^{1}H NMR Study of the Effect of G-T Mismatches on Dynamics and Stability of d(GCGTGCGC)$_2$ and Its Berenil Complex

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The effects of G-T mismatches on thermal stability, the base-pair lifetime and the global structure of a d(GCGTGCGC)$_2$ duplex were studied by using ^{1}H NMR, UV and CD spectroscopy. The existence of G-T mismatches was found to cause a noticeable change in the chemical environment of imino protons associated with significant decrease in the base-pair lifetime at the mismatched site as well as in thermal stability of the duplex itself. The melting transition of d(GCGTGCGC)$_2$ was not cooperative at all at 100 mM or lower concentration of NaCl, but became cooperative at 500 mM or higher NaCl concentration. The melting temperature (T_m) of this duplex was 32 ℃ at 500 mM concentration of NaCl, which is much lower than that of d(GCGCGCGC)$_2$ at the same NaCl concentration. This suggests that the decrease in stability may be ascribed to the decrease in the base-pair lifetime and the deviation from the normal structure due to the G-T mismatches. Adding berenil to d(GCGTGCGC)$_2$ caused no observable change in the global structure but the large decrease in the base-pair lifetime and the stability of the duplex.

Introduction

Large progress has been achieved in the understanding of biological functions of deoxyribonucleic acid (DNA) in terms of its structure since the double helical structure of DNA was reported.[1] Recently, it has been recognized that understanding of the unusual DNA structure is also important in relation to the high fidelity found in many biological processes associated with DNA. Various unusual DNA structures, such as base-pair mismatches,[2,3] bent DNA,[4,5] hairpin structures,[6] supercoiled structures,[7] left-handed DNA,[8~10] and triple helix DNA[11,12] have been proposed as possible structures of DNA. In particular, the base-pair mismatch is considered to be very important for understanding of mutation occurring in the process of DNA replication because the geometry and effect on the DNA structure of the mismatched base pair seem to be the important factors in the recognition and repair by enzymes.

The incorporation of mismatched base pairs into DNA during replication occurs rarely due to the excellent mechanisms of proofreading by DNA polymerases and repair enzymes.[13,14] However, base-pair mismatches can be formed as intermediates of substitution mutation through the transition or the transversion process.[2,15] Among possible base-pair mismatches, a G-T mismatch can be introduced quite stably into DNA during replication and is regarded as slightly less stable than the standard Watson-Crick G-C or A-T pairs.[2,15] In addition, studies of oligonucleotides containing mismatched base pairs can provide the unique means of perturbing a DNA structure in order to correlate the structure with its stability. The structural perturbation also alters the alignment of water molecules around the DNA duplex.[3] For better understanding of the perturbed local structure of the DNA duplex caused by the incorporation of mismatched base pairs, a large number of studies have been peformed with various synthetic oligonucleotides, such as d(CGCGTG)$_2$ containing G-T mismatches,[16] d(ATGAGCGAATA) containing G-A mismatches,[17] d(CGGGAATTCGCG)$_2$ containing G-G mismatches.[18] The X-ray crystallographic data show that the G-T mismatches result in the slight deviations from the normal structure in the base stacking and the sugar-phosphate backbone at the mismatched site but no significant perturbation in other regions.[3,19] In contrast to this, the A-C mismatches were reported to cause the structural perturbation noticeably near the mismatched site.[15] Therefore, perturbation by the G-T mismatch may be construed entirely as the local effect at the mismatched site. And the G-T mismatch appears to shift the thymine base slightly toward the major groove and, at the same time, the guanine toward the minor groove, exposing the heteroatomic groups of the bases to surrounding water molecules, thus allowing for some unusual hydrogen bonds.[16] The alterations caused by the mismatch are considered to be important features in the discrimination of the Watson-Crick base pairs from the mismatched ones by proofreading activities during replication and repair. Thus, as has been stated above, studies on the structure and dynamics of the DNA duplex containing mismatches can provide us many valuable informations about the properties of hydrogen bonding involving the base-pairing, the effects of the mismatches on the local and global structure of the DNA duplex, and the relationship between the structure and the sequence context of the mismatch as well as the efficiency of the mismatch repair.

Berenil, a trypanosidal drug, was chosen as a ligand for the binding interaction to model oligonucleotides in the research and is known to bind to the minor groove of the A-T base pair rich regions with a strong affinity. But the result from a previous study showed that berenil also gave an effect on the stability of a d(GCGCGCGC)$_2$ duplex upon binding,[20] therefore, the effect on the stability of the mismatched d(GCGTGCGC)$_2$ by complexation with berenil is of in-

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terest.

In this paper we present some results showing the effects of the G-T mismatches in d(GCGTGCGC)$_2$ and its berenil complex on the structure, thermal stability, thermodynamic properties of duplex formation, and dynamics of the duplex. The experimental results were obtained by using one- and two-dimensional NMR spectroscopy primarily and also by some other spectroscopic methods such as UV and circular dichroic (CD) spectroscopy.

