Spectroscopic Studies on Binding of Berenil to Double Helix of d(GCTTAAGC)$_2$ and d(GCAATTGC)$_2$

Sungho Huh* and Eunhee Kim

Department of Biochemistry, College of Natural Sciences, Chungnam National University, Taejon 305-764, Korea

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Thermodynamic parameters for double helix formation of d(GCTTAAGC)$_2$, d(GCAATTGC)$_2$ and their complexes with berenil were obtained by observing absorbance at 260 nm during the DNA melting process. Two oligodeoxynucleotides are self-complementary and have identical base pairing but different base sequence in the central four bases. From the CD spectral and UV melting data, they revealed noticeable difference in their conformation as well as thermodynamic property. The melting transition of d(GCTTAAGC)$_2$ was more or less enthalpic, but rather entropic for that of d(GCAATTGC)$_2$. And berenil bound to d(GCAATTGC)$_2$ more tightly and increased the melting temperature of the oligodeoxynucleotide from 30 ℃ to 38 ℃, but the overall conformation was maintained. Binding of berenil to the d(GCTTAAGC)$_2$ duplex did not increase melting temperature of the oligodeoxynucleotide, but caused a conformational change. The experimental results showed that ΔH° and ΔS° could be used to explain the sequence specificity appeared in the binding interaction between each model oligodeoxynucleotide and berenil.

Introduction

Most of the biological functions of the DNA in living cells are regulated by binding of protein or other ligand to the specific site on the DNA. In binding interactions, the ligand or protein recognizes the specific binding site on the DNA and the specificity is thought to be associated with several factors, such as base pairing, base sequence, local conformation, and environmental conditions, etc. Among them, base sequence is considered to be a primary factor. But it is still unclear how protein or the ligand recognizes its binding site on the DNA specifically. In addition, the binding interaction between the DNA and the ligand is regarded as a physicochemical process.[1,2] And the binding interaction will be affected by stability of the DNA duplex, which is related to thermodynamics of double helix formation from a single stranded state.

In this study, two synthetic oligodeoxynucleotides, d(GCTTAAGC)$_2$ and d(GCAATTGC)$_2$, were used as the model DNA (Figure 1). Two oligodeoxynucleotides are self-complementary and palindromic. They have identical base pairings but different base sequence in the central four bases. They are considered to be a good model to elucidate any relationship between the base-sequence specificity and thermodynamics of the double helix formation of the DNA. Berenil [4,4'-(1-triazene-1,3-diyl)bis(benzamidine)] was chosen as the ligand having positively charged amidine groups at terminal positions and has been used as an antitrypanosomal drug. This ligand is known to be a minor-groove binding drug with a strong affinity to A-T base-pair rich regions of the DNA duplex (Figure 2).[3,4] Thermodynamic study of double helix formation has been done with melting experiments of model oligodeoxynucleotides and their complexes with berenil by observing UV absorbance at 260 nm. Thermodynamic parameters obtained from the DNA melting experiments were based on the assumption of the all-or-none model.

*To whom correspondence should be addresses.

5' - G C T T A A G C - 3'

3' - C G A A T T C G - 5'

5' - G C A A T T G C - 3'

3' - C G T T A A C G - 5'

Figure 1. The sequence of model oligodeoxynucleotides.

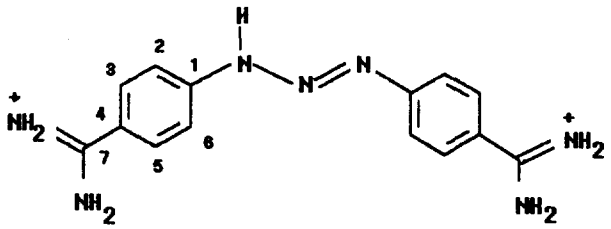

Figure 2. Chemical structure of berenil.

Experimental

Materials. The model oligodeoxynucleotides, d(GCTTAAGC)$_2$ and d(GCAATTGC)$_2$, were synthesized with a ABI DNA synthesizer 380B by β-cyanoethylphosphoramidite chemistry in solid phase and purified with an ion exchange column and lyophilized. Berenil diaceturate was purchased from Sigma Chemical Co. and other chemicals were at least reagent grade and used without further purification.

