Stability of DNA

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A–T Base Pairs are More Stable Than G–C Base Pairs in a Hydrated Ionic Liquid**

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The canonical DNA structure is a B-form duplex consisting of Watson–Crick base pairs.[1] In physiologically relevant buffered solutions, G–C base pairs are more stable than A–T base pairs.[1] This stability difference affects the formation of local and long-range DNA structures. DNAs have enormous potential in the fields of nanobiotechnology and biomedical technology because single DNA strands can recognize and hybridize with complementary sequences through highly specific base-pairing interactions.[2] DNAs can undergo structural transitions in response to environmental stimuli.[2a–g] Such stimuli include pH,[2a,b] small molecules (e.g., cosolutes and ions),[2c–e] macromolecules (e.g., oligonucleotides and proteins),[2f] and electrical signals.[2g] Researchers have taken advantage of the switching properties of DNA that depend on stimuli to develop DNA-based materials such as sensors,[2a,c] logic devices,[2b] circuits,[2e] and drugs.[2 h] We have developed logic devices that respond to input molecules (e.g., pH and cations) that monitor the change in DNA structure from G-quadruplexes to duplexes, and these are of considerable interest in DNA computing.[2b,d] Certain ions are known to preferentially stabilize certain base pairs. For example, alkylammonium ions bind to A–T base pairs in the minor groove of a DNA duplex,[3] stabilizing regions of A–T base pairs.[3a] Some osmolytes, such as glycine betain which has alkylammonium derivative ions, destabilize G–C rich DNA duplexes by binding to unpaired guanines, at high salt concentrations.[4] However, environmental stimuli that can drastically control Watson–Crick base pair stabilities have not been reported.

In this study, we evaluated the effect of an ionic liquid (IL) on base pair stability. ILs provide favorable environments for a wide range of chemical reactions.[5] A representative IL, choline dihydrogen phosphate (choline dhp),[6] dissolved in a small amount of water ensures long-term stability of biomolecules like DNA[6a] and proteins.[6b,c] As choline dhp contains alkylammonium ions, we reasoned that this IL might impact the DNA duplex stability. The water activity in the hydrated ILs is low.[6d] We have shown that water activity is an important factor for DNA stability.[7] The solute behavior can be regulated by the combination of anions and cations of ILs.[5a,b] In this study, we have quantitatively investigated the thermodynamic stability of DNA in hydrated choline dhp.

First, we investigated the sequence dependence of DNA stability in solutions of hydrated choline dhp by evaluation of ultraviolet (UV) melting curves. The melting temperatures (T_ms) of 10-mer DNA duplexes with different A–T base pair contents were measured in a solution of 4 M choline dhp (80 wt % choline dhp) and in a solution of 4 M NaCl (Table 1). The A–T base pairs in ODN1 to ODN6 are consecutive. In ODN7, ODN8, ODN9, and ODN10 the sequences are random. We also evaluated the stability in 4 M NaCl as previous quantitative analyses of DNA duplex stabilities have been performed in NaCl solutions.[8a–e] Typical UV melting curves are shown in Figure 1. In NaCl solution, the T_m values of DNA duplexes with consecutive A–T base pairs decreased from 45.3 to 30.7 °C as the A–T content increased (Figure 1 a and Table 1). In contrast, in choline dhp a reverse trend for the stabilities of DNA duplexes was observed. The T_m values of the DNA duplexes in choline dhp solution increased from 33.3 to 53.3 °C as the A–T content increased (Figure 1 b and Table 1). To confirm the effect of choline dhp, we also measured the T_m values of DNA duplexes ODN7, ODN8, ODN9, and ODN10 (Table 1). The DNA duplexes with the highest A–T contents had the highest T_ms in a solution of choline dhp. Intriguingly, ODN7, composed only of A and T, was more stable in choline dhp solution than in NaCl solution, whereas ODN8, composed only of G–C base pairs, was destabilized in choline dhp relative to the NaCl solution. Finally, ODN7 was more stable than ODN8 in choline dhp solution with T_m values of 37.7 and 33.1 °C, respectively. The ΔT_m was calculated by subtracting the T_m value obtained in NaCl solution from that in the choline dhp solution. Figure 2 a shows that the correlation between ΔT_m values and A–T content of the duplexes is linear. The DNA duplexes with more than 61 % A–T content were stabilized in choline dhp solution, whereas the DNA duplexes with less than 61 % A–T content were destabilized. A similar trend was also seen for 12-mer DNA duplexes of different sequences (see Figure S1 and Table S1 in the Supporting Information).