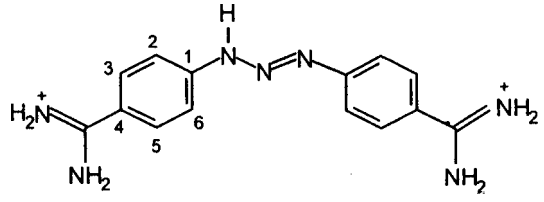
Materials and Methods

Materials. The model oligodeoxynucleotides, d(GCGTGCGC) and d(GCGCGCGC), were synthesized with a DNA synthesizer (ABI 391 model) by β-cyanoethyl phosphoramidite chemistry in solid phase. They were purified with an ion exchange column and then lyophilized. Berenil diacetate was purchased from Sigma Chemical Co. All other chemicals used were at least of reagent grade and used without further purification.

Method. The freeze-dried oligodeoxynucleotide was dissolved in 20 mM phosphate buffer of pH 6.9 with 100 mM of NaCl. The concentration of each oligodeoxynucleotide in solution was determined by measuring its absorbance at 260 nm. When dissolved in the same phosphate buffer, the molar absorption coefficients for d(GCGTGCGC)$_2$ and d(GCGCGCGC)$_2$ at 260 nm were 103,000 and 79000 $M^{-1}cm^{-1}$, respectively.[21] All the imino proton 1D NMR and 2D NOESY spectra were obtained on a Bruker DMX 600 NMR spectrometer in the Korea Basic Science Institute. The samples were prepared in 20 mM phosphate buffer (pH 6.9) with 100 mM of NaCl. For the imino proton spectra, the Jump and Return (1-$\bar{1}$) solvent suppression pulse sequence was used with the carrier frequency set at the water resonance.[22] Two-dimensional NOESY spectra were obtained by using the field gradient NOESY pulse sequence in the TPPI mode at 10 ℃.[23] The mixing time was 400 ms, and the data size were 400

5′→GCGCGCGC→3′
3′←CGCGCGCG←5′
1 2 3 4 4 3 2 1
(A)

5′→GCGTGCGC→3′
3′←CGCGTGCG←5′
1 2 3 4 4 3 2 1
(B)



(C)

Figure 1. Base sequences of a Watson-Crick duplex d(GCGCGCGC)$_2$ (A), a d(GCGTGCGC)$_2$ duplex containing mismatched G-T pairs(B) and a chemical structure of berenil(C).

and 4 K for t_1 and t_2 dimension, respectively. The DNA melting experiments were carried out with a HP-8452 UV-VIS spectrophotometer equipped with a Peltier temperature controller. DNA melting experiments of the d(GCGTGCGC)$_2$ and d(GCGCGCGC)$_2$ duplexes in 20 mM phosphate buffer (pH 6.9) containing 100 mM of NaCl were performed by monitoring their UV absorbances at 260 nm every 2 ℃ while temperature being increased from 15 ℃ to 85 ℃ at a rate of 0.5 ℃/min. No corrections were made for volume expansion. The experiments were repeated at two other elevated NaCl concentrations, 500 and 1000 mM. Melting experiments with the complex of each oligonucleotide and equimolar concentration of berenil were also performed in the same way as described above. Circular dichroic spectra of two oligodeoxynucleotides and their berenil complexes were observed with a JASCO 600 spectropolarimeter which was equipped with a water-jacketed cell holder maintained at 20 ℃ and interfaced to a PC computer for data collection and analysis.

Thermodynamic parameters were determined by using the van't Hoff method. If all-or-none model (two-state transition) is assumed for double helix formation of the self-complementary oligonucleotides from the single-stranded state, the equilibrium constant for the transition $2A \rightleftharpoons A_2$ can be written as follows:

$$K=\frac{\alpha}{2(1-\alpha)^2C_T} \tag{1}$$

Here α and C_T are, respectively, the fraction of oligonucleotides in the double helical state and the total single strand concentration. The value of α at any temperature can be obtained from the DNA melting curve prepared by the method reported previously.[24] The equilibrium constant K may also be put into the following form:

$$\ln K=-\frac{\Delta H^\circ}{R}\left(\frac{1}{T}\right)+\frac{\Delta S^\circ}{R} \tag{2}$$

Therefore, thermodynamic parameters, ΔH°, ΔS° and ΔG° for double helix formation from the single-stranded state can be determined from the plot of ln K *vs.* $1/T$. Of course, this is not a direct method to measure the transition enthalpy.[24] But if the upper and lower baselines for the melting curve are adjusted properly, one can make a fairly accurate estimation of the thermodynamic parameters for the melting transition of the oligonucleotide duplex. The transition enthalpy can also be obtained by using the relationship between $1/T_m$ and lnC_t, where T_m and C_t represent the melting temperature and the total oligonucleotide concentration in the single-stranded state, respectively. At the melting temperature, since $\alpha=1/2$, Eqs. (1) and (2) can be combined to yield

$$\frac{1}{T_m}=\frac{R}{\Delta H^\circ}\ln C_t+\frac{\Delta S^\circ}{\Delta H^\circ} \tag{3}$$

Thus the plot of $1/T_m$ *vs.* ln C_t can yield ΔH° and ΔS° from its slope and Y-intercept, respectively. In contrast to the plot of ln K *vs.* $1/T$, ΔH° determined from this plot is immune to the baseline-dependent errors and has been reported to show a good agreement with that of the calorimetric result.[25]

Results and Discussions

^{1}H NMR spectra of d(GCGTGCGC)$_2$ and its berenil complex. One-dimensional ^{1}H NMR spectra of the d(GCGTGCGC)$_2$ duplex and its berenil complex are shown in Figure 2. Adding berenil to the d(GCGTGCGC)$_2$ duplex caused no noticeable change in the spectrum except for the linewidth of resonance signals, indicating that the presence of berenil around the d(GCGTGCGC)$_2$ duplex exerted no observable effect on the conformation of DNA.

