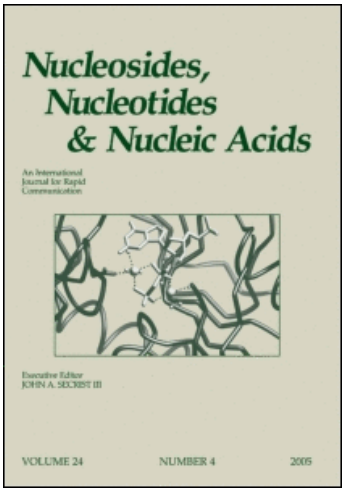


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# Comparative Studies of the Thermodynamic Stabilities Between Sheared A:G and Watson-Crick A:U(T) Base Pairs in RNA and DNA

Masato Katahira[a]; Jun-ichi Saeki[a]; Mayumi Kanagawa[a]; Masashi Nagaoka[a]; Seiichi Uesugi[a]
[a] Department of Bioengineering, Faculty of Engineering, Yokohama National University, Yokohama, Japan

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NUCLEOSIDES & NUCLEOTIDES, 15(1-3), 585-598 (1996)

# COMPARATIVE STUDIES OF THE THERMODYNAMIC STABILITIES BETWEEN SHEARED A:G AND WATSON-CRICK A:U(T) BASE PAIRS IN RNA AND DNA#

Masato Katahira, Jun-ichi Saeki, Mayumi Kanagawa, Masashi Nagaoka and Seiichi Uesugi*
Department of Bioengineering, Faculty of Engineering,
Yokohama National University, 156 Tokiwadai, Hodogaya-ku,
Yokohama 240, Japan

**ABSTRACT:** Thermodynamic parameters for duplex formation were determined from CD melting curves for r(GGACGAGUCC)$_2$ and d(GGACGAGTCC)$_2$, both of which form two consecutive 'sheared' A:G base pairs at the center [Katahira et al. (1993) Nucleic Acids Res. **21**, 5418-5424; Katahira et al., (1994) Nucleic Acids Res. **22**, 2752-2759]. The parameters were determined also for r(GGACUAGUCC)$_2$ and d(GGACTAGTCC)$_2$, where the A:G mismatches are replaced by Watson-Crick A:U(T) base pairs. Thermodynamic properties for duplex formation are compared between the sheared and the Watson-Crick base pairs, and between RNA and DNA. Difference in the thermodynamic stability is analyzed and discussed in terms of enthalpy and entropy changes. The characteristic features in CD spectra of RNA and DNA containing the sheared A:G base pairs are also reported.



## INTRODUCTION

Since the discovery of ribozymes, studies to elucidate how ribozymes exert their enzymatic activities on the basis of their structures have been carried out.[1-11] It has been suggested that an A:G mismatch base pair is formed in some ribozymes and that the structure derived from this base pairing could play a crucial role in their enzymatic activities.[12,13]

First we have demonstrated[14] that the unique 'sheared' A:G base pairs[12,15-24] are formed in the DNA duplexes, d(GGACGAGTCC)$_2$ and d(GGACGACATC):d(GATGGAGTCC), the base sequences of which are modelled after both a hammerhead ribozyme[25] and a lead ribozyme.[26] In the case of d(GGACGCATC):d(GATGAGTCC) where the central two consecutive A:G mis-

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#This paper is dedicated to Dr. Yoshihisa Mizuno on the occasion of his 75th birthday.

matches of d(GGACGACATC):d(GATGGAGTCC) are replaced by a single A:G mismatch, on the other hand, another kind of A:G base pairing, presumably a "head to head" A:G base pair,[27-29] occurs.[30] Thus, sequence dependent polymorphism of the structure of the A:G base pair is observed.

Secondly, we have demonstrated that the sheared A:G base pairs are formed in $r(GGACGAGUCC)_2$, the first six bases of which are identical to those of part of the hammerhead ribozyme.[31] This work together with the one by Turner's group[32] have strongly suggested formation of the sheared A:G base pairs in hammerhead ribozymes. Quite recently the sheared A:G base pairs have been found in the crystal structure of a hammerhead ribozyme by McKay's group.[33]

As the next step, it is important to evaluate the thermal stability of the unique sheared A:G base pair in order to assess its biological role. As mentioned above, we have already studied the structures of $r(GGACGAGUCC)_2$ (A:G-RNA) containing a partial sequence of the ribozyme and a corresponding DNA version, $d(GGACGAGTCC)_2$ (A:G-DNA). A difference between the RNA and DNA duplexes in the stacking interaction involving the sheared A:G base pairs is pointed out.[31] Here we have investigated the thermodynamic properties of the two duplexes. As controls, $r(GGACUAGUCC)_2$ (A:U-RNA) and $d(GGACTAGTCC)_2$ (A:T-DNA) were also studied. The A:G mismatches in the central two base pairs are replaced by standard Watson-Crick A:U(T) base pairs in these duplexes. The number of hydrogen bonds for the central two base pairs, two hydrogen bonds per base pair, is not changed by the replacement. The thermodynamic parameters were determined from CD melting curves for these four duplexes. The thermodynamic parameters are compared between RNA and DNA, and between the sheared A:G base pairs and the standard Watson-Crick A:U(T) base pairs. Difference in the thermodynamic properties is analyzed.

Additionally, the CD spectra of the four duplexes are compared together with those of d(GGACGACATC):d(GATGGAGTCC) and d(GGACGCATC):d(GATGAGTCC). The characteristic features of the CD spectra of the duplexes containing the sheared A:G base pairs are pointed out.

