# Solvent Effect on the Hydrogen Bonds between Guanine and Cytosine and between Hypoxanthine and Cytosine

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The solvent effect on the hydrogen bonds between guanine and cytosine and between hypoxanthine and cytosine was studied by means of solution calorimetry and ab initio MO calculations. The experimental enthalpy changes for guanine-cytosine and hypoxanthine-cytosine systems in aqueous solution were estimated to be about −10 and −14 kJ mol$^{-1}$, respectively. In order to explain these experimental results, the electronic structures of hydrated bases and hydrated base pairs were calculated, assuming two types of hydration schemes. In the first type, it was expected that the complementary base pairs were destabilized in aqueous solution. In the second type, the stabilization energies in aqueous solution were calculated to be −11.9 kJ mol$^{-1}$ for the hydrated guanine-cytosine system and −18.5 kJ mol$^{-1}$ for hypoxanthine-cytosine one, respectively, which agreed with the experimental results. This suggests that water molecules bound to base pairs contribute to the stabilization/destabilization of hydrogen bonds between bases. The present calculation showed the possibility of C-H$^{\delta+}$···O hydrogen-bond formation between the complementary base pair and $H_2O$.

It is well-known that deoxyribonucleic acid (DNA) is a macromolecule with a double-stranded helical structure formed by a complementary interaction between purine and pyrimidine bases,[1] bringing about a helix-coil transition by a change in the environmental conditions, such as pH, ionic strength, or temperature.[2]

In previous papers,[3,4] in order to clarify the stabilization scheme of DNA molecule in aqueous solution, the enthalpy changes of duplex formations by an equimolar mixture of poly(riboguanylic acid)-(poly(G)) with poly(ribocytidylic acid) (poly(C)), and of poly(riboinosinic acid)(poly(I)) with poly(C) solutions with a 0.1 mol dm$^{-3}$ Tris-HCl buffer solution at pH 7.60 were measured using LKB batch-type microcalorimeter at 298.15 K. Poly(I) contains hypoxanthine, which is a deamino analogue of guanine. We reported that the enthalpy changes accompanying duplex formation were about −9 kJ (mol of base pair)$^{-1}$ for the poly(G)·poly(C) duplex, and about −21 kJ (mol of base pair)$^{-1}$ for the poly(I)·poly(C) duplex, respectively. The estimated enthalpy changes between polynucleotides may correspond to the total energy containing various types of interactions such as the hydrogen bonding between bases, the solvent effect of water, the stacking effect, conformational change, and the dissociation of self-associated poly(G) and poly(I).[3-19] Especially, the hydrogen bonds between bases may mainly contribute to both the stability and conformation of polynucleotides.

Recently,[20] we reported on theoretical and experimental studies concerning the Watson-Crick and the Hoogsteen pairs of adenine-uracil hydrogen-bonded systems, and pointed out that not only the hydrogen bonding between bases, but also the solvent effect of water played an important role in the stability of base pairs in aqueous solution.

Clementi and Corongiu[19] investigated theoretically the hydration schemes of guanine, cytosine, and the guanine-cytosine pair by means of isoenergy contour map analysis and Monte Carlo simulations.

In the present study, in order to elucidate the solvent effect of water on the stability of a base pair, the energies of nonhydrated and hydrated complementary base pairs (Watson-Crick pairing) of guanine with cytosine and of hypoxanthine with cytosine were calculated by an ab initio MO method, by assuming two types of hydration schemes. These results were compared with the results of solution calorimetric experiments.

## Experimental

9-Methylguanine, cytosine (Cyt), and hypoxanthine (Hyp) were purchased from Tokyo Kasei Kogyo Co., Ltd., Japan, and used without further purification. 9-Methylguanine, instead of guanine (Gua), was used because of the requirement of higher solubility in aqueous solution.

Water was purified by an ordinary method,[21] and finally distilled by the use of a long column.

A 0.1 mol dm$^{-3}$ Tris-HCl (tris(hydroxymethyl)aminomethane-hydrochloric acid) solution was used as the buffer solution (pH 7.0).

In this study, an LKB batch-type microcalorimeter was used (described in a previous paper[22]). The measured temperature was 298.15±0.005 K. For measurements of the heat of mixing, equal volumes (about 2 ml) of $5\times10^{-4}$ mol dm$^{-3}$ Gua or Hyp and Cyt solutions were mixed.

## Calculations

Ab initio MO calculations were carried out with IMSPACK[23] and GAUSSIAN 80[24] programs using STO-3G minimal basis set.[25] The geometry of Hyp was optimized (see Fig. 1). The geometries of Gua and Cyt were taken from Del Bene[26] and Matsushita et al.[27] respectively.

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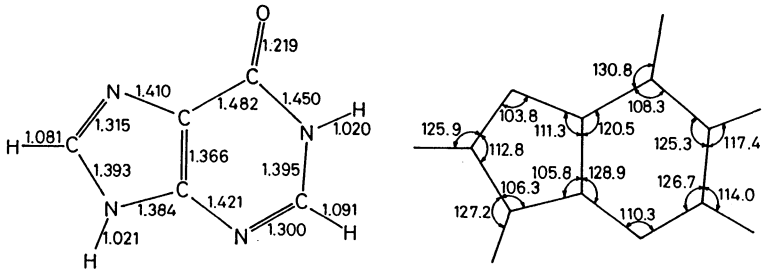


Fig. 1. The optimized structure of Hyp. Units are angstroms and degrees.

Since the hydrogen bond is much weaker than the usual covalent bond, it is expected that the geometries of bases might be almost unchanged by hydrogen-bond formation. Therefore, we optimized only the hydrogen-bonding regions of the nonhydrated base pairs, and the hydrated bases and base pairs.

For the calculation of intermolecular interaction energy, an extended basis set, such as 4-31G or 6-31G*, is recommended. However, since the number of basis sets for hydrogen-bonded systems in the present work was very large, the STO-3G minimal basis set was used. The purpose of this work was to perform both comparative and qualitative studies of the effects of hydrogen bonding and hydration on the physicochemical properties of DNA. For this purpose, the STO-3G minimal basis set proved to be useful.