Method. The freeze-dried oligodeoxynucleotide was dissolved in 20 mM phosphate buffer solution with 100 mM of NaCl and pH 7.0. The concentration of each oligodeoxynucleotide in solution was determined by measuring absorbance at 260 nm. When dissolved in the same phosphate buffer, the molar absorption coefficients for d(GCTTAAGC)$_2$ and d

$(GCAATTGC)_2$ at 260 nm were 106,800 $M^{-1}cm^{-1}$ and 105,600 $M^{-1}cm^{-1}$, respectively, as determined by phosphorus analysis.[5] The DNA melting experiments were carried out with a HP-8452 UV-VIS spectrophotometer equipped with a temperature controlled cell holder, interfaced to a PC-AT computer for acquisition and analysis of experimental data. DNA melting transitions of $d(GCTTAAGC)_2$ and $d(GCAATTGC)_2$ duplexes in 20 mM phosphate buffer (pH 7.0) containing 100 mM of NaCl were observed by monitoring UV absorbance at 260 nm *vs.* temperature every 2 ℃, from 15 ℃ to 85 ℃. The temperature was incremented at a rate of 0.5 ℃/min by using a circulating water bath. No corrections were made for volume expansion. Melting experiments were repeated at 500 and 1000 mM of NaCl. Melting experiments with complexes of each oligodeoxynucleotide and equimolar concentration of berenil were also performed in the same way as describe above. CD spectra of two oligodeoxynucleotides and their complexes with berenil were obtained with a JASCO 600 spectropolarimeter equipped with a water-jacketed cell holder at 20 ℃, interfaced to a PC-AT computer for data collection and analysis.

Thermodynamic parameters were obtained using the van't Hoff method. If all-or-none model (two-state transition) is assumed for double helix formation of a self-complementary oligonucleotide from the single-stranded state, an equilibrium constant for the transition where the equilibrium is $2A \rightleftarrows A_2$ can be given as following.

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

Here α and C_T are the fraction of oligonucleotides in the double helical state and total strand concentration, respectively. The value of at any temperature can be obtained from the DNA melting curve by the method previously reported.[6~8] The equilibrium constant K can also be given as following.

$$K=\exp\left[\left(-\Delta\frac{H^\circ}{RT}\right)+\frac{\Delta S^\circ}{R}\right]$$

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

Thermodynamic parameters, ΔH°, ΔS° and ΔG° for double helix formation from single-stranded state can be determined from a van't Hoff plot of $\mathrm{Ln}(K)$ *vs.* $(1/T)$. This is not a direct method to measure the transition enthalpy.[9] But if the proper upper and lower baselines were used for calibration of absorbance at any temperature, this method can produce an acceptable result for understanding thermodynamics of melting transition of oligodeoxynucleotides between the double helical state and the single-stranded state.

Results and Discussion

Melting transition of $d(GCTTAAGC)_2$ and $d(GCAATTGC)_2$. The melting curves of two oligodeoxynucleotides at various salt concentrations are shown in Figures 3 and 4. No cooperative melting pattern appeared in the melting curve of the $d(GCTTAAGC)_2$ duplex at 100, 500 and 1000 mM of NaCl concentration. This meant that melting transition of this oligodeoxynucleotide could not be explained by the all-or-none model, in another words, there existed considerable amount of intermediates during melting transition. The melting temperature (T_m) for $d(GCTTAAGC)_2$ could not be obtained. In contrast, a clear cooperative melting pattern was shown in the melting curve of the $d(GCAATTGC)_2$ duplex at 100, 500, and 1000 mM of NaCl. The T_m values of the $d(GCAATTGC)_2$ duplex at various NaCl concentrations were obtained from the plot of (fraction of strands in double helical state) *vs.* temperature where $\alpha=0.5$ and they were 31, 39 and 42 ℃ at 100, 500 and 1000 mM of NaCl, respectively. The clear dependency of T_m on the salt concentration was shown. The CD spectra of two oligonucleotides also showed a difference in their conformation (Figure 5). According to CD spectral data, the double helical structure of $d(GCAATTGC)_2$ was regarded to have a typical B-type conformation and $d(GCTTAAGC)_2$ showed the B-type conformation basically, too. But the optical properties partially associated to A-DNA also appeared in the CD spectrum of the $d(GCTTAAGC)_2$ duplex. This indicated that the $d(GCTTAAGC)_2$ duplex has a locally perturbed structure deviated from the

