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NMR Study on Thermal Stability of the Double Helical Structures of $d(CGAATTCG)_2$, $d(CGTATACG)_2$ and their berenil complexes

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Abstract : We prepared two oligonucleotides containing same base pairing, but different base sequence in the middle region, d(CGAATTCG) and d(CGTATACG). NMR and UV absorbance data represented that such variation in base sequence could cause a significant difference in melting temperature and dynamics between $d(CGAATTCG)_2$ and $d(CGTATACG)_2$ duplexes, which are regarded to be associated with the stacked structure and the width of the minor groove of them. The latter showed poor stability compared to the former, because of poor stacking of bases. And berenil could bind to the minor groove of $d(CGAATTCG)_2$ which is relatively narrow, more strongly than $d(CGTATACG)_2$ and this gave rise to large improvement in thermal stability of the $d(CGAATTCG)_2$ duplex, compared to $d(CGTATACG)_2$.

key words: NMR, DNA, berenil, stability, base pairing, base sequence

INTRODUCTION

Much progress has been achieved in understanding biological functions of DNA in terms of its structure since the double helical structure of DNA was reported.[1] Recent studies on structure and conformation of DNA have afforded new insights on the atomic level.[2-4] But an important question in understanding of the biological features and activity of DNA remains with the relation between its functions and structural features. The biological function of DNA in gene expression is controlled by interactions with regulatory proteins

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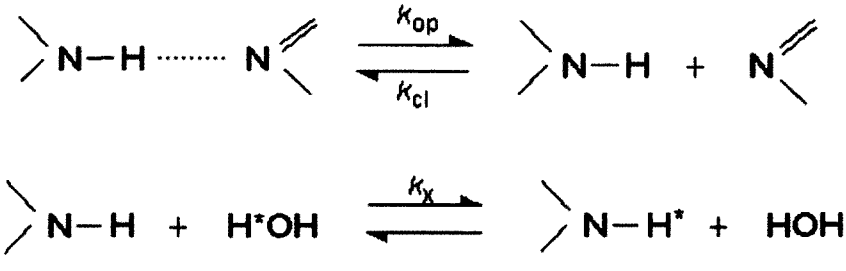
and other molecules. In many cases, these molecules show very high specificity in binding interactions with DNA,[5,6] which is thought to originate primarily from the variations in the base sequence of DNA. Since the natural DNA molecule is too large for detailed analysis by NMR spectroscopy, short oligonucleotides have been used frequently in the hope and expectation that various structural features of these short DNA fragments can be extrapolated to those of much larger native DNA.

In this study we have chosen two synthetic oligonucleotides d(CGAATTCG) and d(CGTATACG) as model DNA fragments (Fig. 1). The model oligonucleotide d(CGAATTCG) contains the recognition sequence of EcoRI type II resrtriction endonuclease. They are self-complementary and have same base pairings but different base sequences, which are considered to serve as good models for the purpose of studying effects of base sequence on the properties of a duplex including stability. Most structural and dynamic data we utilzed in this study were obtained primarily by means of proton nuclear magnetic resonance spectroscopy. For measuring data on dynamics of DNA duplexes, in particular, the linewidths of resonance signals of imino protons in guanine and thymine bases of DNA were observed as described elsewhere.[7-10] The signal linewidth of an imino proton is known to depend on both its spin-spin relaxation and exchange rate between the bases of DNA and surrounding water molecules or neighboring groups containing exchangeable protons. The imino proton exchange process can be described by the scheme given below.[11]



Scheme

Here k_x is the rate constant for the exchange of the imino proton with the proton of water or neighboring groups. For the internal base-pairs, k_x was reported to be much faster than k_{cl} .[12] Therefore, almost every time the internal base-pair opens, the imino proton is expected to exchange with a neighboring exchangeable proton. This is an opening- limited process. In contrast, terminal base-pairs open and close faster than the proton exchange rate, which is an exchange-limited process. Besides the NMR data, UV melting and circular dichroic data have also been obtained. From UV melting data we could gain information on thermodynamic properties of duplex formation of two oligonucleotides and their complexes with berenil.