## Results and Discussion

**Hydrogen-Bonding Interactions of Gua with Cyt and of Hyp with Cyt.** Since it is well-known experimentally that Watson-Crick pairing is the most stable structure in possible hydrogen-bonding schemes of Gua with Cyt,[28] we calculated this type of base pair. The Watson-Crick pairing of Hyp with Cyt was also calculated. The optimized geometric parameters for the hydrogen-bonding region of Gua-Cyt and Hyp-Cyt pairs are shown in Fig. 2, together with the experimental values of the 9-ethylguanine-1-methylcytosine pair determined by X-ray diffraction crystallography (in parenthesis[28]). As can be seen in Fig. 2a, the calculated intermolecular distances are somewhat underestimated. This is a general tendency as long as one uses STO-3G minimal basis set.

The calculated results of the hydrogen-bonding energies ($E_{HB}$), the magnitude of the charge transfer from pyrimidine to purine bases ($\Delta q$), and dipole moments ($\mu$) for Gua-Cyt and Hyp-Cyt pairs are summarized in Table 1, together with the vector sum of the dipole moments of monomers ($\mu_{SUM}$). As can be seen in Table 1, $E_{HB}$ was estimated to be −111.3

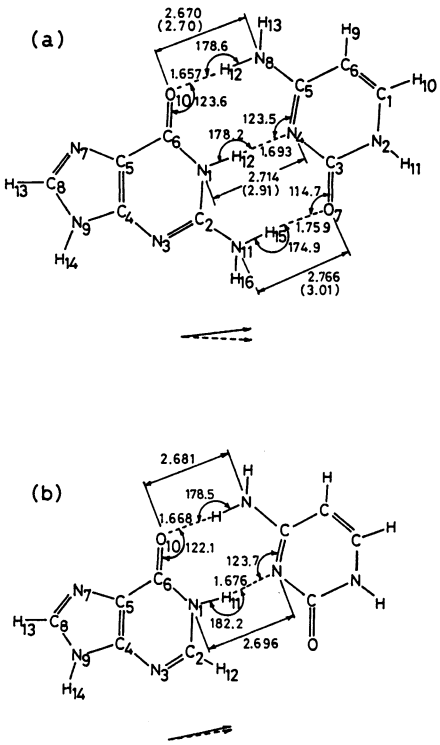


Fig. 2. The optimized geometric parameters of (a) Gua-Cyt and (b) Hyp-Cyt pairs. Units are angstroms and degrees. The solid and broken arrows show the direction of dipole moments for base pairs and of the vector sum of dipole moments for monomers, respectively.

Table 1. The Calculated Results of the Hydrogen-Bonding Energies, $E_{HB}$, the Magnitude of the Charge Transfer from Pyrimidine to Purine Bases, $\Delta q$, Dipole Moments of Base Pairs, $\mu$, and the Vector Sum of Dipole Moments of Monomers, $\mu_{SUM}$, for Gua-Cyt and Hyp-Cyt Pairs

| | $E_{HB}$/kJ mol$^{-1}$ | $\Delta q$/e[a] | $\mu$[b]/Debye | $\mu_{SUM}$[b]/Debye |
|---|---|---|---|---|
| Gua-Cyt pair | −111.3 | 0.06 | 6.53 | 4.78 |
| Hyp-Cyt pair | −82.4 | 0.02 | 4.13 | 3.11 |

a) e=−1.6×10$^{-19}$ C. b) The direction refers to Fig. 2.

kJ mol$^{-1}$ for Gua-Cyt pair and −82.4 kJ mol$^{-1}$ for Hyp-Cyt one, respectively. Hereafter, we call the hydrogen bonds between the complementary bases the internal hydrogen bonds. Therefore, $E_{HB}$ is the energy of the internal hydrogen bonds. The calculated result for the Gua-Cyt pair is comparable with the experimental one reported by Yanson et al. ($\Delta H°$=−87.9 kJ mol$^{-1}$)[6,7] and with the calculated one by Forner et al. ($E_{HB}$=−124.1 kJ mol$^{-1}$).[8] Since the calculated dipole moment of the Gua-Cyt pair is larger than that of the Hyp-Cyt one, it is expected that in a polar solvent, the Gua-Cyt pair may be more stable than that of Hyp-Cyt due to a dipole-dipole interaction between solute and solvent molecules.

In order to obtain informations concerning the nature of the internal hydrogen bonds of Gua-Cyt and Hyp-Cyt pairs, the calculated hydrogen-bonding energies were decomposed into physically meaningful energy components such as the electrostatic ($E_{ES}$), the exchange repulsion ($E_{EX}$), the polarization ($E_{PL}$), and the charge-transfer ($E_{CT}$) energies, according to the Kitaura-Morokuma method.[29] The results are summarized in Table 2, where $E_{CT(X\rightarrow Y)}$ and $E_{CT(Y\rightarrow X)}$ refer to the charge-transfer interaction energies from Gua and Hyp to Cyt, respectively. As can be seen in Table 2, the dominant energy components for both Gua-Cyt and Hyp-Cyt pairs are the electrostatic and the charge-transfer interactions, as in the usual hydrogen-bonded systems.[30]

It should be noted that for the Hyp-Cyt pair, $E_{CT(Y\rightarrow X)}$ (−50.6 kJ mol$^{-1}$) is almost the same as $E_{CT(X\rightarrow Y)}$ (−49.8 kJ mol$^{-1}$), though the Gua-Cyt pair, $E_{CT(Y\rightarrow X)}$ (−88.3 kJ mol$^{-1}$), is much larger than $E_{CT(X\rightarrow Y)}$ (−51.5 kJ mol$^{-1}$). This result is because in the Gua-Cyt pair, two hydrogen bonds ($N_1$-$H_{12}$···$N_4$ and $N_{11}$-$H_{15}$···$O_7$) contribute to $E_{CT(Y\rightarrow X)}$, while only one hydrogen bond ($O_{10}$···$H_{12}$-$N_8$) contributes to $E_{CT(X\rightarrow Y)}$. On the other hand, in the Hyp-Cyt pair, one hydrogen bond ($O_{10}$···$H_{12}$-$N_8$) contributes to $E_{CT(X\rightarrow Y)}$ and one hydrogen bond ($N_1$-$H_{11}$···$N_4$) to $E_{CT(Y\rightarrow X)}$.