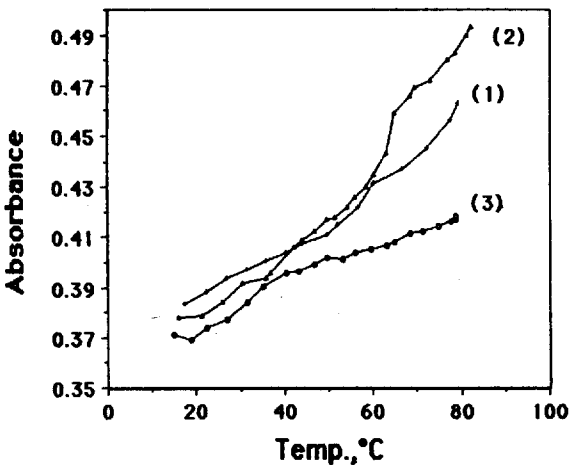

Figure 3. Melting curves of $d(GCTTAAGC)_2$ in 20 mM phosphate buffer (pH 7.0) at various NaCl concentrations: (1) 100 mM, (2) 500 mM, and (3) 1000 mM.

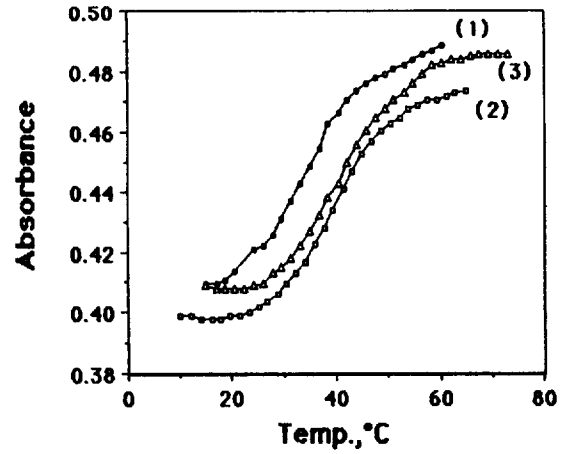

Figure 4. Melting curves of $d(GCAATTGC)_2$ in 20 mM phosphate buffer (pH 7.0) at various NaCl concentrations: (1) 100 mM, (2) 500 mM, and (3) 1000 mM.

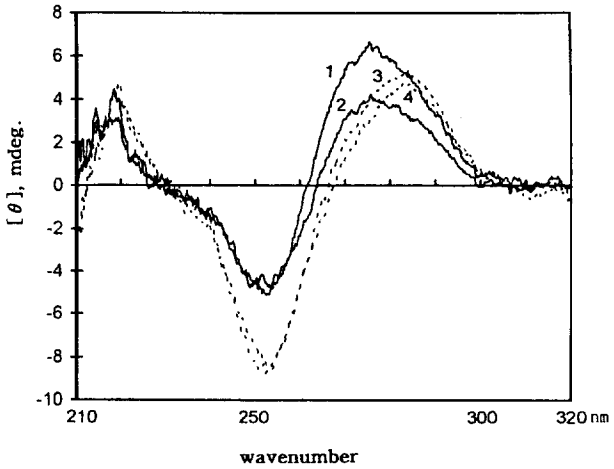

Figure 5. CD spectra of $d(GCTTAAGC)_2$, $d(GCTTAAGC)_2$ and their complexes with berenil in 20 mM phosphate buffer (pH 7.0) with 100 mM of NaCl at 20 ℃. 1. $d(GCTTAAGC)_2$; 2. $d(GCTTAAGC)_2$-bernil complex; 3. $d(GCAATTGC)_2$; 4. $d(GCAATTGC)_2$-berenil complex.