MATERIALS AND METHODS

The model oligonucleotides d(CGAATTCG) and d(CGTATACG) were chemically synthesized by means of an ABI 391 DNA synthesizer using β-cyanoethylphosphoramidite method in solid phase. They were dialyzed with dialysis tubings with the molecular weight cut-off limit of 1000 and then lyophilized. Each lyophilized oligonucleotide was dissolved into 0.5 mL of 20 mM phosphate buffer(pH 6.9) containing 100 mM of NaCl and was transferred into an NMR tube. Berenil (4,4'-diamidinobenzene diaceturate), which is an antitrypanosomal drug containing positively charged amidino groups at both termini, was chosen as a ligand for binding interaction (Fig. 1). NMR spectra of the oligonucleotides and their berenil complexes were obtained on a Bruker DMX 600 NMR spectrometer operating at 600 MHz for ^{1}H. The jump-and-return pulse sequence was used for effective suppression of the solvent water signal.[13] For observing the temperature-dependence of the linewidths of the imino protons, ^{1}H NMR experiments were performed at various temperatures ranging from -6 ℃ to 55 ℃. The imino proton signals of d(CGAATTCG)$_2$ and d(CGTATACG)$_2$ duplexes and their complexes with berenil have been observed at various temperatures and assigned based on the temperature-dependence of line broadening, 2D NOESY data, and the NMR data of DNA duplexes with similar sequences reported elsewhere (Fig. 2).[14]

(A)

	1	2	3	4	5	6	7	8
5′→	C	G	A	A	T	T	C	G
3′←	G	C	T	T	A	A	G	C

(B)

	1	2	3	4	5	6	7	8
5′→	C	G	T	A	T	A	C	G
3′←	G	C	A	T	A	T	G	C

(C)

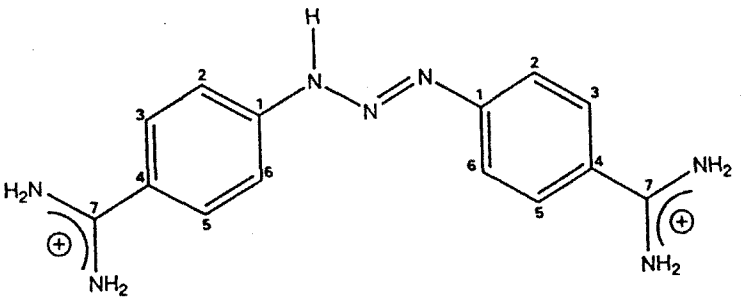


Fig. 1. Model oligonucleotides d(CGAATTCG)$_2$ (A), d(CGTATACG)$_2$ (B), and berenil(C).