The imino proton resonance signals in d(GCGTGCGC)$_2$. The imino proton spectrum in d(GCGTGCGC)$_2$ was found to substantially differ from that of d(GCGCGCGC)$_2$ (Figure 3),[27] suggesting that incorporation of the G-T mismatches into the positions 4 and 5 of the d(GCGCGCGC)$_2$ duplex can induce considerable changes in the environment around the imino protons involved in the base pairings. As can be seen from Figure 3A, three resonance signals for the imino protons appear respectively at 13.21, 11.87, and 10.75 ppm in the spectrum taken at −6 ℃. They were assigned to the imino protons of G3NH, T4NH and G4NH, respectively, based on the two-dimensional Nuclear Overhauser Effect (2D NOE) data and their temperature-dependent linewidths.

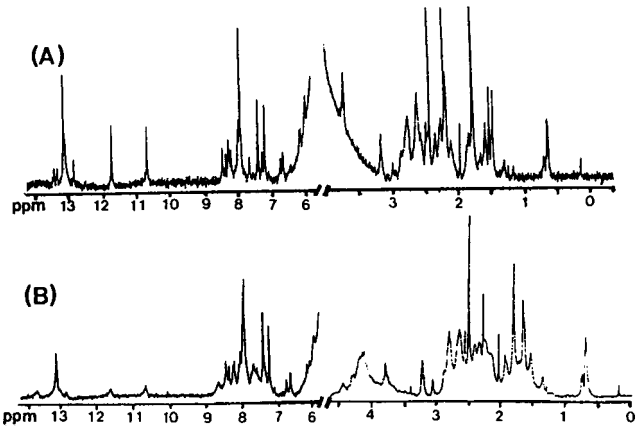


Figure 2. ^{1}H NMR spectra of d(GCGTGCGC)$_2$ (A) and its berenil complex (B) in 20 mM phosphate buffer with 100 mM NaCl and pH 7.

Figure 3A also shows that the linewidths of these resonance signals decrease with increasing temperature over the range of −6 ℃ to 19 ℃ but they become broadened significantly at 30 ℃ and finally disappear at 43 ℃. In contrast to this, imino proton signals in d(GCGCGCGC)$_2$ can be clearly recognized even at 55 ℃, indicating that this duplex has much better thermal stability than d(GCGTGCGC)$_2$. The imino protons of the terminal guanines did not show up due to their fast fraying rate at −6 ℃. The G2NH signal appears at 13.13 ppm but readily broadens out. Three tiny resonances marked with the asterisks near the G3NH turned out to arise from resonances due to impurities which were not yet accounted for. All the imino signals showed upfield shifts with increasing temperature, indicating that the proton exchange occurs between the base imino group and water. The appearance of two imino signals of T4NH and G4NH in a upfield region means that there should exist stable base pairs between thymine and guanine bases via non-standard Watson-Crick hydrogen bonding (Figure 4) in accordance with the reports by other authors.[16,26]

Imino proton signals in the d(GCGTGCGC)$_2$-berenil complex. The NMR spectrum given in Figure 3B shows the imino proton signals in the d(GCGTGCGC)$_2$-berenil complex. In contrast to the berenil-free d(GCGTGCGC)$_2$, the imino proton signals of G2NH and G3NH are resolved

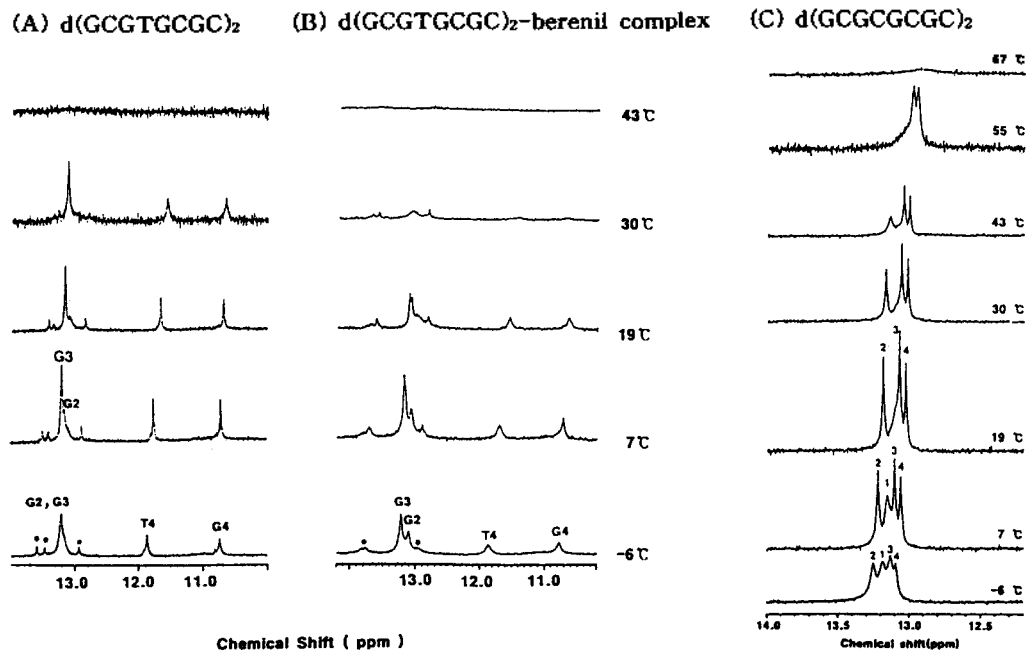
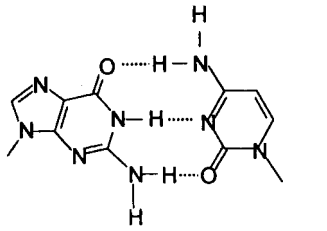
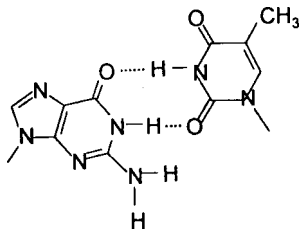


