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12. Preparation of compound **2**: To a solution of 29 mg (0.041 mmol) of C_{60} in 30 mL of toluene of 7 mg (0.082 mmol) of sarcosine and 31 mg (0.082 mmol) of **1** were added. After the resulting mixture was stirred at room temperature for 20 min under nitrogen, the mixture was heated to reflux under nitrogen for 43 hr. The solvent was removed in vacuo. The crude solid product was purified by flash chromatography on silica gel using a mixture n-hexane and toluene as eluent. The yield of **2** was 55% (based on the consumed C_{60}). Preparation of compounds **5** and **7**: The compounds were prepared by procedure as compound **2** (yield 77%). Physical Measurements UV-visible spectra were recorded in n-hexane at room temperature on a BECKMAN DU7500 Spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra of **2** and **5** were obtained in $CDCl_3$ and of **7** were obtained in $CDCl_3/CS_2=1:2$(v/v) on an FT-NMR 400 MHz JEOL Spectrometer. MALDI-MS spectra were recorded on a Shimazu Compact MALDI II Spectrometer. IR spectra were recorded on a Nicolet 5DXB FT-IR Spectrometer. CV and DPV were obtained in 1 : 3 acetonitrile-toluene (0.1 M $(n\text{-}Bu)_4NPF_6$) at room temperature on a BAS 100B/W potentiostat. HPLC conditions; RP-18 Column, acetonitrile-toluene (6 : 4 v/v) (compound **2**); SIL-5B Column, methanol-dichloromethane (1 : 9 v/v) (compound **5**); SIL-5B Column, methanol-dichloromethane (1 : 9 v/v) (compound **7**).[13] MALDI-MS for crude samples: 408 (M^+-720) (base peak), 1128 (M^+), 1536 (bisadduct), 1940 (trisadduct), and 2344 (tetraadduct) (compound **2**); 562 (M^+-720) and 1282 (M^+) (base peak) (compound **5**); 720 (C_{60}^+), 961 (M^+), 1097 (bisadduct-FeCp) (base peak), and 1205 (bisadduct) (compound **7**). Purified samples by HPLC show molecular ion peaks as base peaks.
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15. UV-visible spectra(n-hexane) of **2** and **5** show the pyrrolidine derivative retains the main electronic features of C_{60}, except for a new band at 307 nm. FT-IR (dispersed on KBr plate), 1728 (C=O), 1243 (C-O), 1349 (C-N), 2931, 2853, 1595, 1433, 1180, 1117, 1053, 758, 716, 667, 561 and 519 cm^{-1}. (compound **2**).
16. ^{1}H NMR (400 MHz) of **2**: δ 8.25 (ring CH), δ 4.03 (OCH 2), δ 4.0 (ring CH2), δ 3.93 (CO_2Me), δ 2.98 (N-CH_3), δ 1.76 (CH_2-pyrrolidine, broad), δ 1.25 ((CH_2)$_8$, multiplet). ^{13}C NMR of **2**: δ 159.1, δ 122.7, δ 119.8, δ 131.6 (ring C), δ 166.2 (-CO_2-), δ 52.3 (-Methyl), δ 68.5 (ring CH_2), δ 70.3 (ring CH), δ 40.0 (-NCH_3), the signals of C_{60} are scattered between 142.1 ppm and 146.5 ppm. ^{1}H NMR and ^{13}C NMR of **5**: similar signal pattern was appeared. Regarding **7**, results matched the previously published data well.

NMR Study on Dynamics of Double Helical d(GCGCGCGC)$_2$ and Its Complex with Berenil

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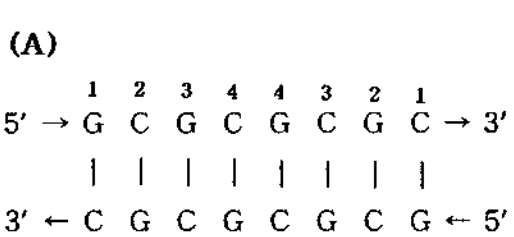
Since Pohl and Jovin reported that poly(dG-dC)·poly(dG-dC) showed quite a different CD spectrum from that of a regular B-DNA structure at high NaCl concentration,[1,2] lots of studies have been carried out in order to investigate the effects of the base sequences and the buffer conditions on the transition between B- and Z-DNA.[3-8] The GpC or CpG rich region appears frequently in the genomic DNA and the structural flexibility of this region is regarded to play an important role in gene expression.[9,10]