Table 2. Hydrogen-Bonding Energies and Their Components for the Gua-Cyt and Hyp-Cyt Pairs

| | | Gua-Cyt pair /kJ mol$^{-1}$ | Hyp-Cyt pair /kJ mol$^{-1}$ |
|---|---|---|---|
| $E_{HB}$ | | −111.3 | −82.4 |
| $E_{ES}$ | | −141.0 | −112.1 |
| $E_{EX}$ | | 212.5 | 166.5 |
| $E_{PL}$ | | −13.0 | −9.2 |
| $E_{CT}$ | | −140.2 | −101.3 |
| $E_{CT}$ | $E_{CT(X\rightarrow Y)}$[a] | −51.5 | −49.8 |
| | $E_{CT(Y\rightarrow X)}$[a] | −88.3 | −50.6 |

a) X refers to Gua for Gua-Cyt pair and to Hyp for Hyp-Cyt one. Y refers to Cyt.

## The Results of Calorimetric Measurements

The heats of mixing of Gua and Cyt solutions and Hyp and Cyt solutions were measured using an LKB batch-type microcalorimeter at 298.15±0.005 K. The experimental results were exothermic, suggesting that the mixed systems were stabilized by complementary base-pair formation, if we assumed that the heat of dilution of each base was negligible.

The obtained results are summarized in Table 3, together with the $\Delta H°$-values reported by other investigators.[5-7] As can be seen in Table 3, it is of interest to note that the stability ($\Delta H°=H°$(base pair A-B)−$H°$(base A)−$H°$(base B)) of the Gua-Cyt system decreases with an increase in the polarity of solvents. From the point of view of a dipole-dipole interaction,

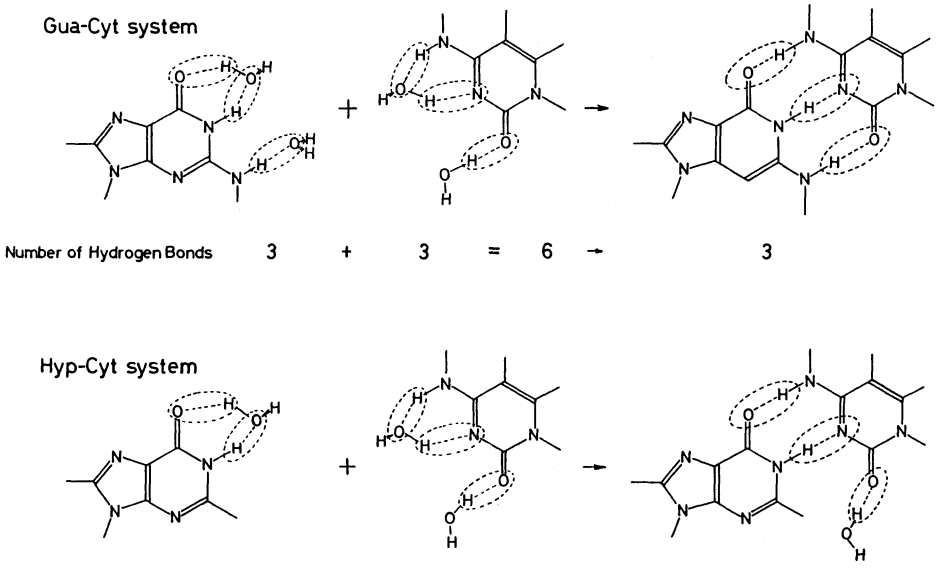

Table 3. Experimental Enthalpy Changes of the Hydrogen-Bond Formation between Gua and Cyt and between Hyp and Cyt

| Solvent | Enthalpy change, $\Delta H^\circ$ /kJ (mol base pair)$^{-1}$ | | Ref. |
|---|---|---|---|
| | Gua-Cyt pair | Hyp-Cyt pair | |
| $H_2O$[a] | −10 | −14 | This work |
| $CHCl_3$[b] | −42—−48 | −38 | 5 |
| Gas phase | −87.9 | | 6,7 |

a) The concentration of $5\times10^{-4}$ mol dm$^{-3}$. b) Guanosine and Cytisine for Gua-Cyt pair, and 2′,3′-*O*-benzylidine-5′-*O*-tritylinosine and cytisine for Hyp-Cyt pair.

the stability of complementary base pairs is expected to increase with an increase in the polarity of solvents, and also with an increase in the dipole moment of the base pair. Actually, in $CHCl_3$ solutions, the Gua-Cyt system (calculated dipole moment $\mu$=6.53 Debye) is more stable than the Hyp-Cyt system ($\mu$=4.13 Debye) (see Table 3). Therefore, the decrease of stability in the aqueous solution comes from destabilization due to the dehydration of a hydrated base in the process forming a base pair. That is, in the Gua-Cyt system, the number of hydrogen bonds decreases from six to three(hydrated four $H_2O$ molecules are removed and three internal hydrogen bonds are formed in Gua-Cyt pair), while in the Hyp-Cyt system, the number of hydrogen bonds decreases from five to three (see figure given below). As a result, the stability of the base pair in aqueous solution is reversed; that is, the order of stability is Hyp-Cyt pair>Gua-Cyt pair.

**Hydration Effect on the Internal Hydrogen Bonds of Gua-Cyt and Hyp-Cyt Pairs.** In order to elucidate the hydration effect on the stability of internal hydrogen bonds of the complementary base pair, hydrated bases and base pairs were calculated by ab initio MO methods, by assuming various degrees of hydration.

To consider possible hydration schemes for Gua, Hyp, and Cyt, the net charges (Mulliken population of atoms) were calculated. The results are shown in Fig. 3. As can be seen, there are eight hydrogen-bonding sites (proton donating or accepting site) in Gua and six sites in Hyp and Cyt, respectively, at which hydration is possible. We distinguish the hydrogen-bonding site and the hydration region. The possible hydration regions of bases are shown in Fig. 3. There are five hydration regions ((A)—(E)) in Gua, four regions ((F)—(I)) in Hyp, and four regions ((J)—(M)) in Cyt.

