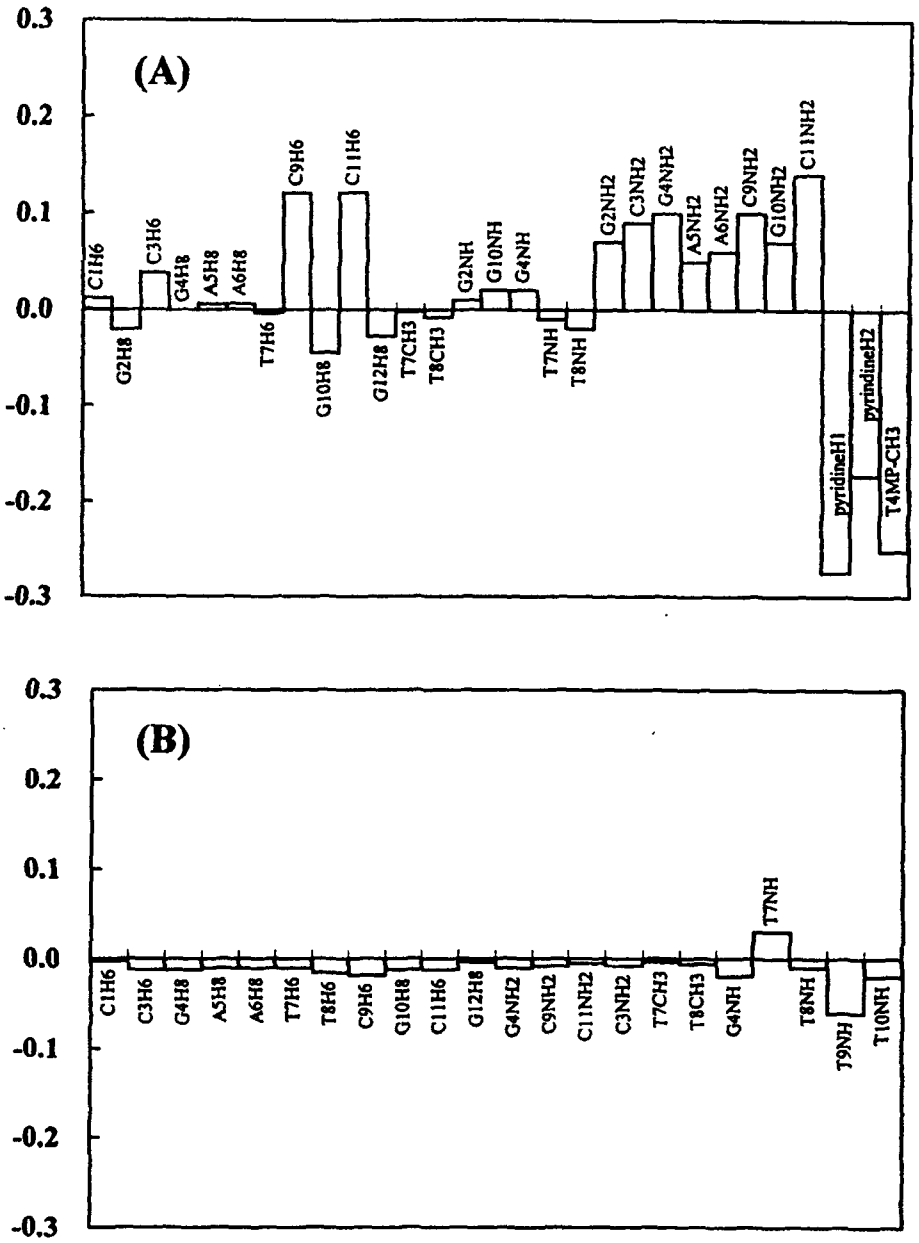


Fig. 10. ^{1}H chemical shift changes of base protons caused by binding T4MPyP (A) and TMAP (B) to d(CGCGAATTCGCG).

CONCLUSION

Judging from UV and CD results, binding of T4MPyP to d(CGCGAATTCGCG)$_2$ was considered to be a single mode and partial intercalation, but NMR data did not show the clear evidence for intercalation. TMAP showed a quite different pattern for binding to the same DNA. T4MPyP was considered to position more closely to base protons than TMAP because of the significant effect on chemical shifts of many base protons upon binding to d(CGCGAATTCGCG)$_2$. Guliaev et al. and Bennet et al. proposed most probable models for their samples based on X-ray or NMR data, but our results did not agree with these models and showed quite different binding pattern. Therefore, we are trying to obtain more data including two-dimensional NMR data to figure out a detailed model for each of d(CGCGAATTCGCG)$_2$-T4MPyP and d(CGCGAATTCGCG)$_2$-TMAP complexes.

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