In this study, we have prepared a self-complementary, synthetic oligonucleotide d(GCGCGCGC) containing the GpC sequences associated with the B-Z transition and have studied the dynamics of the d(GCGCGCGC)$_2$ double helix and its

(A)



(B)

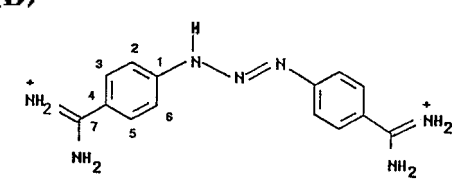


Figure 1. structure of the duplex d(GCGCGCGC)$_2$ (A) and berenil(B).

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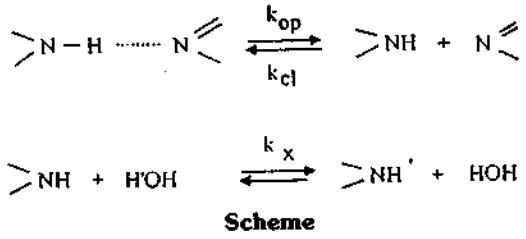
complex with an antitrypanosomal drug berenil by using NMR spectroscopy (Figure 1). In addition, the effect of berenil-binding to d(GCGCGCGC)$_2$ on the base-pair opening has been also revealed from the results. The linewidths of the resonance signals of the imino protons depend on the opening rate of the base-pairing and the exchange rate of the protons between the DNA duplex and water molecules, in addition to the spin-spin relaxation. Base-pair opening has been regarded to be very important in replication and transcription. For the initiation of these biological processes the specific part of a DNA double helix has to be separated. Since the physiological temperature is lower than the DNA melting temperature, the kinetics of the base-pair opening has to be investigated for the better understanding of separation of the DNA double helix. Imino proton exchange between the base-pairs of DNA and water molecules can be described by the scheme given below.[11]



Scheme

Here k_x is the rate constant for the base(or acid)-catalyzed exchange of the imino proton with solvent water. According to many experimental results, k_x is much larger than k_d for the internal base-pairs.[12] Therefore every time an internal base-pair opens, an imino proton exchanges with a water proton. This is the opening-limited process. In contrast, the terminal base-pairs open and close faster than the exchange rate. Therefore, this is the exchange-limited process.

The oligodeoxynucleotide d(GCGCGCGC) was chemically synthesized by an ABI 391 DNA synthesizer using β-cyanoethylphosphoramidite method in solid phase. The synthesized sample was dialyzed with a dialysis tubing of molecular weight cut-off of 1000 and then was lyophilized. The lyophilized oligonucleotide was dissolved into 0.5 mL of 20 mM phosphate buffer (pH 7.0) containing 100 mM of NaCl and was transferred into an NMR tube. Berenil (4,4'-diamidinobenzene diaceturate) which contains positively charged amidino groups at both termini was chosen as a ligand for binding interaction with a duplex d(GCGCGCGC)$_2$ (Figure 1). Even though berenil is known to prefer the 5'-AATT site for binding, the binding data of berenil to d(GCGCGCGC)$_2$ would be very useful in understanding the dynamics and the B-Z transition of DNA. All the NMR spectra of the exchangeable and the nonexchangeable protons of the oligonucleotide and its berenil complex were obtained on a Bruker DMX600 NMR spectrometer operating at 600 MHz for ^{1}H nucleus. The Jump-and-Return pulse sequence was used for effective suppression of the solvent water signal.[13] For observing the temperature-dependence of the chemical shifts and the linewidths of the imino protons, ^{1}H NMR experiments were performed at various temperatures from −6 ℃ to 60 ℃ with an accuracy of ±0.1 ℃.