At the hydration regions, two types of hydration schemes are possible. The first type is as follows. Since each proton-donating or -accepting site in a base can bind with one $H_2O$ molecule, two $H_2O$ molecules can be coordinated at each bay-like region (A), (C), (D), (H), (I), (J), or (L) shown in Fig. 3. In this case,

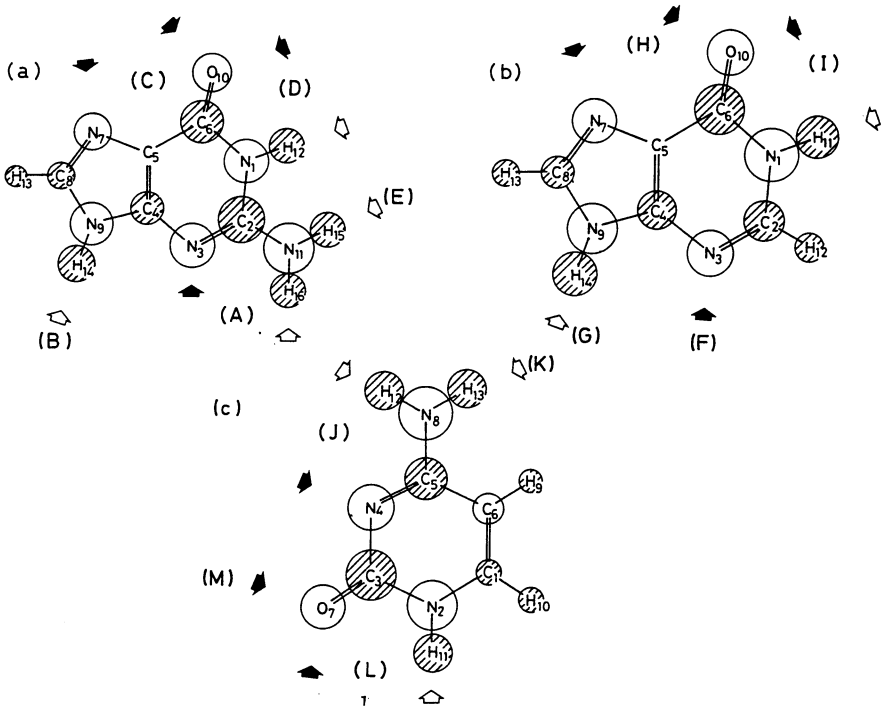


Fig. 3. The net charges of (a) Gua, (b) Hyp, and (c) Cyt. The open and shaded circles represent the negative and positive net charges, respectively. The area of the circle shows the relative magnitude of the net charge. The open and shaded arrows indicate the location of proton donating and accepting sites. The uppercase alphabetical letters represent the hydration regions.

there are some interactions between hydrated $H_2O$'s in these regions, such as hydrogen bonding, dipole-dipole interactions and steric hindrance. Therefore, we optimized each hydrogen-bonding moiety of hydrated bases. At other regions ((B), (E), (F), (G), (K), and (M)), one $H_2O$ molecule is coordinated. Furthermore, there are some direct interactions between hydration regions (A) and (B), between (D) and (E), between (F) and (G), and between (J) and (M). Thus, geometry optimization was performed at these regions simultaneously. The calculated results are shown in Figs. 4a, b, and c.