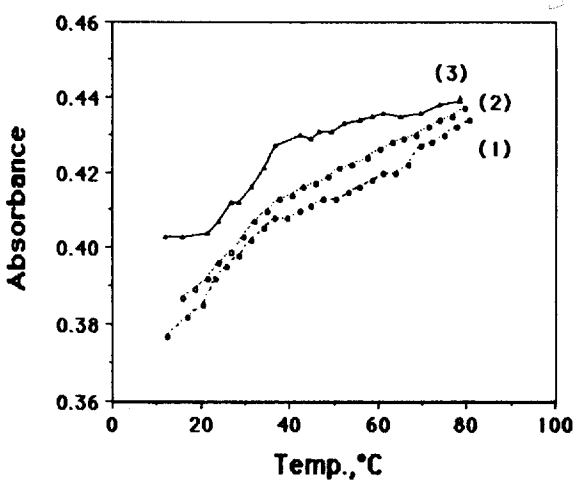

Figure 6. Melting curves of the $d(GCTTAAGC)_2$-berenil complex (molar ratio of 1 to 1) in 20 mM phosphate buffer (pH 7.0) at various NaCl concentrations : (1) 100 mM, (2) 500 mM, and (3) 1000 mM.

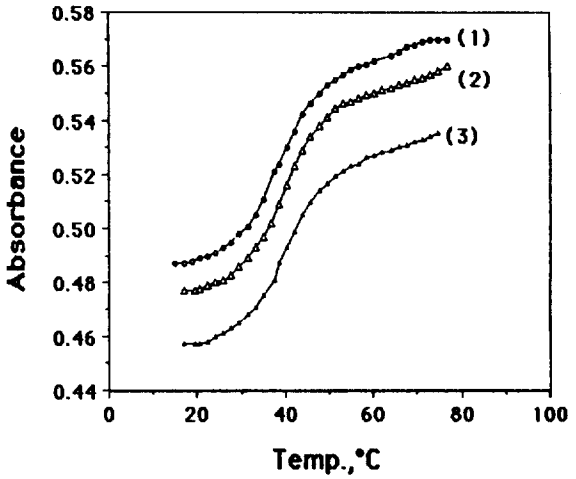

Figure 7. Melting curves of $d(GCAATTGC)_2$-berenil complex in 20 mM phosphate buffer (pH 7.0) at various NaCl concentrations : (1) 100 mM, (2) 500 mM, and (3) 1000 mM.

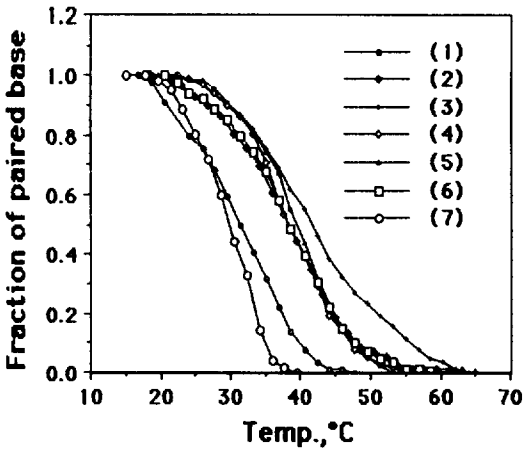

Figure 8. Fraction of paired base *vs.* temperature: $d(GCAATTGC)_2$ at 100 mM (1), 500 mM (2) and 1000 mM of NaCl (3); $d(GCAATTGC)_2$-berenil complex at 100 mM (4), 500 mM (5) and 1000 mM (6); $d(GCTTAAGC)_2$-berenil complex at 1000 mM of NaCl (7).

standard B-DNA conformation.