Figure 3. Imino proton spectra of d(GCGTGCGC)$_2$ (A), its berenil complex (B) and d(GCGCGCGC)$_2$ (C) in 20 mM phosphate buffer with 100 mM NaCl and pH 7.



G-C base pair



G-T mismatch

Figure 4. Mismatched G-T base pairing.

well at −6 ℃. Splitting of G3NH signal can be seen at 19 ℃, which might be caused by the slight disruption of the two-fold symmetry in the duplex structure due to the presence of berenil nearby. All the imino proton signals in the d(GCGTGCGC)$_2$-berenil complex show the same temperature-dependence as those in d(GCGTGCGC)$_2$, but are broadened readily with increasing temperature.

Estimation of the base-pair lifetime. The broadening of the imino proton signals may be explained in terms of the spin-spin relaxation of imino protons as well as the proton exchange between the oligonucleotide base and environment including solvent water. If the proton exchange may be assumed as a slowly proceeding two-site first order reaction, then the linewidth is given as[27,28]

$$\pi\nu_{1/2} = 1/T_2 + 1/\tau \quad (4)$$

where $\nu_{1/2}$ is the signal linewidth in Hz, and T_2 and τ are the spin-spin relaxation time and the base-pair lifetime, respectively. The melting temperature of the d(GCGTGCGC)$_2$ duplex is around 32 ℃ at the 500 mM of NaCl concentration, consequently a considerable amount of the oligonucleotide exist in the open state at 30 ℃. Under this circumstance, the contribution of $1/\tau$ to the linewidth is thought to be dominant over that of $1/T_2$,[28,29] therefore, we can simplify Eq. (4) as following.

$$\pi\nu_{1/2} \approx 1/\tau \quad (5)$$

Temperature-dependent linewidths of the imino proton signals of the d(GCGTGCGC)$_2$ duplex and its berenil complex, as shown in Figure 5, indicate the proton exchanges indeed proceed slowly on the NMR time scale.

The method of estimating the lifetimes of the hydrogen-bonded imino protons of the base pairs just described is not deemed suitable for the terminal and their neighboring bases because for these bases the proton exchange rates are very fast but could reasonably be applied to estimate the lifetimes of G3NH, T4NH, and G4NH, which are involved in base pairing and the results are listed in Table 1. The

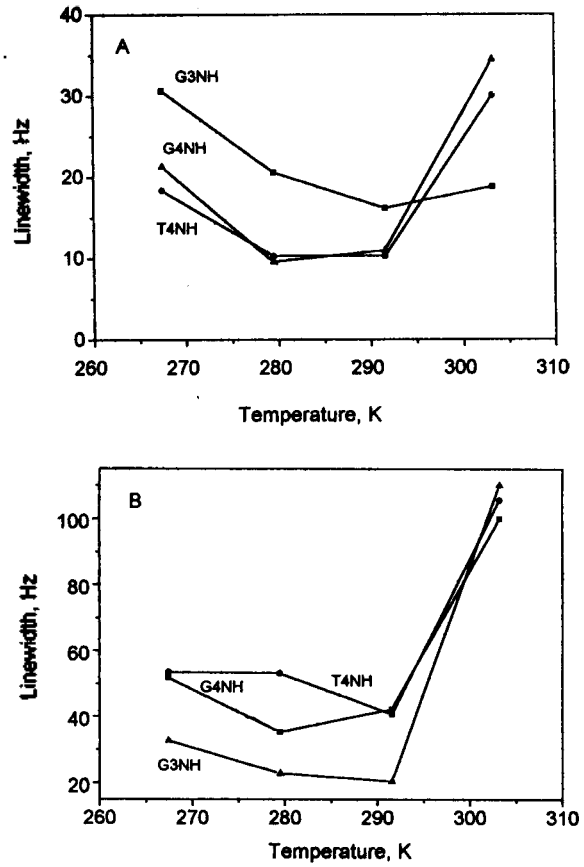


Figure 5. Temperature-dependence of linewidths of imino protons of d(GCGTGCGC)$_2$ (A) and its berenil complex (B).