Figure 2 shows the ^{1}H NMR spectra of the imino protons of the duplex d(GCGCGCGC)$_2$ and its complex with berenil

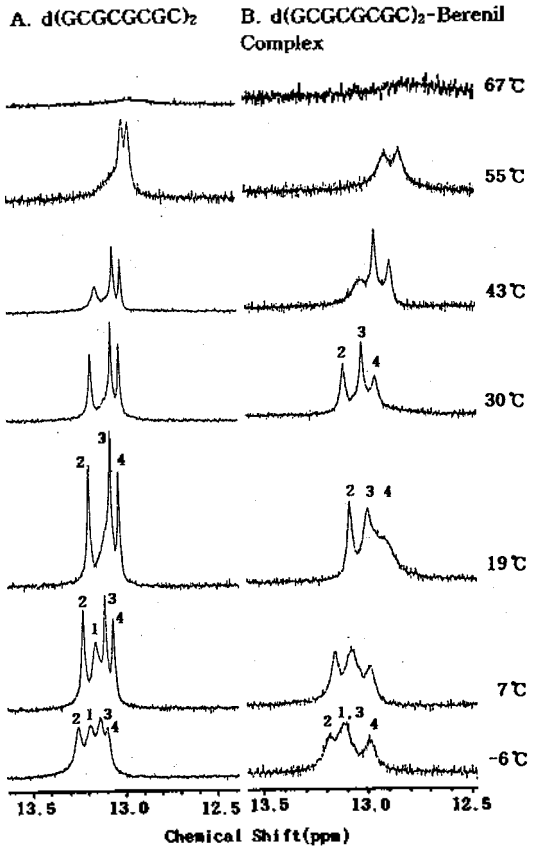


Figure 2. Imino resonance signals of d(GCGCGCGC)$_2$ (A) and its berenil complex(B) at various temperature.

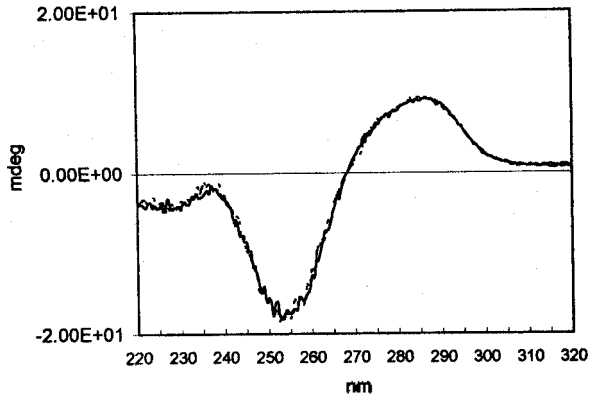


Figure 3. CD spectra of d(GCGCGCGC)$_2$ (···) and its berenil complex (—) in 20 mM of phosphate buffer containing 100 mM of NaCl (pH 7.0).

at various temperatures. The imino proton signals have been assigned based on the temperature-dependence of line broadening and the NMR data of DNA duplexes with similar sequences which were reported previously.[14] First of all, two-fold symmetry of the d(GCGCGCGC)$_2$ duplex was conserved even after berenil binding. This indicated that base-pair opening and berenil binding did not perturb the two-fold symmetry of the conformation of the oligonucleotide duplex. This was also supported by CD spectroscopic data of the duplex which show no change after binding with berenil (Figure