The energy due to base pairing through hydrogen bonds of two oligodeoxynucleotide duplexes could be regarded to be same because the number and the type of base pairings are identical for the $d(GCTTAAGC)_2$ and the $d(GCAATTGC)_2$ duplex. When two oligodeoxynucleotides were assumed to belong to the B-DNA family, the stacking energy calculated quantum mechanically showed that the $d(GCAATTGC)_2$ duplex has a little higher stacking energy than the $d(GCTTAAGC)_2$ duplex by about 2 or 3 kcal/mol.[10] Therefore it seemed that the difference in the energy due to hydrogen bonding and base stacking is not considered to be large enough to explain quite different melting behavior between two oligodeoxynucleotides, which have identical base pairing but different base sequences. Therefore other contribution to stability of double helical structure, such as entropic contribution should be considered additionally. In fact, well-developed zig-zag string of water molecules along -AATT- sequence in the minor groove of the $d(CGCGAATTCGCG)_2$ duplex has been observed by X-ray crystallography.[11,12]

Melting transition of oligodeoxynucleotide complexes with berenil. The melting curves of a $d(GCTTAAGC)_2$-berenil complex at three different NaCl concentrations are shown in Figure 6. The cooperative melting pattern did not appear at 100 and 500 mM of NaCl, but appeared at 1000 mM of NaCl. The melting temperature (T_m) of the $d(GCTTAAGC)_2$-berenil complex at 1000 mM of NaCl was determined from the plot of fraction of base paired (α) *vs.* temperature and it was 30 ℃ (Figure 8). Because T_m of the $d(GCTTAAGC)_2$ duplex could not be obtained readily, the difference in melting temperature between $d(GCTTAAGC)_2$ and its complex with berenil could not be observed. But T_m of the $d(GCTTAAGC)_2$ duplex at 1000 mM of NaCl could be roughly estimated to be about 30 ℃ from its melting curve, the melting temperature of the $d(GCTTAAGC)_2$ duplex

Table 1. T_m and thermodynamic parameters for double helix formation of oligodeoxynucleotides and their complexes with berenil

Sample	T_m (℃)	ΔH^o (kcal/mol)	ΔS^o (cal/mol·K)	ΔG^o (kcal/mol)
d(GCTTAAGC)$_2$; [NaCl]=1000 mM[a]	30	−86	−262	−6.8
d(GCTTAAGC)$_2$-Berenil; [NaCl]=1000 mM[a]	30	−96	−293	−7.0
d(GCAATTGC)$_2$; [NaCl]=100 mM	31	−58	−167	−7.1
d(GCAATTGC)$_2$; [NaCl]=500 mM	39	−57	−158	−7.2
d(GCAATTGC)$_2$; [NaCl]=1000 mM	42	−51	−138	−7.7
d(GCAATTGC)$_2$-Berenil; [NaCl]=100 mM	38	−73	−211	−7.5
d(GCAATTGC)$_2$-Berenil; [NaCl]=500 mM	39	−74	−213	−7.3
d(GCAATTGC)$_2$-Berenil; [NaCl]=1000 mM	38	−61	−173	−7.3

[a]Roughly estimated thermodynamic parameters from melting curves.

and its complex with berenil seemed to be almost same. In contrast, the melting curve became cooperative clearly after berenil binding in the presence of 1000 mM of NaCl. This indicated that berenil binding to d(GCTTAAGC)$_2$ looked so weak and did not increase the thermal stability of oligodeoxynucleotide, but caused some change related with the cooperative melting pattern. The CD spectral data showed a change in the double helical structure of this oligodeoxynucleotide after berenil binding. Binding berenil to the d(GCTTAAGC)$_2$ duplex shifted its conformation toward a typical B-DNA conformation (Figure 5). This meant that the double helical structure of d(GCTTAAGC)$_2$ was changed toward the typical B-DNA structure by reducing its conformational property associated to A-DNA through binding with berenil. This conformational change, as mentioned above, did not nearly affect on its T_m, but changed the melting pattern from a noncooperative pattern into cooperative one.