Table 1. Base-pair lifetime of imino protons of d(GCGTGCGC)$_2$ and its berenil complex, determined from the linewidth of the resonance signals at 30 ℃

	Temp.,	d(GCGTGCGC)$_2$		d(GCGTGCGC)$_2$-Berenil Complex	
	℃	Linewidth[a] (Hz)	Life time (ms)	Linewidth[a] (Hz)	Life time (ms)
G3NH	30	19	17	110	3
T4NH	30	30	11	106	3
G4NH	30	34	9	100	3

[a]The linewidth values measured from resonance signals contain the relative errors within ±5%.

results show that the imino protons of the mismatched G-T pairs have a relatively short lifetime compared with those of the G-C pairs at the position 3. Also, our unpublished data indicate that the lifetimes of hydrogen-bonded imino protons in d(GCGCGCGC)$_2$ and d(CGAATTCG)$_2$ are around 40 and 25 ms at 30 ℃, respectively, which are longer than those of the imino protons of the mismatched G-T pair. This may be ascribable to the fact that the mismatched G-T pairs would come under broader contact with surrounding water molecules because of the structural perturbation induced by incorporation of two G-T mismatches. And this structural

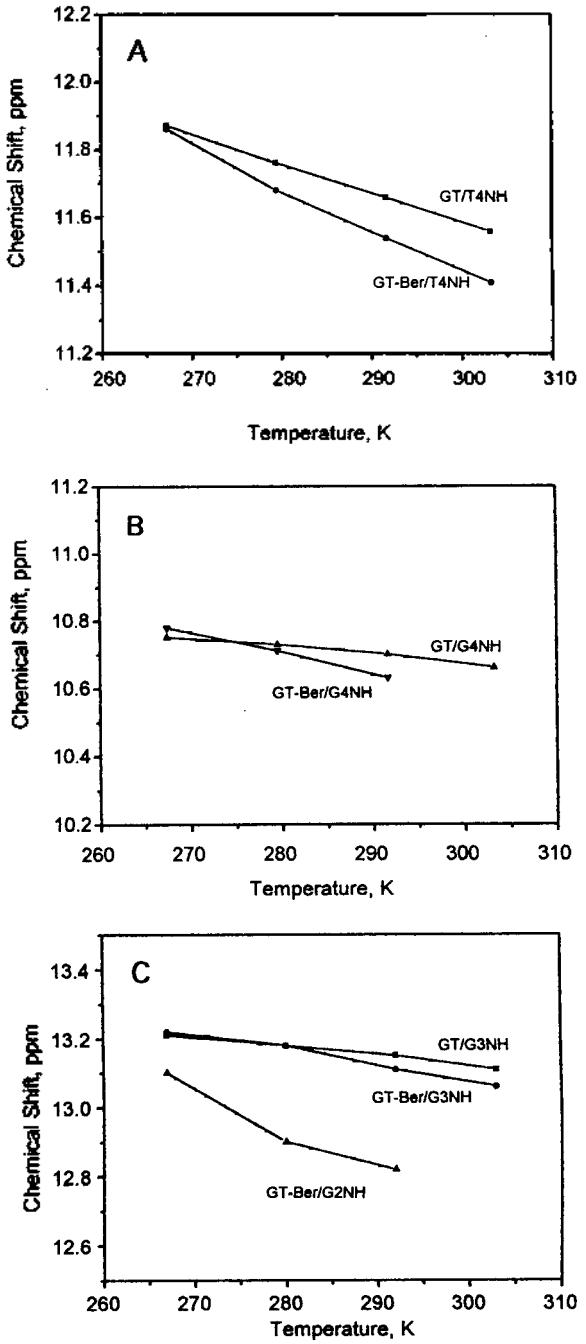


Figure 6. Temperature-dependence of chemical shifts of imino protons of T4NH(A), G4NH(B) and G3NH(C) of d(GCGTGCGC)$_2$ and its berenil complex.

perturbation at the G-T mismatched site seem to affect the lifetimes of the hydrogen-bonded imino protons of the neighboring base pairs.

Addition of berenil resulted in a large decrease in the lifetimes of all the imino protons, notably for G3NH, so that no difference could be observed in the lifetimes of the hydrogen-bonded imino protons between the G-C pair at the position 3 and the mismatched G-T base pair at the position 4.

Temperature dependence of imino proton chemical shifts. Figure 6 shows the temperature dependence of the chemical shift of imino protons in the mismatched pair. The thymine imino proton (T4NH) shows a substantially strong temperature dependence while the guanine imino protons in the standard Watson-Crick G-C pair(G3NH) and the mismatched G-T pair(G4NH) show only a relatively weak temperature dependence. This fact may reflect that the thymine of the mismatched G-T pair can be stacked less stably than the guanine base of the G-T pair. Besides, addition of berenil clearly makes the chemical shifts a little more temperature dependent for both guanine and thymine imino protons.