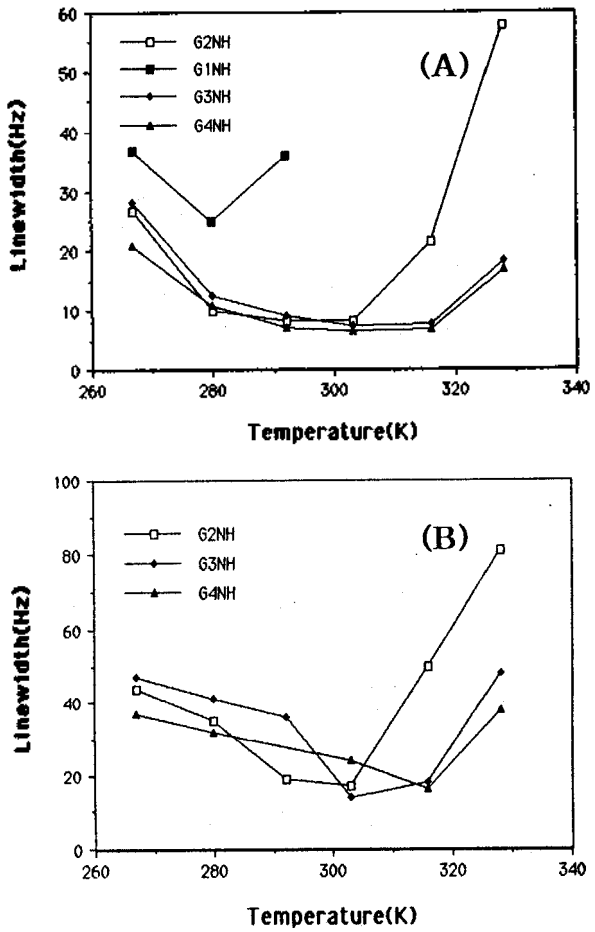


Figure 4. Changes in linewidth of the imino proton signals of d(GCGCGCGC)$_2$ (A) and its berenil complex(B) with increase in temperature.

3). But berenil binding caused a considerable effect on dynamics of the duplex (Figure 2, 4). The increase in linewidth would be caused by spin-spin relaxation as well as by the exchange of protons between the oligonucleotide bases and environment such as neighboring water molecules, buffer molecules or the proton acceptors at DNA bases, etc. If the proton exchange reaction is assumed to be the two-site first order exchange, then the linewidth is given by an equation (1).[15~18]

$$\pi\Delta_{1/2}=\frac{1}{T_2}+\tau^{-1} \quad (1)$$

Here, $\Delta_{1/2}$ is the linewidth of an imino proton signal, T_2 is the spin-spin relaxation time, and τ is the life time of base-pairing. Since the contribution of $1/T_2$ to the linewidth can be assumed to be very small compared to τ^{-1} at 40 ℃ or above, the linewidth is governed by the life time of the base-pair at higher temperature than 40 ℃. In order to obtain the pure contribution of exchanging processes of the protons between DNA and water to the linewidth, the minimum value of the linewidths over the entire temperature range was subtracted from the linewidths at higher than 40 ℃ for each proton. This is because the contribution of $1/T_2$ to the linewidth is considered to be not only very large

Table 1. Base-pair life times determined from the linewidths of the resonance signals of the imino protons

	Temp., ℃	d(GCGCGCGC)$_2$	d(GCGCGCGC)$_2$-Berenil
G2NH	43	15 ms	unavailable
	57	6 ms	5 ms
G3NH	43	40 ms	unavailable
	57	29 ms	9 ms
G4NH	43	45 mg	unavailable
	57	32 ms	14 ms

Table 2. Thermodynamic property for double helix formation of d(GCGCGCGC)$_2$ and its berenil complex in 20 mM phosphate buffer containing 100 mM of NaCl (pH 7.0)

	T_o (℃)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (eu)	$\Delta G°$ (kcal/mol)
d(GCGCGCGC)$_2$	60.8	−62	−162	−13
d(GCGCGCGC)$_2$-Berenil	61.1	−68	−180	−14

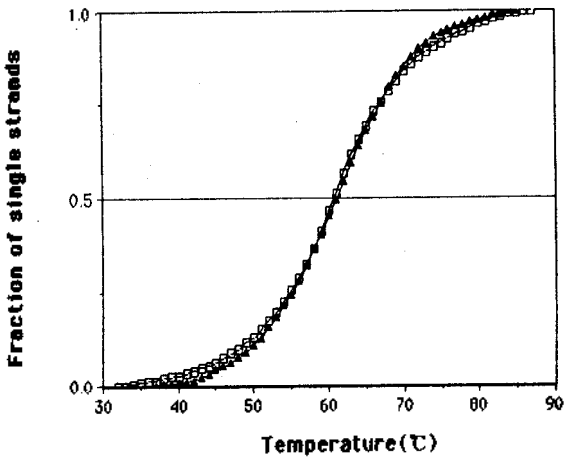


Figure 5. Melting curves of d(GCGCGCGC)$_2$ (□) and its berenil complex (▲) in 20 mM of phosphate buffer containing 100 mM of NaCl (pH 7.0).