According to melting curves for d(GCAATTGC)$_2$ and d(GCAATTGC)$_2$-berenil complex, berenil binding did not cause any change in the melting pattern of the d(GCAATTGC)$_2$ duplex (Figure 7). CD spectral data of the d(GCAATTGC)$_2$ duplex and its complex with berenil also showed no difference in the overall structure between them. Binding berenil to d(GCAATTGC)$_2$ increased the T_m value of oligonucleotide from 30 ℃ to 38 ℃ at 100 mM of NaCl, but did not influence on T_m at higher NaCl concentration (Figure 8). This might mean that the double helical structure of d(GCAATTGC)$_2$ was stabilized considerably through berenil binding even at 100 mM of NaCl. But under the condition of high NaCl concentration, like 500 or 1000 mM, berenil binding did not contribute to stability of the d(GCAATTGC)$_2$ duplex any more. At about 500 mM of NaCl, this oligonucleotide was thought to be able to form a very stable conformation, so berenil binding would not cause additional contribution to thermal stability of the d(GCAATTGC)$_2$ duplex. Also it was shown that binding of berenil to the d(GCAATTGC)$_2$ duplex also contributed considerably to stability of a double helical structure of the oligodeoxynucleotide at 100 mM of NaCl, so the additional increase in concentration of NaCl did not make any difference in the thermal stability (T_m) of the duplex. The CD spectral data showed that berenil binding did not cause any change in conformation of d(GCAATTGC)$_2$ duplex. The increase in T_m after complexation with berenil might be caused by formation of direct hydrogen bonds or indirect hydrogen bonds by way of water molecules between amidine groups of berenil and adenine nitrogen N3 of the internal adenines.[3]

The d(GCTTAAGC)$_2$-berenil complex showed much lower T_m than the d(GCAATTGC)$_2$-berenil complex by about 10 ℃ at 1000 mM of NaCl, which said berenil could stabilized the double helical structure of the d(GCAATTGC)$_2$ duplex more effectiviely than the d(GCTTAAGC)$_2$ duplex. These two oligonucleotides have exactly identical base pairing, but a different base sequence in the central four bases. So it was clear that the large difference in T_m between them was caused from the difference in their base sequence. The d(GCAATTGC)$_2$ duplex can form an very ordered zig-zag string of water molecules along -AATT- sequence in the minor groove, which is called 'spine of hydration'.[11,12] According to the earlier result published elsewhere,[13,14] TA sequence could not make well stacked form because this sequence lies in poor overlap which contrasts the favorable overlap in the AT sequence.

Thermodynamics of melting transition of two oligodeoxynucleotides and their complexes with berenil. The van't Hoff plots for the model oligodeoxynucleotides and their complexes with berenil showed a good linear relationship between Ln K and $1/T$. The plots at 1000 mM of NaCl are shown in Figure 9. Thermodynamic parameters for double helix formation of the model oligodeoxynucleotides and their complexes with berenil at different salt concentrations were determined from the plots. A noticeable result was that both $-\Delta H^o$ and $-\Delta S^o$ values of the d(GCTTAAGC)$_2$-berenil complex at 1000 mM of NaCl were much larger than those of the d(GCAATTGC)$_2$ duplex as well as its complex with berenil. But ΔG^o values for all cases did not show any considerable difference.

Earlier 2-D NOESY (Two-dimensional Nuclear Overhauser Effect Spectroscopy) data showed that hydrogen atoms of berenil had more contacts with hydrogens of bases and sugars of d(GCAATTGC)$_2$ through dipolar coupling in the minor groove than those of d(GCTTAAGC)$_2$ in their complexes.[3,4] In addition, a strong evidence for formation of the additional hydrogen bond between berenil amidine proton and purine nitrogen N3 of the internal adenine of d(GCAATTGC)$_2$ has been reported,[3] but no evidence for the hydrogen bond

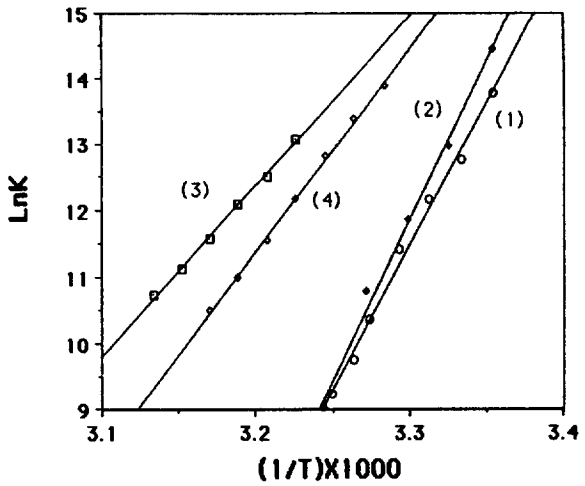

Figure 9. The van't Hoff plots of ln K *vs.* $1/T$ for d(GCTTAAGC)$_2$ (1), d(GCTTAAGC)$_2$-berenil complex (2), d(GCAATTGC)$_2$ (3), and d(GCAATTGC)$_2$-berenil complex (4) at 1000 mM of NaCl.