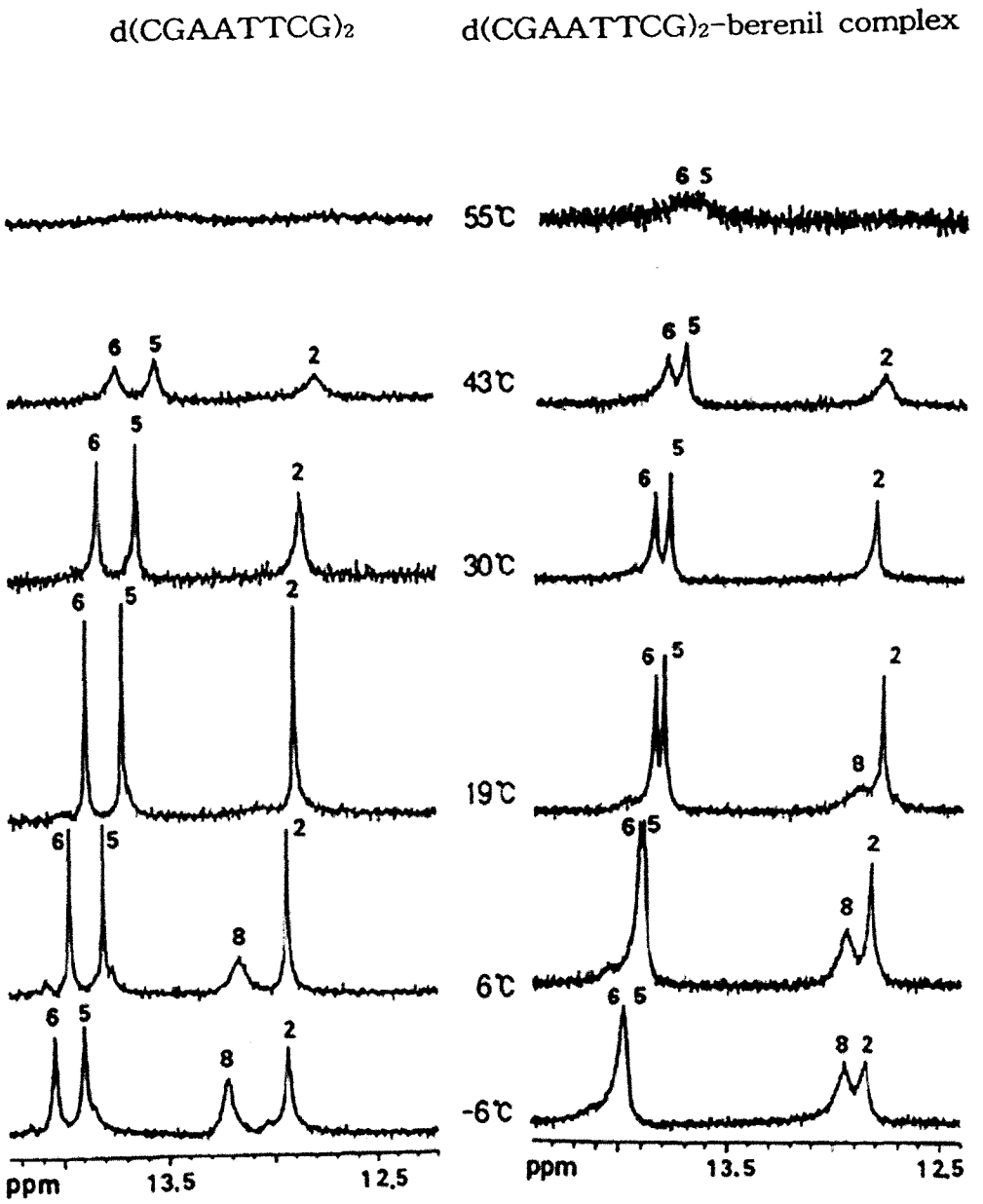


Fig.2. Imino resonance signals of d(CGAATTCG)$_2$, d(CGTATACG)$_2$ and their berenil complexes at various temperature.