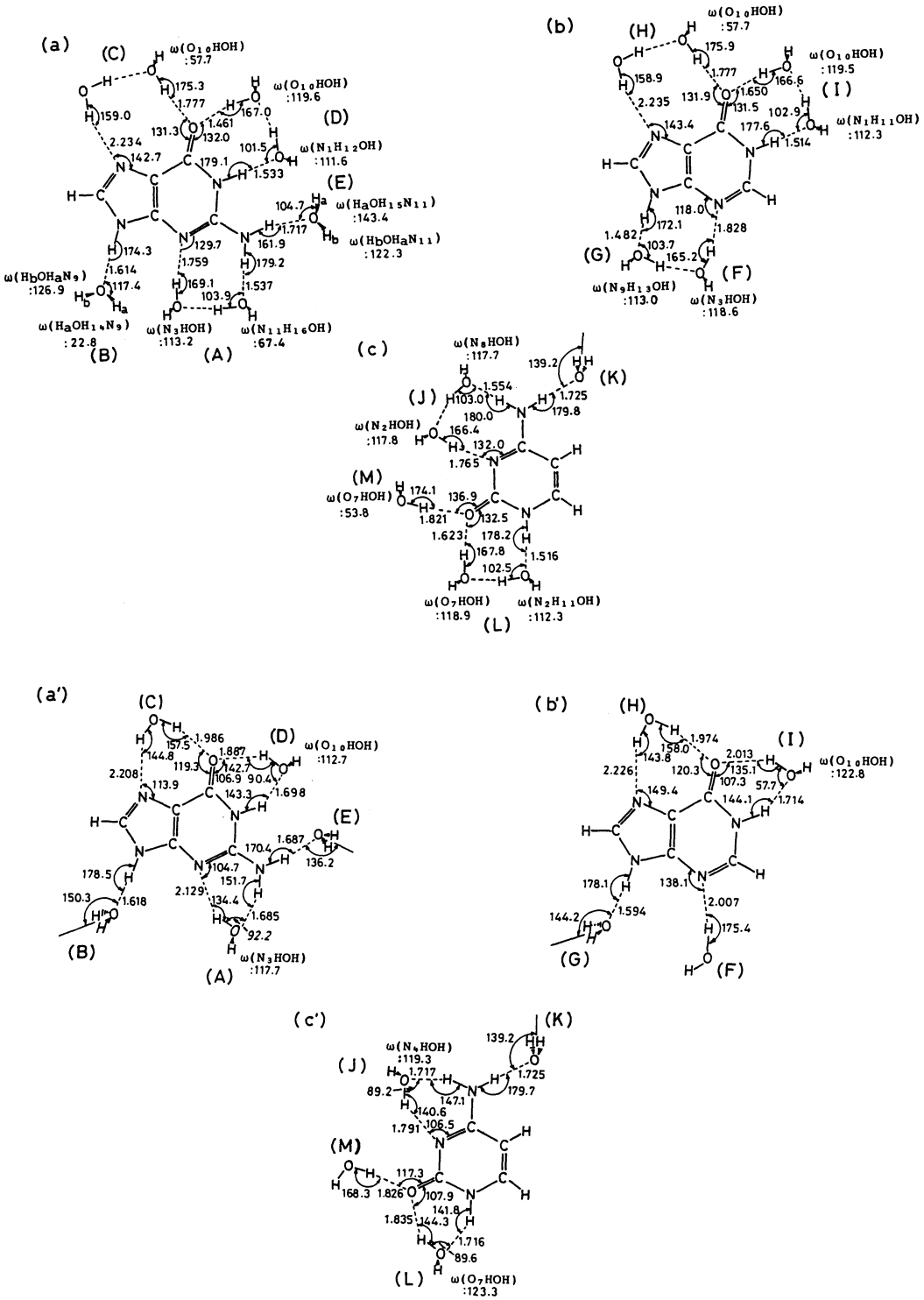


Fig. 4. The optimized geometric parameters of (a) and (a′) hydrated Gua, (b) and (b′) hydrated Hyp, and (c) and (c′) hydrated Cyt. Units are angstroms and degrees. Dihedral angles are given as follows; for an example, the dihedral angle between $N_3$-H-O and H-O-H planes at region (A) in the hydrated Gua(figure a′) is given by $\omega(N_3HOH)$: 117.7.

In the second type of hydration scheme, one $H_2O$ molecule is bound between proton-donating and -accepting sites in each bay- like region shown in Figs. 4a′, b′, and c′. Optimized geometric parameters are shown n Figs. 4a′, b′, and c′.

The hydration scheme of Gua shown in Fig. 4a′ is rather similar to that of Pullman et al.[12] and that of Clementi and Corongiu.[19] The hydration scheme of Cyt, shown in Fig. 4c′, is similar to that of Clementi and Corongiu calculated by Monte Calro simulations.[19] However, it is somewhat different from that of Pullman et al.[12]

The electron density changes due to internal hydrogen-bond formation of Gua-Cyt and Hyp-Cyt pairs were calculated (Fig. 5). As can be seen in Fig. 5, it is of interest to note that the charge densities on the $H_9$ and $H_{10}$ atoms of the C-H bonds of Cyt in the Gua-Cyt pair and those on the $H_{12}$ atom of the C-H bond of Hyp, and $H_9$ and $H_{10}$ atoms of Cyt in the Hyp-Cyt pair considerably decreased by the formation

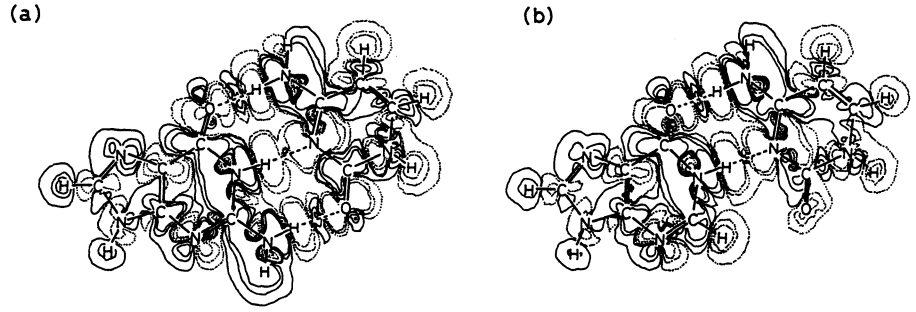


Fig. 5. The electron density changes due to the hydrogen-bond formation of (a) Gua-Cyt and (b) Hyp-Cyt pairs. Full and dotted lines indicate the density increase and decrease, respectively. The values of these lines are ±0.0001, ±0.0005, and ±0.001 (Bohr)$^{-3}$, successively.

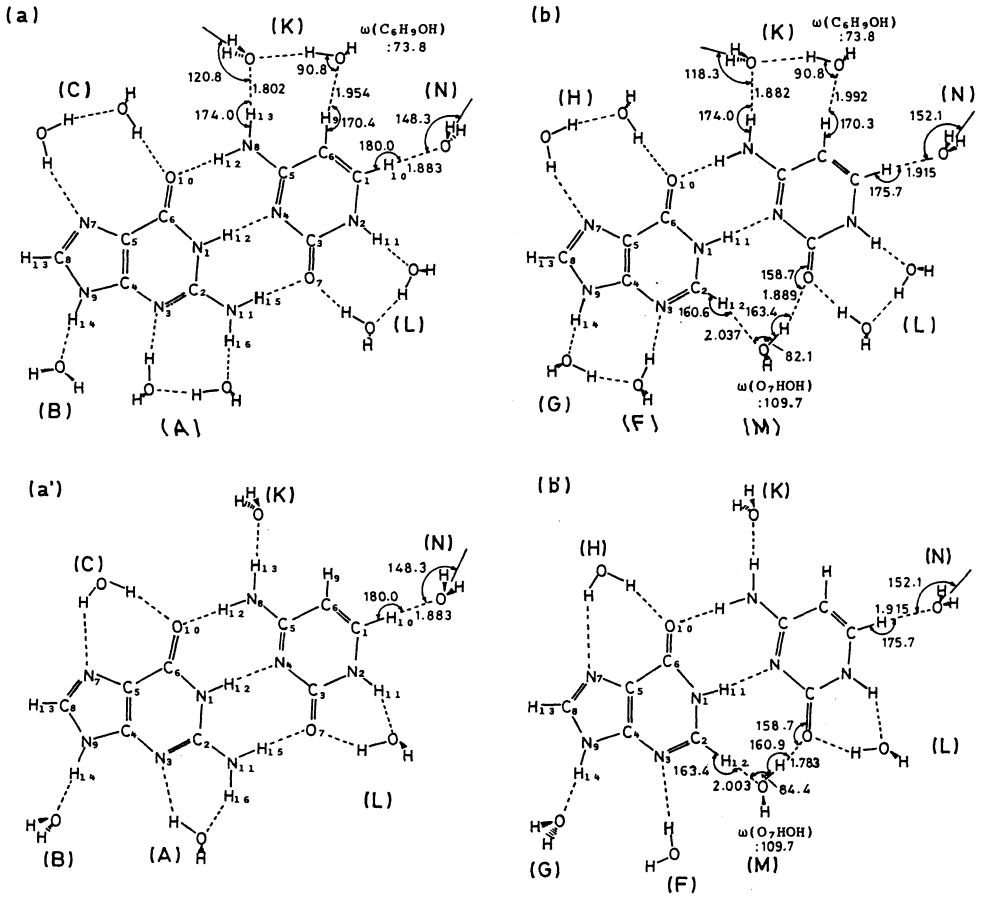


Fig. 6. The hydration schemes and optimized geometric parameters between base pair and water molecule of (a) and (a′) Gua-Cyt and (b) and (b′) Hyp-Cyt pairs. Units are angstroms and degrees.

of internal hydrogen bonds. Therefore, these hydrogen atoms come to have unusual, positive net charges. This result expects an unusual C-$H^{\delta+}$···O hydrogen-bond formation with $H_2O$ at $H_{12}$ of Hyp in the Hyp-Cyt pair. Also, there is a possibility of a similar type hydrogen bonding between $H_2O$ and $C_6$-$H_9$ or $C_1$-$H_{10}$ of Cyt in both Gua-Cyt and Hyp-Cyt pairs.