To gain insight into how choline dhp altered the stabilities of DNA duplexes, we measured the thermodynamic parameters for ODN9 and ODN10 (Table 2). ODN9 and ODN10 show a two-state transition in thermal melting,[8] and structures were not changed drastically by the solution conditions (0.1, 0.5, 1, 2, and 4 M NaCl or choline dhp; see Figure S2 in

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Table 1: Sequences used in this study and melting temperatures for the formation of duplexes in the presence of 4 M NaCl or 4 M choline dhp (IL).

Sequence name[a]	Sequence	T_m [°C][b] NaCl	IL
ODN1(10)	5′-AAAAAAAAAA-3′/5′-TTTTTTTTTT-3′	30.7	53.3
ODN2(9)	5′-AAAAAAAAAC-3′/5′-GTTTTTTTTT-3′	31.5	48.6
ODN3(8)	5′-AAAAAAAACC-3′/5′-GGTTTTTTTT-3′	36.0	46.6
ODN4(7)	5′-AAAAAAACCC-3′/5′-GGGTTTTTTT-3′	38.7	42.7
ODN5(6)	5′-AAAAAACCCC-3′/5′-GGGGTTTTTT-3′	40.0	36.3
ODN6(5)	5′-AAAAACCCCC-3′/5′-GGGGGTTTTT-3′	45.3	33.3
ODN7(10)	5′-AAATATATTT-3′/5′-AAATATATTT-3′	17.4	37.7
ODN8(0)	5′-GGGCGCGCCC-3′/5′-GGGCGCGCCC-3′	61.0	33.1
ODN9(8)	5′-TTATAACCTA −3′/5′-TAGGTTATAA −3′	26.7	34.8
ODN10(2)	5′-CGGCAAGCGC-3′/5′-GCGCTTGCCG-3′	47.2	30.5

[a] The number of A–T base pairs in the DNA duplex is shown in parentheses. [b] The melting temperature was calculated at a strand concentration of 5 μM.

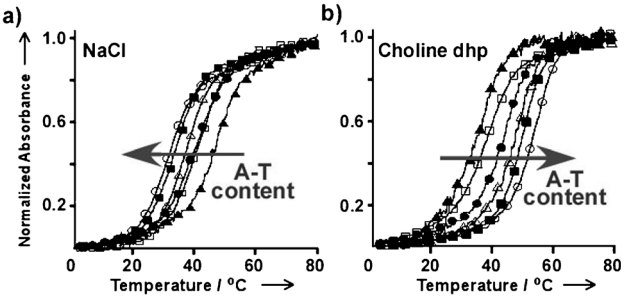

Figure 1. Normalized UV melting curves at 260 nm for ODN1 (○), ODN2 (■), ODN3 (△), ODN4 (●), ODN5 (□), and ODN6 (▲) in a solution of 50 mM MES (pH 6.0), 1 mM Na_2EDTA, and a) 4 M NaCl or b) 4 M choline dihydrogenphosphate. The concentration of the DNA strands was 5 μM (MES = 2-morpholinoethanesulfonic acid and EDTA = ethylenediaminetetraacetic acid).

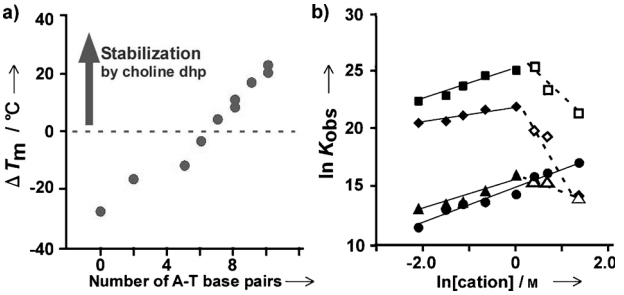

Figure 2. a) The correlation between ΔT_m values and A–T content of the duplexes. ΔT_m was calculated by subtracting the T_m value obtained in NaCl solution from that in the choline dhp solution. b) Dependencies of the $\ln K_{obs}$ values for ODN9 (triangles) and ODN10 (squares) on the NaCl concentration or for ODN9 (circles) and ODN10 (diamonds) on the choline dhp concentration. The data points that deviated from a linear plot (solid line) are indicated with open symbols. Slopes plots for data from ODN9 and ODN10 in NaCl and ODN9 and ODN10 in choline dhp are 1.28 ± 0.23, 1.27 ± 0.15, 1.47 ± 0.11, and 0.68 ± 0.10, respectively.