between berenil and d(GCTTAAGC)$_2$ has been observed.[4] The NMR data and T_m values indicated that berenil bound to the d(GCAATTGC)$_2$ duplex via the minor groove more tightly than to the d(GCTTAAGC)$_2$ duplex. Therefore the large -ΔH^o value for melting transition of the d(GCTTAAGC)$_2$-berenil complex at 1000 mM of NaCl was regarded to be caused from the enthalpic contribution other than formation of additional hydrogen bonds between berenil and d(GCTTAAGC)$_2$, such as electrostatic interactions between positive amidine groups of berenil and negative phosphate groups of oligodeoxynucleotide or some polar atoms like O4' of deoxyribose sugar.

For the d(GCAATTGC)$_2$ duplex, the ΔG^o value stayed almost same as NaCl concentration changed. Both -ΔH^o and -ΔS^o values did not show any considerable difference until NaCl concentration approached to 1000 mM and then decreased. But T_m increased continuously. In case of the d(GCAATTGC)$_2$-berenil complex, -ΔH^o and -ΔS^o values showed the same pattern, but T_m stayed same. Decrease in -ΔH^o at the NaCl concentration more than 1000 mM has been observed elsewhere,[6] and which was explained in such a way that the population of intermediate state increased during melting transition at higher NaCl concentration, therefore the ΔH^o derived from UV melting data based on the assumption of the all-or-none model would become inaccurate. But more detailed study is necessary to resolve this ambiguity. Binding of berenil to this duplex was considered to make the enthalpic contribution for double helix formation much favorable over the entropic contribution. When berenil bound to the d(GCAATTGC)$_2$ duplex, new hydrogen bonds were made between berenil and oligodeoxynucleotide and many water molecules consisting of the spine of hydration localized at the -AATT- site in the minor groove were replaced by berenil. Therefore much less water molecules and counter ions would be released from the complex during its melting transition, compared to the d(GCAATTGC)$_2$ duplex.

From the experimental results, we could find that ΔG^o values for duplex formation of two oligodeoxynucleotides and their complexes with berenil did not respond sensitively to the change in the base sequence of them used for the research as well as in the environmental conditions. Therefore as far as these two oligodeoxynucleotides are concerned, ΔG^o can not be used to obtain the information on base sequence specificity. Contrast to this, the clear difference in -ΔH^o and -ΔS^o values for double helix formation between d(GCTTAAGC)$_2$ and d(GCAATTGC)$_2$ have been observed.

Conclusion

Thermodynamic parameters obtained from UV melting experiments and CD spectral data clearly showed the differences in thermodynamic property and conformation between d(GCTTAAGC)$_2$ and d(GCAATTGC)$_2$, having identical base paring but different base sequences in the central four bases. The melting transition of the d(GCAATTGC)$_2$ duplex showed greater extent of entropic contribution compared to that of the d(GCTTAAGC)$_2$ duplex. Berenil bound to d(GCAATTGC)$_2$ preferentially and increased its melting temperature by 8 ℃. But the overall conforamtion of oligodeoxynucleotide was maintained after binding of berenil. In contrast, binding of berenil to d(GCTTAAGC)$_2$ did not increase melting temperature and melting transition of this complex was also regarded to be enthalpic rather than entropic. The overall conformation of d(GCTTAAGC)$_2$ changed noticeably after binding of berenil. And binding of berenil to the model oligonucleotides decreased the extent of entropic contribution for melting transition, because of displacement of water and counter ions in the minor groove with berenil. The experimental results showed that ΔH^o and ΔS^o could be utilized to understand the specific binding of berenil to oligodeoxynucleotides.