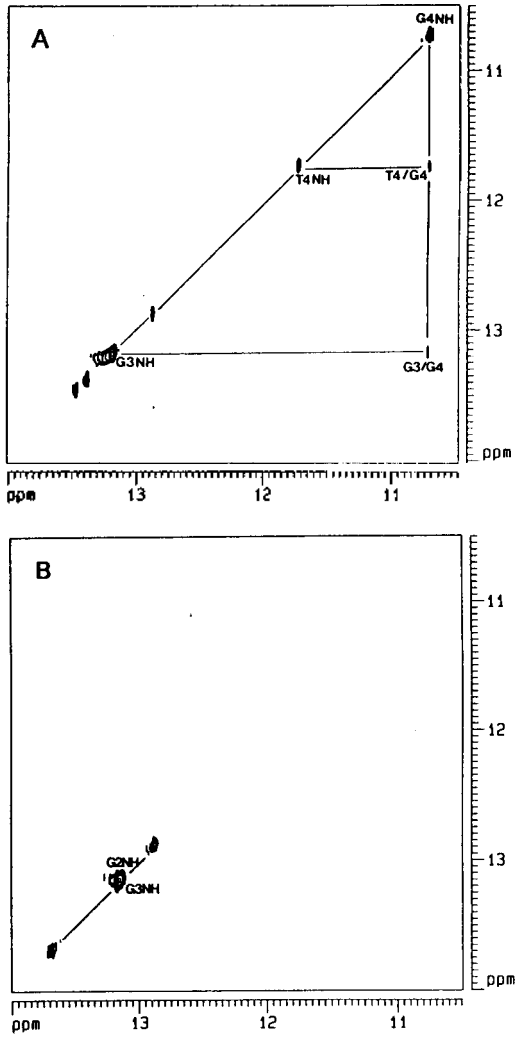


Figure 7. Two-dimensional Nuclear Overhauser Effect spectra of imino protons of d(GCGTGCGC)$_2$ (A) and its berenil complex (B).

Two-Dimensional NMR Spectral Data. Two cross peaks, one between G3NH and G4NH and the other between G4NH and T4NH, in Fig 7A provides the evidence for the existence of the non-Watson-Crick pairing between guanine and thymine bases at the positions 4 and 5. This wobble base pairing is considered to be formed by two hydrogen bonds, one between imino hydrogen H1 of G4 and C2 carbonyl oxygen of T4 of the complementary strand and the other

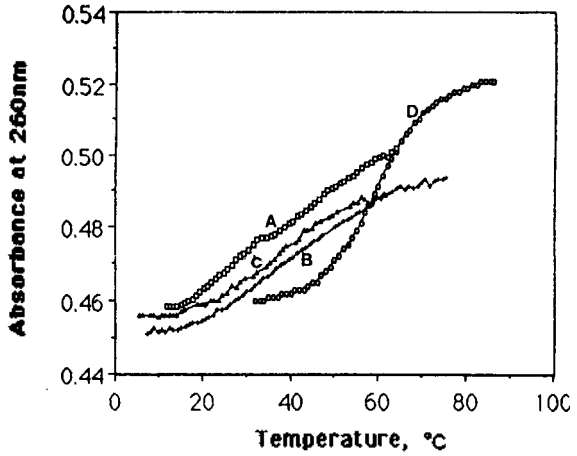


Figure 8. UV Melting curves of d(GCGTGCGC)$_2$ in 20 mM phosphate buffer (pH 6.9) with 100 mM NaCl (A), 500 mM NaCl (B), its berenil complex in 20 mM phosphate buffer (pH 6.9) with 500 mM NaCl (C) and d(GCTGCGCGC)$_2$ in 20 mM phosphate buffer (pH 6.9) with 100 mM NaCl (D).

between imino hydrogen H3 of T4 and C6 carbonyl oxygen of G4 of the complementary strand (Figure 4). No cross peak between G3NH and T4NH could be seen. This means that the helical twist between the G-C pair at the position 3 and the G-T mismatch at the position 4 became larger than that between the standard G-C pairs, thereby increasing the distance between G3NH and T4NH beyond the limit that a NOE contact is feasible. Keepers et al. reported that the amino protons of the guanine were exposed in the minor groove on the basis of their work with the duplex d(CGTGAATTCGCG)$_2$, which is consistent with our result.[30] In the 2-D NOE spectrum of the berenil complex, these cross peaks did not show up because of the fast exchange rate accelerated by the presence of berenil (Figure 7B). In this case we could not observe any change in the chemical shifts of the G4 and T4 imino protons, meaning that berenil produced no effect on the local structure of the region containing the G-T mismatches.

Melting curve of d(GCGTGCGC)$_2$ and its berenil complex. The melting curve of d(GCGTGCGC)$_2$ in 20 mM of phosphate buffer with 100 mM of NaCl (pH 6.9) shows a quite different pattern from that of d(GCGCGCGC)$_2$ (Figure 8). Almost linear melting pattern of d(GCGTGCGC)$_2$ over the whole temperature range indicates that a large number of intermediates are involved in the melting process. Therefore, the two-state model (all-or-none model) could not be applied for the transition between the duplex and the single-stranded state for this oligonucleotide. But the melting curve became sigmoidal at higher NaCl concentrations of 500 and 1000 mM, implying the cooperative melting transition.