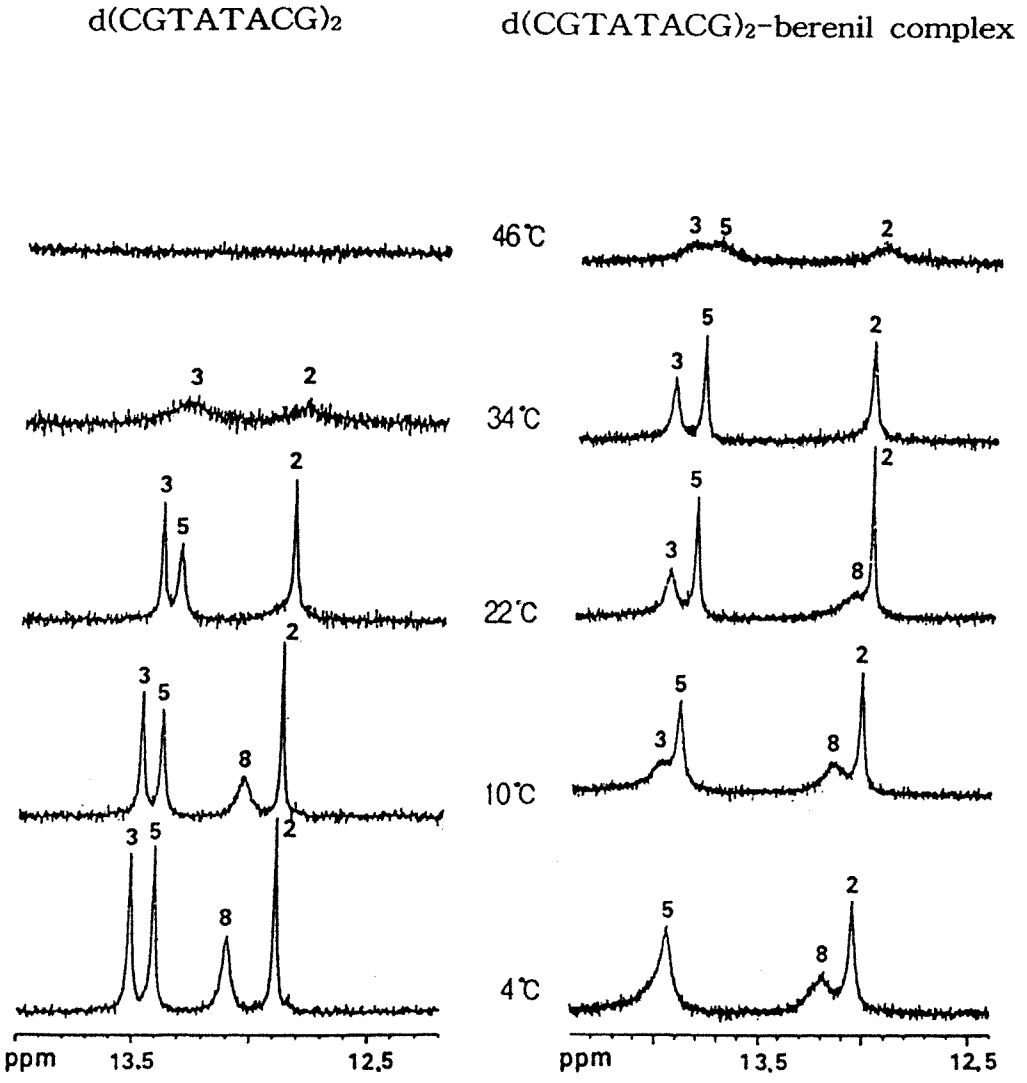


Fig.2(continued).

RESULTS AND DISCUSSIONS

First of all, two-fold symmetry of double helical structures of the two DNA duplexes was evident from these data. We also could readily recognize the differences in the chemical shifts and thermal stability between them. The $d(CGAATTCG)_2$ duplex showed better thermal stability than $d(CGTATACG)_2$, which is consistent with the UV melting results (Fig. 3). Melting temperature (T_m) of the former was observed to be higher than the latter. In the middle of $d(CGTATACG)_2$, TA, AT and TA steps appear back to back in order. According to X-ray data, the TA step had low propeller twist and high helical twist while the AT step had high propeller twist and low helical twist.[4,15] Therefore, TA and AT steps adjacent to each other can lead to poor base stacking and local structural perturbation at the TATA sequence, resulting in weakening of interstrand hydrogen bonds for base pairing. Binding of berenil to $d(CGAATTCG)_2$ increased melting temperature significantly. By contrast, $d(CGTATACG)_2$ did not cause the large increase in melting temperature upon binding to berenil. In contrast to the TATA sequence, the AATT sequence showd the narrow minor groove in double helical structure[3,4], therefore berenil molecule could fit well into it, forming two hydrgen bonds between its terminal amidines and N3 of adenines at position 3 and 4.[16,21]

Dynamics of oligonucleotide duplexes and their complexes with berenil has been studied by observing the change in linewidths of imino protons participating in base pairing at various temperatures. If the proton exchange is assumed to be the first-order two-site jump process, then the linewidth can be expressed as follows:[17-20]

$$\pi \nu_{1/2} = 1/T_2 + \tau^{-1}$$

Where $\nu_{1/2}$ is the linewidth at half-height of the imino proton signal, T_2 the spin-spin relaxation time, and τ the lifetime of base pairing. At the temperature higher than 30℃, the contribution by $1/T_2$ to the linewidth was known to be very small compared to τ^{-1} and nearly temperature- independent,[10] and therefore the linewidth can be determined primarily by the lifetime of the base pairing. The method of estimating the lifetimes of the hydrogen-bonded imino protons just described could resonably be applied to estimate the lifetimes of the internal bases and the results are listed in Tables 1, 2, although it is not deemed a