In order to investigate this type of interesting C-$H^{\delta+}$···O hydrogen bonding, we carried out ab initio MO calculations, while assuming various possible hydration schemes (Figs. 6a, b, a′, and b′). The optimized geometrical parameters are shown in these figures. Our result shows that C-$H^{\delta+}$···O hydrogen bonds are possible in both Gua-Cyt and Hyp-Cyt pairs.

Goldblum et al. investigated the hydration scheme of the Gua-Cyt pair using an overlap multipole procedure.[11] However, their scheme is somewhat different from ours. On the other hand, the hydration scheme of the Gua-Cyt pair shown in Fig. 6a is rather similar to that of Clementi and Corongiu studied by Monte Carlo simulations.[19]

In order to estimate the effect of hydration on the internal hydrogen bonds of complementary base pairs, Gua-Cyt and Hyp-Cyt pairs with various degrees of hydration were calculated. The calculated results are summarized in Table 4. For the Gua-Cyt pair, the internal hydrogen bonds are stabilized by hydration at regions (C), (K), and (N), but destabilized by that at regions (A), (B), and (L) in both hydration schemes (Figs. 6a and 6a′). Especially, $H_2O$ molecules which arehydrated to an amino group (region (A)) brought about a significant destabilization of internal hydrogen bonds of the Gua-Cyt pair. It is of interest to note that the same result was also found in the hydration to the amino group of the adenine-uracil pair.[20] On the other hand, for the Hyp-Cyt pair, hydration at regions (H), (K), (N), and (M) causes a stabilization of the internal hydrogen bonds, while that at region (L) brings about destabilization in both hydration schemes (Figs. 6b and 6b′).

When all of the possible donating and accepting sites are hydrated, the Hyp-Cyt pair is considerably stabilized, while the Gua-Cyt pair is slightly destabilized (see Table 4). This result can be explained as follows: The charge-transfer interaction at the internal hydrogen bonds of the Gua-Cyt pair is weakened, while that of the Hyp-Cyt pair is strengthened, due to a change in the HOMO and LUMO energies by hydration. Both the Gua-Cyt and Hyp-Cyt pairs are stabilized by C-$H^{\delta+}$···O hydrogen-bond formation.

The experimental results show that the enthalpy changes due to complementary base-pair formation in aqueous solution were rather small compared with those in $CHCl_3$ or the gas phase. As mentioned in the previous section, this is due to destabilization by the dehydration of hydrated bases in the process of complementary base-pair formation.

In order to explain the experimental results quantitatively, the energy differences between the hydrated bases and base pairs were evaluated by the following energy cycles. For the first type of hydration scheme

Table 4. The Calculated Hydrogen-Bonding Energies between Hydrated Gua and Cyt, and between Hydrated Hyp and Cyt for Various Hydration

| Gua-Cyt pair | | | Hyp-Cyt pair | | |
|---|---|---|---|---|---|
| Region[a] | $E_{HB}$ / kJ mol$^{-1}$ | $\Delta E_{HB}$[b] / kJ mol$^{-1}$ | Region[a] | $E_{HB}$ / kJ mol$^{-1}$ | $\Delta E_{HB}$[c] / kJ mol$^{-1}$ |
| Nonhydrated | −111.3 | | Nonhydrated | −82.4 | |
| (Hydration schemes shown in Figs. 6a and b) | | | | | |
| A | −100.0 | +11.3 | F+G | −86.0 | −3.6 |
| B | −108.8 | +2.5 | | | |
| C | −112.0 | −0.7 | H | −82.7 | −0.3 |
| K | −118.5 | −7.2 | K | −86.8 | −4.4 |
| L | −108.0 | +3.3 | L | −81.1 | +1.3 |
| N | −115.0[d] | −3.7 | N | −84.2[d] | −1.8 |
| | | | M | −84.9[d] | −2.5 |
| A+B+C+K+L+N | −109.8[d] | +1.5 | F+G+H+K+L+N+M | −102.8[d] | −20.4 |
| (Hydration schemes shown in Figs. 6a′ and b′) | | | | | |
| A | −103.5 | +7.8 | F | −84.1 | −1.7 |
| B | −107.9 | +3.4 | G | −79.1 | +2.7 |
| C | −114.3 | −3.0 | H | −84.6 | −2.2 |
| K | −112.7 | −1.4 | K | −82.7 | −0.3 |
| L | −110.3 | +1.0 | L | −81.8 | +0.6 |
| N | −115.0[d] | −3.7 | N | −84.2[d] | −1.8 |
| | | | M | −88.1[d] | −5.7 |
| A+B+C+K+L+N | 110.1[d] | +1.2 | F+G+H+K+L+N+M | −93.3[d] | −10.9 |

a) Refer to Fig. 6. b) $\Delta E_{HB}=E_{HB}-(-111.3)$. c) $\Delta E_{HB}=E_{HB}-(-82.4)$. d) The values contain the C-$H^{\delta+}$···O hydrogen-bonding energies.

of the Gua-Cyt system (Fig. 6a), the energy cycle (Cycle 1) can be expressed as follows:

$$\begin{array}{ccc} (\text{Gua} + 8\text{H}_2\text{O}) & \xrightarrow[\Delta E_1]{} & (\text{Gua-8H}_2\text{O}) \\ + & & + \\ (\text{Cyt} + 6\text{H}_2\text{O}) & \xrightarrow[\Delta E_2]{} & (\text{Cyt-6H}_2\text{O}) \\ \downarrow \Delta E_5 & & \downarrow \Delta E_3 \\ (\text{Gua-Cyt} + 14\text{H}_2\text{O}) & \xrightarrow[\Delta E_4]{} & (5\text{H}_2\text{O-Gua-Cyt-5H}_2\text{O}) + 2(\text{H}_2\text{O})_2 \end{array}$$