compared to that of τ^{-1} at this temperature, but also nearly temperature-independent at higher temperatures.[19] The life times of the imino protons of G-C pairs at 2, 3 and 4 positions of the d(GCGCGCGC)$_2$ double helix were determined to be 6, 29 and 32 ms at 55 ℃, respectively. But those of the complex with berenil were about 5, 9, and 14 ms, respectively (Table 1). Among them, the internal imino protons at 3 and 4 positions showed a large decrease in the life time of base-pairing upon binding with berenil. But it is still unclear whether the decrease in base-pair life time is caused by acid catalysis by berenil in the exchange reaction or by the delicate perturbation in DNA conformation due to complexation with berenil.

According to Figure 2, binding of berenil to the d(GCGCGCGC)$_2$ double helix at 100 mM of NaCl did not cause any change in the thermal stability of the double helical structure

of the d(GCGCGCGC)$_2$. Thermodynamic data obtained from UV melting transition of d(GCGCGCGC)$_2$ double helix and its complex with berenil also supported this result (Table 2, Figure 5). Basically, berenil is known to bind strongly to the 5'-AATT site in the minor-groove *via* two hydrogen bonds: one between amidino proton and thymine carbonyl oxygen, and the other between amidino proton at the other site and adenine N3.[20] Very recently, Pilch *et al.* reported that berenil could bind poly[d(G-C)]$_2$ *via* intercalation as well as complexation in the minor-groove,[21] but our NMR data did not show any evidence for intercalation. More detailed studies about the effect of berenil binding on the base-pair life time of the d(GCGCGCGC)$_2$ double helix is under progress by using 2-Dimensional NMR spectroscopy.

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[3,3]Sigmatropic Rearrangement of Dihydropyran Derivatives

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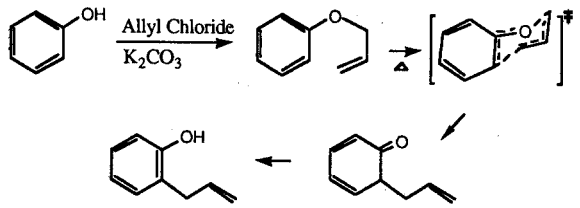
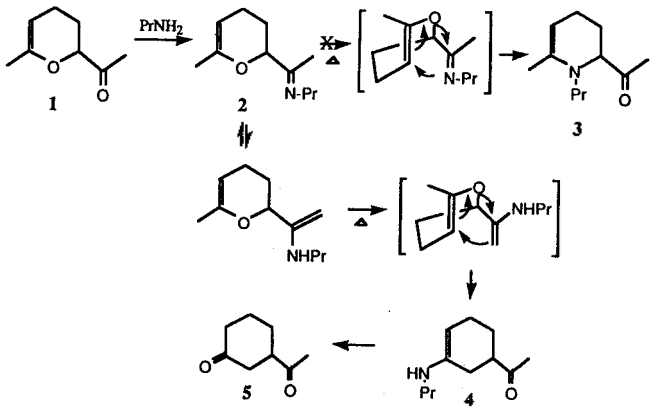
[3,3]sigmatropic shift, especially Claisen rearrangement, has been utilized for introduction of allyl group on benzene ring (Scheme 1).[1] This reaction has been known to proceed as stereoselective concerted mechanism *via* chair cyclohexane transition state.[2] Buchi has taken advantage of this [3,3]sigmatropic shift to gain entry into substituted cyclohexene system.[3] We now wish to introduce [3,3]sigmatropic rearrangement of dihydropyran derivatives to other structures, otherwise which are not readily available.

Dihydropyran **1**, was prepared from methyl vinyl ketone,[4] was converted to the imine **2** by mixing a slight excess of propylamine over molecular sieves in ether solution (Scheme 2). The propyl imine **2** could be purified by distillation, but because of rapid deterioration it had to be used within a few days after purification. Heating the imine at 250 ℃ re-



Scheme 1.



Scheme 2.