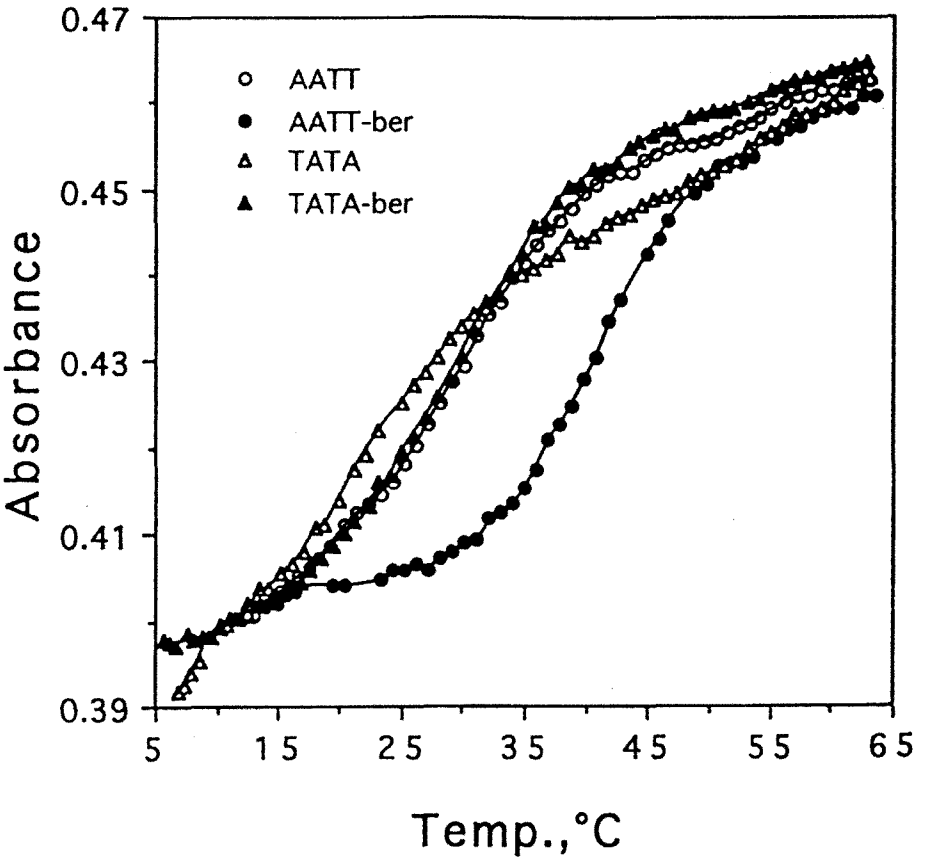


Fig. 3. Melting curves of model oligonucleotides and their berenil complexes in 20 mM of phosphate buffer containing 100 mM of NaCl(pH 7.0);
AATT: $d(CGAATTCG)_2$, AATT-ber: $d(CGAATTCG)_2$-berenil complex
TATA: $d(CGTATACG)_2$, TATA-ber: $d(CGTATACG)_2$-berenil complex

suitable means for the terminal bases where both base-pair opening and proton exchange with the surroundings are very fast. The results show that the internal base pairs in $d(CGAATTCG)_2$ have quite long lifetimes compared to those in $d(CGTATACG)_2$, regardless of the presence of berenil, which means that shuffling the base sequence of internal four