The d(GCGTGCGC)$_2$-berenil complex also shows a sigmoidal melting curve at 500 mM of NaCl, but its melting temperature is lower than that of the berenil-free duplex. High NaCl concentration may be thought of as making the melting behavior of d(GCGTGCGC)$_2$ cooperative by improving thermal stability of the duplex through countering destabilizing effect by negatively charged phosphate groups. Addition of berenil to the duplex was found to lower the melting temperature by 4 ℃ at 500 mM NaCl and by 6 ℃ at 1 M NaCl (Table 2), but it did not affect the cooperativity of melting transition at all. Besides, the melting temperature (T_m) of d(GCGTGCGC)$_2$ was lower than that of d(GCGCGCGC)$_2$ by as much as 30 ℃,[20] indicating that incorporation of two mismatched G-T pairs at positions 4 and 5 of d(GCGCGCGC)$_2$ was accompanied by enormous decrease in the thermal stability.

Table 2. Melting temperature and thermodynamic parameters for double helix formation of d(GCGTGCGC)$_2$ and its berenil complex, determined from plots of ln K *vs* $1/T$ (Values in parentheses were determined from a plot of $1/T_m$ *vs.* ln C_t)

Sample [NaCl], mM	T_m (℃)	$\Delta H°\Delta S°$ (Kcal/mol)	$\Delta G°$ (eu)	(Kcal/mol)
d(GCGTGCGC)$_2$ [NaCl]=500 mM	32	−45	−125	−8
d(GCGTGCGC)$_2$ [NaCl]=1000 mM	36	−47 (−52)	−129 (−146)	−9 (−9)
d(GCGTGCGC)$_2$-berenil complex [NaCl]=500 mM	28	−65	−193	−7
d(GCGTGCGC)$_2$-berenil complex [NaCl]=1000 mM	30	−65	−193	−8

Determination of the thermodynamic parameters. In case of the d(GCGTGCGC)$_2$ duplex at 1000 mM of NaCl, $\Delta H°$, $\Delta S°$ and $\Delta G°$ values determined by two different methods, one by the plot of ln K *vs.* $1/T$ and the other by the plot of $1/T_m$ *vs.* ln C_t, all agreed well with each other within the error bounds of ± 15%. This indicates that our method of adjusting the upper and lower base lines of the melting curve is reasonably adequate for the estimation of $\Delta H°$, $\Delta S°$ and $\Delta G°$. The results listed in Table 2 show that thermodynamic parameters $\Delta H°$, $\Delta S°$ and $\Delta G°$ for the d(GCGTGCGC)$_2$ duplex are smaller in magnitude than those for the d(GCGCGCGC)$_2$ duplex.[31] The difference in $\Delta H°$ by *ca.* 20 Kcal/mol can be ascribed to the shorter lifetime of the mismatched G-T base pairs and weaker stacking interactions between the guanine and the thymine of the 5′-GTG-3′ sequence. Patel *et al.*[16] observed that the transition enthalpy for d(CGTGAATTCGCG)$_2$ was nearly the same as that of d(CGCGAATTCGCG)$_2$. On the basis of this observation they proposed that the lower T_m value originated solely from the entropic contribution. But Keepers *et al.* argued, based on their works with the same oligonucleotide, that it was not clear whether the lower T_m value was due to the entropic contribution or not.[30] They also showed that the strand-separation energy estimated for d(CGTGAATTCGCG)$_2$ was lower than that for d(CGCGAATTCGCG)$_2$ by *ca.* 10 Kcal/mol. Furthermore, according to the result obtained by Hilbers *et al.*,[31] the single G-T base-pair mismatch incorporated at any one of the positions 6, 7, 12 in d(TATTAATATCAAGTTG)·d(CAACTTGATATTAATA) lowers $\Delta H°$ by more than 10 Kcal/mol. In view of these, our transition enthalpy data obtained in the present study do not seem unjustifiably wrong.

Effect of mismatches on Circular Dichroic spectra.

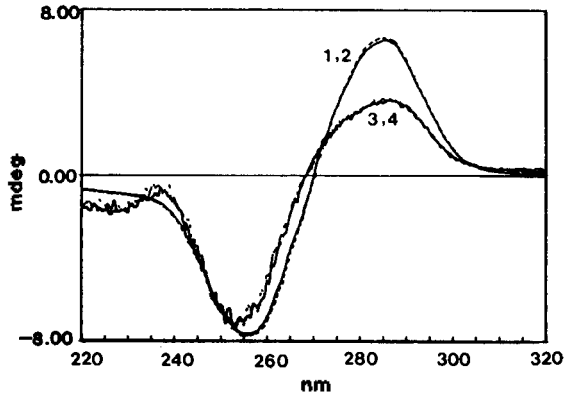


Figure 9. CD spectra of d(GCGTGCGC)$_2$ and d(GCGCGCGC)$_2$ in 20 mM phosphate buffer (pH 6.9) containing 100 mM NaCl. (1) d(GCGTGCGC)$_2$; (2) d(GCGTGCGC)$_2$-berenil complex; (3) d (GCGCGCGC)$_2$; (4) d(GCGCGCGC)$_2$-berenil complex.

Figure 9 shows the circular dichroic (CD) spectra of d(GCGTGCGC)$_2$ and d(GCGCGCGC)$_2$ taken at 20 ℃, with and without berenil, respectively, in 20 mM sodium phosphate buffer containing 100 mM of NaCl (pH 6.9). The difference between the spectra for two berenil-free duplexes can be clearly recognized in the long wavelength region, which indicates that incorporation of two G-T mismatches into d(GCGCGCGC)$_2$ brings on the conformational perturbation via the wobble base pairing between the guanine and the thymine noticeably at the mismatched sites. By contrast, additions of berenil to these duplexes produce no noticeable effect on their global structure.