For the second type hydration scheme (Fig. 6a'), it (Cycle II) can be expressed as follows:

$$\begin{array}{ccc} (\text{Gua} + 5\text{H}_2\text{O}) & \xrightarrow[\Delta E_1]{} & (\text{Gua-5H}_2\text{O}) \\ + & & + \\ (\text{Cyt} + 4\text{H}_2\text{O}) & \xrightarrow[\Delta E_2]{} & (\text{Cyt-4H}_2\text{O}) \\ \downarrow \Delta E_5 & & \downarrow \Delta E_3 \\ (\text{Gua-Cyt} + 9\text{H}_2\text{O}) & \xrightarrow[\Delta E_4]{} & (3\text{H}_2\text{O-Gua-Cyt-3H}_2\text{O}) + \frac{3}{2}(\text{H}_2\text{O})_2 \end{array}$$

Similarly, for the first type hydration scheme of the Hyp-Cyt system (Fig. 6b), it (Cycle III) is represented as follows:

$$\begin{array}{ccc} (\text{Hyp} + 6\text{H}_2\text{O}) & \xrightarrow[\Delta E_1]{} & (\text{Hyp-6H}_2\text{O}) \\ + & & + \\ (\text{Cyt} + 6\text{H}_2\text{O}) & \xrightarrow[\Delta E_2]{} & (\text{Cyt-6H}_2\text{O}) \\ \downarrow \Delta E_5 & & \downarrow \Delta E_3 \\ (\text{Hyp-Cyt} + 12\text{H}_2\text{O}) & \xrightarrow[\Delta E_4]{} & (4\text{H}_2\text{O-Hyp-Cyt-5H}_2\text{O}) + (\text{H}_2\text{O})_2 \\ & & \quad \diagdown \text{H}_2\text{O} \diagup \end{array}$$

For the second type of hydration scheme (Fig. 6b'), it (Cycle IV) is given by

$$\begin{array}{ccc} (\text{Hyp} + 4\text{H}_2\text{O}) & \xrightarrow[\Delta E_1]{} & (\text{Hyp-4H}_2\text{O}) \\ + & & + \\ (\text{Cyt} + 4\text{H}_2\text{O}) & \xrightarrow[\Delta E_2]{} & (\text{Cyt-4H}_2\text{O}) \\ \downarrow \Delta E_5 & & \downarrow \Delta E_3 \\ (\text{Hyp-Cyt} + 8\text{H}_2\text{O}) & \xrightarrow[\Delta E_4]{} & (3\text{H}_2\text{O-Hyp-Cyt-3H}_2\text{O}) + \frac{1}{2}(\text{H}_2\text{O})_2 \\ & & \quad \diagdown \text{H}_2\text{O} \diagup \end{array}$$

where, it was assumed that the removed $H_2O$ molecules formed the dimer. The dimerization energy was calculated to be $-21.3$ kJ mol$^{-1}$.

The calculated values are summarized follows:

Cycle I: $\Delta E_1 = -373.8$ kJ mol$^{-1}$
$\Delta E_2 = -287.4$ kJ mol$^{-1}$
$\Delta E_4 = -445.2$ kJ mol$^{-1}$
$\Delta E_5 = -111.3$ kJ mol$^{-1}$

Table 5. The Calculated Energy Differences by Hydrogen-Bond Formation between Hydrated Gua and Cyt and between Hydrated Hyp and Cyt, and the Experimental Enthalpy Changes in Aqueous Solution

| | $\Delta E_3$[a] | $\Delta E_3$[b] | $\Delta H^\circ$[c] |
|---|---|---|---|
| | kJ mol$^{-1}$ | kJ mol$^{-1}$ | kJ mol$^{-1}$ |
| Gua-Cyt pair | +104.7(Cycle I) | −11.9(Cycle II) | −10 |
| Hyp-Cyt pair | +97.5(Cycle III) | −18.5(Cycle IV) | −14 |

a) The first type of hydration schemes. b) The second type of hydration schemes. c) Data from calorimetric experiment (see Table 3).

Cycle II: $\Delta E_1 = -191.1$ kJ mol$^{-1}$
$\Delta E_2 = -144.4$ kJ mol$^{-1}$
$\Delta E_4 = -236.1$ kJ mol$^{-1}$
$\Delta E_5 = -111.3$ kJ mol$^{-1}$

Cycle III: $\Delta E_1 = -305.9$ kJ mol$^{-1}$
$\Delta E_2 = -287.4$ kJ mol$^{-1}$
$\Delta E_4 = -413.4$ kJ mol$^{-1}$
$\Delta E_5 = -82.4$ kJ mol$^{-1}$

Cycle IV: $\Delta E_1 = -138.7$ kJ mol$^{-1}$
$\Delta E_2 = -144.4$ kJ mol$^{-1}$
$\Delta E_4 = -219.2$ kJ mol$^{-1}$
$\Delta E_5 = -82.4$ kJ mol$^{-1}$

$\Delta E_5$ is equal to $E_{HB}$ (the internal hydrogen-bonding energy of complementary base pair in vacuo). The values are given in Table 1. Comparing the hydration energy of Gua with that of Hyp in both Cycles ($\Delta E_1$), we can see that the hydrated Gua is more stable than the hydrated Hyp. This is due to the existance of an amino group at which stable hydration occurs.

$\Delta E_3$ is the energy difference, which may correspond to the mixing process in the calorimetric experiment. It can be estimated by using the energies in the energy cycles mentioned above. The obtained results are summarized in Table 5, together with the $\Delta H^\circ$-values determined from calorimetric experiments. The magnitudes of the calculated energy differences ($\Delta E_3$), based on Cycles II and IV, are in good agreement with the experimental enthalpy change, while those based on Cycles I and III disagree with the experiment. It is of interest to note that the order of the $\Delta E_3$-values of hydrated Gua-Cyt and Hyp-Cyt pairs in both energy cycles agrees with that of the experimental $\Delta H^\circ$-values.