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Menschutkin-Type Reaction of Substituted Benzyl Arenesulfonates with Substituted N,N-Dimethylanilines

Sung Hong Kim, Soo-Dong Yoh, Mizue Fujio*
Hiroshi Imahori*, Masaaki Mishima*, and Yuho Tsuno*

Department of Chemistry Education, Kyungpook National University, Taegu 702-701, Korea
**Institute for Fundamental Research of Organic Chemistry, Kyushu University, Hakozaki, Fukuoka 812*
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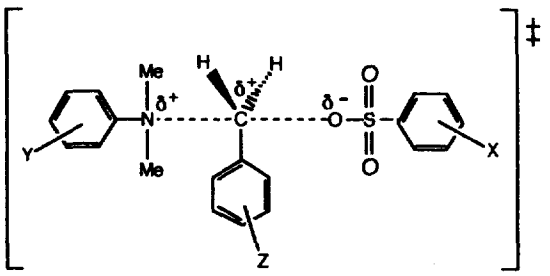
The substituent effects in the second-order reaction of Z-substituted benzyl tosylates and benzyl X-benzenesulfonates with Y-substituted N,N-dimethylanilines (DMAs) were studied in acetonitrile at 35 ℃. Rate constants for the reaction of Z-benzyl tosylates with DMAs exhibited a curved Hammett plots, and the apparent ρ_Z values increased negatively in the following sequence Y=*m*-NO_2>H>*p*-MeO>*p*-NMe_2. This means that the weaker nucleophile magnifies the effect of the benzyl substituent Z. The plot of ρ_Y against log $(k_Z/k_H)_{Y=H}$ showed good linear relation with a slope of 0.57 and it was discussed that the change in transition state structure was mainly attributed to C-N bond formation. The $|\rho_Y|$ value, determined by introducing of electron-withdrawing substituent in the leaving group, was decreased from −1.75 (X=*p*-Me) to −1.58 (X=*p*-NO_2). This result indicates that a poor leaving group requires more nucleophilic assistance.

Introduction

Curved Hammett plot is one of the most characteristic feature of substituent effect in nucleophilic displacement reaction of substituted benzyl system. Although various interpretation have been offered for this borderline behavior,[1~11] the way of mechanistic shift for the curvature in the Hammett plot has not been resolved completely. In order to investigate the variation of the structure of S_N2 transition state for benzyl reaction, it is important to evaluate the contribution between the bond formation of nucleophile-benzyl carbon (C_α) and the bond fission of C_α-leaving group. However, variations in the structure of either the nucleophile or the leaving group would change several properties of the reactant, which could not explain how the structure of the reactants affects the structure of a S_N2 transition state.

The Menschutkin-type reaction of benzyl benzenesulfonates with DMAs is a typical S_N2 displacement reaction and accompanies a considerable change in mechanism with substituent changes. Furthermore, this system is highly appropriate to describe the shift of mechanism in terms of the substituent effects for three variables, X, Y, and Z, in three aryl moieties which are capable of monitoring the degree of bond formation, bond fission, and central charge development, respectively, without any steric change in the fundamental framework of the transition state.

In the foregoing paper,[1] we have analyzed the substituent effect on the reactions of substituted Z-benzyl tosylates with DMA, and pointed out a remarkable mechanistic change with substituent Z in the benzyl substrate. Electron-donating substituents prompted the reaction, indicating positive charge development at the benzyl reaction center in the transition state. The negative ρ_Z values for the whole range of benzyl Z substituents indicate the dominance of the bond fission in the transition state, which becomes looser (or tighter) as the substituent Z becomes more electron donating (or attracting). In this paper, we will concern mainly with the reactions between typical benzyl tosylates and Y-substituted DMAs and an elucidation of the relation between benzyl substituent Z and the contribution to C-N bond formation. The effect of the leaving group on the transition state structure is also discussed.



(1)

Results and Discussion

The reaction rates of Z-substituted benzyl tosylates with Y-substituted DMAs were measured in acetonitrile at 35 ℃ by the increase of the conductance of the quaternary anilinium salts at initial concentration of ester 0.0005 M (M = mol dm^{-3}) and DMAs 0.1 to 0.25 M as described in the