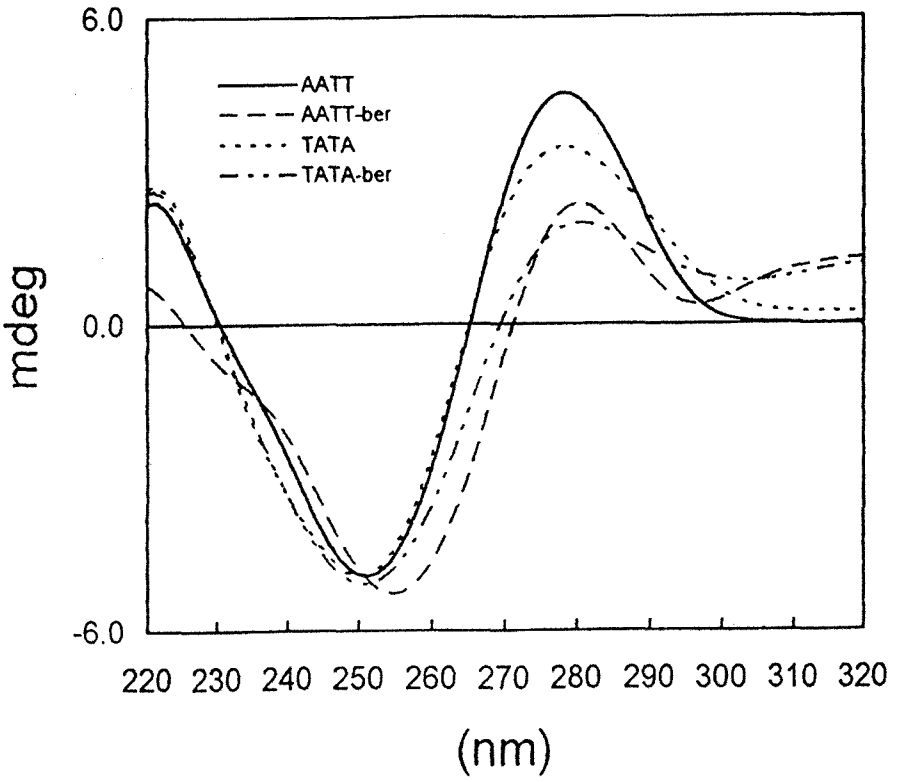


Fig. 4. CD spectra of model oligonucleotides and their complexes in 20 mM of phosphate buffer containing 100 mM of NaCl(pH 7.0).
AATT: $d(CGAATTCG)_2$, AATT-ber: $d(CGAATTCG)_2$-berenil complex
TATA: $d(CGTATACG)_2$, TATA-ber: $d(CGTATACG)_2$-berenil complex

bases, while keeping the overall composition of base-pairings unaltered, could bring about a significant difference in dynamics of the DNA duplex. The circular dichroic data indicate that global structures of the oligonucleotides and their complexes maintain the B-DNA structure (Fig. 4), which in turn means that alteration in the base sequence of internal four bases could exert considerable effect on dynamics of oligonucleotides without causing any substantial change in their global structure. As was mentioned, the TATA sequence gave rise to poor base stacking and caused the local structural perturbation, which resulted in

weakening of interstrand hydrogen bonds involved in base pairing, rendering imino protons more exposed to surrounding water. This rationale could explain nicely why the oligonucleotide $d(CGTATACG)_2$ have very short base-pairing lifetime and low thermal stability, compared to its counterpart $d(CGAATTCG)_2$.

CONCLUSION

As was mentioned, the TATA sequence gave rise to poor stacking and caused the local structural perturbation, which resulted in weakening of interstrand hydrogen bonds involved in base pairing, rendering protons more exposed to surrounding water. This rationale could

Table 1. Base-pair lifetimes determined from the linewidths of the resonance signals of the imino protons of $d(CGAATTCG)_2$ and its berenil complex

	Temp., ℃	$d(CGAATTCG)_2$	$d(CGAATTCG)_2$-berenil
T6NH	30	48 ms	159 ms
	43	15 ms	14 ms
	55	unavailable	3 ms
T5NH	30	72 ms	318 ms
	43	17 ms	16 ms
	55	unavailable	2 ms
G2NH	30	20 ms	80 ms
	43	8 ms	7 ms

Table 2. Base-pair lifetimes determined from the linewidths of the resonance signals of the imino protons of $d(CGTATACG)_2$ and its berenil complex

	Temp., ℃	$d(CGTATACG)_2$	$d(CGTATACG)_2$-berenil
T3NH	35	3 ms	unavailable
	46	unavailable	5 ms
T5NH	46	unavailable	5 ms
G2NH	35	3 ms	45 ms
	46	unavailable	2 ms

explain nicely why the oligonucleotide $d(CGTATACG)_2$ duplex have a very short base-pairing lifetime and low melting temperature, compared to its counterpart $d(CGAATTCG)_2$ duplex. In addition, the TATA sequence formed a relatively wide minor groove compared to the AATT sequence, so berenil could not bind properly to the minor groove of the $d(CGTATACG)_2$ duplex. This caused little increase in melting temperature . By contrast, $d(CGAATTCG)_2$ showed a large increase in the thermal stability as observed in UV absorbance and NMR spectra. Based on the data, we could see that the base sequence of DNA could give a much more significant effect on stability of DNA duplex than the base pairing.

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