Conclusions

The NMR and other spectroscopic data show that the existence of the G-T mismatches at the positions 4 and 5 of d(GCGTGCGC)$_2$ can bring on not only a noticeable change in the structure but a significant decrease in the thermal stability as well, compared with d(GCGCGCGC)$_2$. The melting temperature (T_m) of d(GCGTGCGC)$_2$ was found to be lower than that of d(GCGCGCGC)$_2$ by as much as 30 ℃. The reason for this lowering of T_m still remains elusive but we conjecture that the difference in the base-pair lifetime and the local structure between two oligonucleotides may somehow be responsible for this. Melting transition of d(GCGTGCGC)$_2$ was noncooperative at 100 mM of NaCl but became cooperative at higher salt concentrations of 500 and 1000 mM, while d(GCGCGCGC)$_2$ showed the cooperative melting transition even at 100 mM of NaCl. The thermodynamic data obtained from the melting curve show that the duplex formation of d(GCGTGCGC)$_2$ seems to be entropy-driven, while that of d(GCGCGCGC)$_2$ may be more or less enthalpy-driven. The lifetime of the mismatched G-T pair of d(GCGTGCGC)$_2$ was much shorter than that of G-C pair at the position 3. This indicates that incorporation of the G-T mismatched pair shortens the base-pair lifetime significantly, hence lessening the thermal stability as well. It also produced a noticeable effect on the lifetime of the neighboring base pair. Adding berenil to the d(GCGTGCGC)$_2$ duplex caused no observable change in the global structure; however, it brought on a large decrease in the lifetime of the mismatched G-T pair as well as those of the neighboring G-C pairs in the duplex.

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^{1}H NMR Study of the Inclusion Complexes of Chiral Aromatic Guests with β-Cyclodextrin and Its Derivatives: Discrimination of Aromatic Protons and Chiral Recognition

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The effects of β-CD, Me-β-CD, and biphenyl capped β-CD on ^{1}H NMR spectra of mandelic acid **1**, α-methylbenzylamine **2** and 2-phenylpropionic acid **3** were investigated. Enantiomeric recognition was observed for mandelic acid **1** by all the hosts used, for α-methylbenzylamine **2** by β-CD and Me-β-CD, and for 2-phenylpropionic acid **3** by Me-β-CD. In the presence of biphenyl-capped β-CD, *o*-, *m*-, and *p*-protons of the phenyl groups of the guests are discriminated due to ring current of the capped biphenyl group. The splitting pattern of the phenyl protons indicates that the phenyl group of the guests is inserted into the β-CD cavity from the secondary hydroxyl side and positioned in close proximity to the capped biphenyl ring. The magnitude of the upfield shifts of H3 and H5 protons of β-CD upon binding of guests **1-3** is similar to that caused by ephedrine or pseudoephedrine, suggesting that the substitution at benzylic carbon atom has little effect on the depth of the insertion of the phenyl group into the β-CD cavity and stability of the inclusion complexes.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six(α), seven(β), and eight(γ) D-glucopyranose units linked by α(1,4) linkages and possess hydrophobic cavities. They form inclusion complexes with a variety of substrates.[1,2] It is known that the interaction between CDs and the substrate causes chemical shift changes of both the host and the guest protons in NMR spectra.[2~11] The changes in the chemical shifts provide information on the structure and stability of the CD-guest inclusion complexes.[2~5] Since the CD cavities have chiral environment, CDs and their derivatives can form diastereomeric inclusion complexes with chiral guests. NMR spectroscopy has been used for investigation of chiral recognition properties of CD cavities and elucidation of optical purity of chiral substances.[6~10] β-CD has a better-fitted cavity for aromatic groups and more readily available than the other CDs and thus much of NMR works with CDs were carried out with β-CD. Recently, there have been several reports on the improvement of chiral recognition properties by using functionalized β-CD.[8~11] Uccello-Barretta *et al.* reported that permethylated β-CD is a versatile and promising host for NMR chiral analysis.[10] Here, we report ^{1}H NMR study of the inclusion complexes of chiral aromatic substrates **1-3** with β-CD **4**, methylated β-CD (Me-β-CD) **5**, and biphenyl-capped β-CD **6**. The capped β-CD **6** is used since the compound exhibits enhanced binding affinity to aromatic guests,[12] compared to β-CD itself, and considerable ring current effect of the capped biphenyl ring on the chemical shifts of guest protons is expected. We investigated the ability of the hosts to recognize chirality of the guest molecules, and determined the chemical shift changes of the host and guest protons induced upon complexation. Chiral recognition of the guest molecules by the hosts **4-6**, and discrimination of phenyl protons by the host **6** are demonstrated.

Experimental

Materials. Biphenyl-capped β-CD **6** was synthesized by reacting β-CD with 4,4′-biphenyldisulfonyl chloride in pyridine as described by Tabushi.[13] β-CD (from Aldrich) was used after drying for a day in a vacuum oven at 100 ℃, and 4,4′-biphenyldisulfonyl chloride (from Aldrich) was used after recrystallization in chloroform. Me-β-CD **5** was obtained from Cyclolab in Hungary, and *R*-, *S*-, *R/S*-mandelic acid