## Conclusions

Stabilization due to the formation of hydrogen bonds between Gua and Cyt and between Hyp and Cyt in vacuo and in aqueous solution was investigated by means of ab initio MO calculations and solution calorimetry.

The calculated hydrogen-bonding energies in vacuo were $-111.3$ kJ mol$^{-1}$ for the Gua-Cyt pair and $-82.4$ kJ mol$^{-1}$ for Hyp-Cyt, respectively. Thus, the Gua-Cyt pair was more stable than the Hyp-Cyt pair in vacuo. The main contributions to the internal hydrogen bonds of Gua-Cyt and Hyp-Cyt pairs were both electrostatic and charge-transfer interactions. For the Gua-Cyt pair, the charge transfer from Cyt to Gua through the hydrogen bonds was larger than that from Gua to Cyt. Since the dipole moment of the Gua-Cyt pair was larger than that of Hyp-Cyt, the Gua-Cyt pair was expected to be more stable than the Hyp-Cyt pair in a polar solvent because of the stabilization due to the dipole-dipole interaction between solute and solvent. This expectation agreed with the experiments concerning $CHCl_3$ solutions. However, the calorimetric measurement showed that in aqueous solution, the Hyp-Cyt pair was more stable than Gua-Cyt. That is, the enthalpy changes of the association of Gua with Cyt and of Hyp with Cyt in aqueous solution were estimated to be $-10$ kJ mol$^{-1}$ for the Gua-Cyt system and $-14$ kJ mol$^{-1}$ for the Hyp-Cyt system by using a solution microcalorimeter.

In order to explain this result, we carried out ab initio MO calculations of hydrated bases and base pairs, by assuming two types of hydration schemes. Using the energy cycles, we estimated the energies corresponding to the experimental heats of mixing ($\Delta E_3$). The calculated $\Delta E_3$'s based on the second type hydration scheme agreed with the experiments. Thus, the expermental results for aqueous solutions could be explained by dehydration in the course of complementary base-pair formation.

From the calculated results, it was shown that there was a possibility of C-H$^{\delta+}$···O hydrogen-bond formation between a $H_2O$ molecule and a base pair. Furthermore, it was indicated that a $H_2O$ molecule bound to an amino group of Gua destabilized the internal hydrogen bonds of complementary base pairs, however, that to the amino group of Cyt stabilized the internal hydrogen bonds.

In conclusion, the present work showed that hydration/dehydration had an important effect on the stability of the complementary base pair.

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## References

1) J. D. Watson and F. H. C. Crick, *Nature(London)*, **171**, 737 (1953).

2) W. Saenger, "Principles of Nucleic Acid Structure," Springer-Verlag, New York (1984), Chap. 8.

3) Y. Baba, K. Fujioka, and A. Kagemoto, *Polym. J.*, **10**, 241 (1978).

4) K. Fujioka, Y. Baba, and A. Kagemoto, *Polym. J.*, **12**, 843 (1980).

5) Y. Kyogoku, R. C. Lord, and A. Rich, *Biochim. Biophys. Acta*, **179**, 10 (1969).

6) L. F. Sukhodub and I. K. Yanson, *Nature(London)*, **264**, 245 (1976).

7) I. K. Yanson, A. B. Tepletsky, and L. F. Sukhodub, *Biopolymers*, **18**, 1149 (1979).

8) W. Förner, P. Otto, and J. Ladik, *Chem Phys.*, **86**, 49 (1984).

9) A. Pohorille, S. K. Burt, and R. D. MacElroy, *J. Am. Chem. Soc.*, **106**, 402 (1984).

10) A. Sarai and M. Saito, *Int. J. Quantum Chem.*, **28**, 399 (1985).

11) A. Gordblum, D. Perahia, and A. Pullman, *FEBS Lett.*, **91**, 213 (1978).

12) B. Pullman, S. Miertus, and D. Perahia, *Theor. Chim. Acta*, **50**, 317 (1979).

13) J. T. Powell, E. G. Richards, and W. B. Gratzer, *Biopolymers*, **11**, 235 (1972).

14) J. Langlet, P. Claverie, F. Caron, and J. C. Boeuve, *Int. J. Quantum Chem.*, **19**, 299 (1981).

15) M. Aida and C. Nagata, *Int. J. Quantum Chem*, **29**, 1253 (1986).

16) P. Hobza and C. Sandorfy, *J. Am. Chem. Soc.*, **109**, 1302 (1987).

17) E. Clementi, J. Mehl, and W. Von Niessen, *J. Chem. Phys.*, **54**, 508 (1971).

18) R. Scordamaglia, F. Carallone, and E. Clementi, *J. Am. Chem. Soc.*, **99**, 5545 (1977).

19) E. Clement and G. Corongiu, *J. Chem. Phys.*, **72**, 3979 (1980).

20) Y. Ohta, H. Tanaka, Y. Baba, A. Kagemoto, and K. Nishimoto, *J. Phys. Chem.*, **90**, 4438 (1986).

21) A. Weissberger and E. S. Proskauer, "Organic Solvents," Interscience, New York (1955).

22) Y. Baba, S. Tanaka, and A. Kagemoto, *Makromol. Chem.*, **178**, 2117 (1977).

23) K. Morokuma, S. Kato, K. Kitaura, I. Ohmine, S. Sakai, and S. Obara, IMS Computer Center Program Library, The Institute for Molecular Science, Program No. 372 (1980).

24) J. S. Binkley, R. A. Whiteside, R. Krishnan, R. Seeger P. J. DeFrees, H. B. Schlegel, S. Topiol, L. R. Kahn, and J. A. Pople, *Quantum Chemistry Program Exchange*, **13**, 406 (1981).

25) W. J. Hehre, R. F. Stewart, and J. A. Pople, *J. Chem. Phys.*, **51**, 2657 (1969).

26) J. E Del Bene, *J. Phys. Chem.*, **87**, 367 (1983).

27) T. Matsushita, Y. Baba, and A. Kagemoto, unpublished work.

28) S. Arnott and D. W. L. Hukins, *J. Mol. Biol.*, **81**, 93 (1973).

29) K. Kitaura and K. Morokuma, *Int. J. Quantum Chem.*, **10**, 325 (1976).

30) K. Morokuma and K. Kitaura, "Chemical Applications of Atomic and Molecular Electrostatic Potentials," Plenum, New York (1981), pp. 215—242.