the Supporting Information). The ΔG_{25}° values of ODN9 and ODN10 in 4 M NaCl were −8.4 and −12.7 kcal mol^{-1}, respectively. In 4 M choline dhp, however, the stabilities were reversed: The ΔG_{25}° values of ODN9 and ODN10 were −10.1 and −8.4 kcal mol^{-1}, respectively. The higher A–T content duplex, ODN9, was more stable in choline dhp solution than in NaCl solution because of a favorable enthalpic contribution, whereas ODN10 was destabilized in choline dhp relative to the NaCl solution because of an unfavorable enthalpic contribution. Alkylammonium derivative ions bind to single-stranded DNA, especially unpaired guanines, at high salt concentrations.[4] The destabilization of ODN10 in choline dhp solution was caused by an unfavorable

Table 2: Thermodynamic parameters for DNA duplex formation measured in NaCl or choline dhp.[a]

	ΔH° [kcal mol^{-1}]	$T\Delta S$° [kcal mol^{-1}]	ΔG_{25}° [kcal mol^{-1}]	T_m[b] [°C]
NaCl				
ODN9	−46.6 ± 2.8	−38.2 ± 2.0	−8.4 ± 0.3	38.6
ODN10	−54.7 ± 3.3	−42.0 ± 2.9	−12.7 ± 0.4	63.2
Choline dhp				
ODN9	−66.8 ± 3.4	−56.7 ± 2.7	−10.1 ± 0.4	43.6
ODN10	−47.8 ± 3.7	−39.4 ± 3.4	−8.4 ± 0.5	38.2

[a] All the experiments were carried out in a buffer containing 50 mM MES (pH 6.0), 1 mM Na_2EDTA, and 4 M NaCl or 4 M choline dhp. Thermodynamic parameters were evaluated from the average values obtained from the curve fitting and T_m^{-1} versus $\log(C_t/4)$ plots. [b] The melting temperature was calculated at a strand concentration of 100 μM.

enthalpic effect, implying that choline ions decrease the duplex stability either by destabilizing the formation of hydrogen bonds or by stabilizing single-stranded DNA through preferential binding of choline ions to guanine bases in the single strand.[4,8] As the stabilization of ODN9 in choline dhp solution was enthalpically driven, we hypothesize that choline ions increase duplex stability because of an interaction between choline ions and the ODN9.

To investigate the interaction between cations and DNA duplexes, we measured the number of cations bound to DNA during the formation of DNA duplex structures ($-\Delta n_+$) in solutions of NaCl or choline dhp. The contribution of the counter ion concentration on K_{obs} (observed equilibrium constant for the duplex formation) can be quantified by using Equation (1):[9]

$$(\mathrm{d}\ln K_{obs}/\mathrm{d}\ln[\mathrm{cation}]) = -\Delta n_+ \quad (1)$$

where $-\Delta n_+$ is the number of cations taken up during the formation of duplexes; it represents the magnitude of the counter ion condensation. In general, the stabilities of DNA duplexes increase as the cation concentration increases.[9] For the duplexes studied here, $\ln K_{obs}$ values showed a linear correlation with the natural logarithm (ln) of the NaCl and choline dhp concentration, although this correlation was lost

at concentrations above 1 M (Figure 2b). The $-\Delta n_+$ values per duplex for ODN9 and ODN10 were 1.28 (0.064 per nucleotide), and 1.27 (0.064 per nucleotide), respectively, in agreement with previous reports that the numbers of counter ions bound to a DNA duplex were around 0.040 to 0.079 sodium ions per phosphate group.[9] The plots in Figure 2b were also linear with a positive slope except for data collected in 4 M choline dhp for ODN9 and ODN10. In choline dhp, $-\Delta n_+$ values per nucleotide for ODN9 and ODN10 were 1.47 (0.074 per nucleotide) and 0.68 (0.034 per nucleotide), respectively. The $-\Delta n_+$ value is inversely correlated with the radius of the ion, because large cations cannot physically approach the DNA backbone as closely or as densely as small cations. For example, the $-\Delta n_+$ for sonicated calf-thymus DNA (60% A–T content) of alkylammonium ions ranges from 0.072 to 0.019 ions per phosphate group,which is roughly half the value of $-\Delta n_+$ in NaCl solution.[3c] The $-\Delta n_+$ value of ODN10 in choline dhp solution was half that $-\Delta n_+$ value in NaCl solution. In contrast, $-\Delta n_+$ of ODN9 in choline dhp solution was higher than the $-\Delta n_+$ value in NaCl solution. The slope of the plot shown in Figure 2b for ODN9 in choline dhp solution was linear even at high concentrations of choline dhp. We assume that the linear correlation results because the eight A–T base pairs in this duplex provide many binding sites for choline ions. We also estimated the values of $dT_m/d\ln[\text{cation}]$ for ODN1, ODN2, ODN3, ODN4, ODN5, and ODN6 as these values are related to the number of cations bound upon formation of duplexes.[9] All the DNA duplexes had similar slopes in NaCl solution (see Table S2 and Figure S3a in the Supporting Information). In contrast, the $dT_m/d\ln[\text{choline dhp}]$ values for DNA duplexes increased with A–T content (see Table S2 and Figure S3b in the Supporting Information). Thus, choline ions preferentially bind to A–T base pairs.

At excess salt concentrations of 1 M, the slopes of $d\ln K_{obs}/d\ln[\text{cation}]$ for all DNA duplexes, except for ODN9 in choline dhp solution, were negative (Figure 2b), suggesting that cations were released during the formation of duplexes. The stability of the DNA duplexes probably decreased at high salt concentrtaions because of the binding of cations to bases in the single strands, although the cations generally bind to phosphate groups at lower salt concentrations.[9a] We also measured the thermodynamic parameters for ODN9 and ODN10 (see Table S3 in the Supporting Information) at a salt concentration of 1 M. When the salt concentration was increased from 1 to 4 M, the stability of ODN9 and ODN10 at 25 °C, except for ODN9 in choline dhp solution, decreased because of an unfavorable enthalpic contribution. This results from the destabilization of hydrogen bonds as cations bind preferentially to bases in single-stranded DNAs. Thus, cations were released during the formation of duplexes at high salt concentrations.

We hypothesize that choline dhp binds preferentially to A–T base pairs. To confirm that preferential binding of choline ions to these base pairs contributed to the stability of DNA duplexes, we carried out molecular dynamic calculations of ODN9 and ODN10 and used the CDOCKER program to model binding between one choline ion and ODN9 or ODN10 (see the Supporting Information). Several docking patterns between choline ions and OND9 were obtained (Figure 3 and Figure S4 in the Supporting Information) and the simulations showed that choline ions bound to the major groove of the A–T rich region and formed two

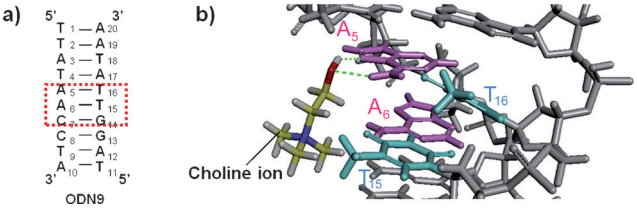

Figure 3. a) Secondary structure of ODN9. The area surrounded by the dashed line was selected as the binding region for the choline ion in the molecular dynamics calculation. b) The predicted interaction between ODN9 (gray) and choline ion (carbon, hydrogen, nitrogen, and oxygen atoms are indicated by yellow, white, blue and red, respectively). Adenine and thymine bases close to choline ions are indicated by pink and light blue, respectively. Hydrogen bonds are rendered in dashed lines (light green).

hydrogen bonds. Figure 3 provides a specific example of the binding of choline ions to ODN9. The hydroxyl group of the choline ion forms two hydrogen bonds with N7 and H6 of A_5, and the methyl group of choline ion is located close to the methyl group of T_{15} on the opposite strand. Interaction of a choline ion with adenosine in a duplex with a different sequence was previously reported.[10] Thus, choline ions bind tightly in the major groove of A–T regions but weakly to the G–C rich regions (see Figure S5 in the Supporting Information). In contrast, choline ions bind preferentially to G–C rich single-stranded DNA, relative to A or T rich strands (see Figure S6 in the Supporting Information). Cations bind preferentially to G,[11] and choline ion binding to guanines effectively destabilizes G–C rich DNA duplexes. The molecular dynamic calculations were carried out under the assumption that a single choline ion binds to a DNA. We do not rule out the possibility that there are other binding modes of the choline ion and DNA at high concentrations (4 M) of choline dhp. Our predicted structure is one of the stable binding styles.

We also estimated the T_m values of ODN9 and ODN10 in 4 M choline chloride, which is not a hydrated IL. ODN10 was more stable than ODN9 in this solution although the stability of ODN9 was increased in choline chloride relative to that in the NaCl solution (data not shown). The water activity of a solution is an important factor contributing to the stability of DNA.[7,12] Solutions with high concentrations of choline dhp (over 4 M) are called hydrated ionic liquids. Hydrated ILs have unique properties such as decreased water activity and vapor pressure and altered ion networks relative to an aqueous buffer without choline dhp. These properties have attracted the attention of researchers in the field of nanobiotechnology. Our results show that the stabilities of DNA duplexes changed drastically at high concentrations of choline dhp in a manner that depends on their content of A–T base pairs. The unique phenomenon observed here, in which A–T base pairs are more stable than G–C base pairs, is due to the special characteristics of the hydrated IL. The data reported

here suggest that it will be possible to develop novel functional materials using DNA base pair switches controlled by hydrated IL as solvent.

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