## MATERIALS AND METHODS

A:G-RNA, A:G-DNA, d(GGACGACATC):d(GATGGAGTCC) and d(GGACGCATC):d(GATGAGTCC) were prepared previously.[14,30,31] A:T-DNA was synthesized

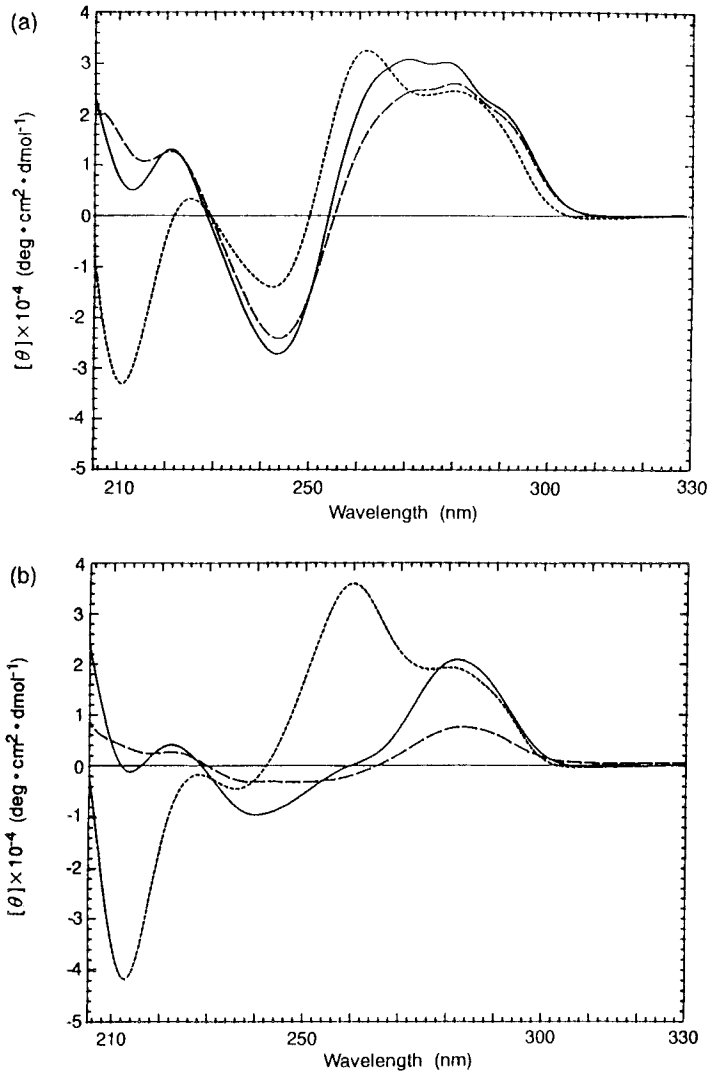


FIG. 1. (a) The CD spectra of A:G-RNA (dotted line), A:G-DNA (solid line) and d(GGACGACATC):d(GATGGAGTCC) (broken line) at 20 °C at the strand concentration of 50 $\mu$M. (b) The CD spectra of A:U-RNA (dotted line), A:T-DNA (solid line) and d(GGACGCATC):d(GATGAGTCC) (broken line) at 20 °C at the strand concentration of 50 $\mu$M.

with a DNA synthesizer (model 392, Applied Biosystems Co.) and purified in the same manner. A:U-RNA was synthesized manually by the solid-phase phosphoramidite method using o-nitrobenzyl groups for 2'-OH protection and purified as described previously.[13]

Each oligomer was dissolved in 20 mM sodium phosphate buffer (pH 7.0) containing 0.15 M NaCl. Oligomer strand concentrations were varied over 100-fold range. The strand concentrations were determined from high-temperature absorbance at 260 nm using the known base compositions of the oligomers and the published extinction coefficients of the individual nucleosides at 260 nm.[34]

CD spectra and thermal CD melting curves were recorded with a JASCO J-720 spectropolarimeter interfaced with an NEC PC-9801 FX personal computer with 0.1 cm and 1.0 cm cells. The temperature of the solution was raised from 5 °C to 85 °C at the rate of 1 °C/min. Changes in CD intensity were monitored at around the $\lambda_{max}$ of each spectrum at 5 °C, at 262 nm for A:G-RNA and A:U-RNA, at 270 nm for A:G-DNA, and at 280 nm for A:T-DNA. The melting temperatures were determined by use of the derivatives of the melting curves.

Thermodynamic parameters for duplex formation, $\Delta H^0$ and $\Delta S^0$, were determined by two methods. In the method (1), sloping base lines are obtained as illustrated in FIG. 2(a). An equilibrium constant, K, at a certain temperature is calculated as follows:

$$K = [D]/[S]^2 = b\cdot(a+b)/2C_T\cdot a^2$$

, where $C_T$ is a total strand concentration, [D] and [S] are the concentrations of double and single strands, respectively, and a and b are values indicated in FIG. 2(a). Plots of reciprocal temperature ($T^{-1}$) versus the logarithm of the equilibrium constant (logK) were fit to equation-1:

$$-2.30R\cdot\log K = \Delta H^0/T - \Delta S^0 \qquad \text{equation-1}$$

, where R=1.99 cal·mol$^{-1}$·K$^{-1}$. Enthalpy and entropy changes obtained from fits of individual melting curves at different total strand concentrations were averaged. In the method (2), plots of reciprocal melting temperature ($Tm^{-1}$) versus the logarithm of the total strand concentration ($\log C_T$) were fit to equation-2:[35,36]

$$Tm^{-1} = (2.30R/\Delta H^0)\log C_T + \Delta S^0/\Delta H^0 \qquad \text{equation-2}$$

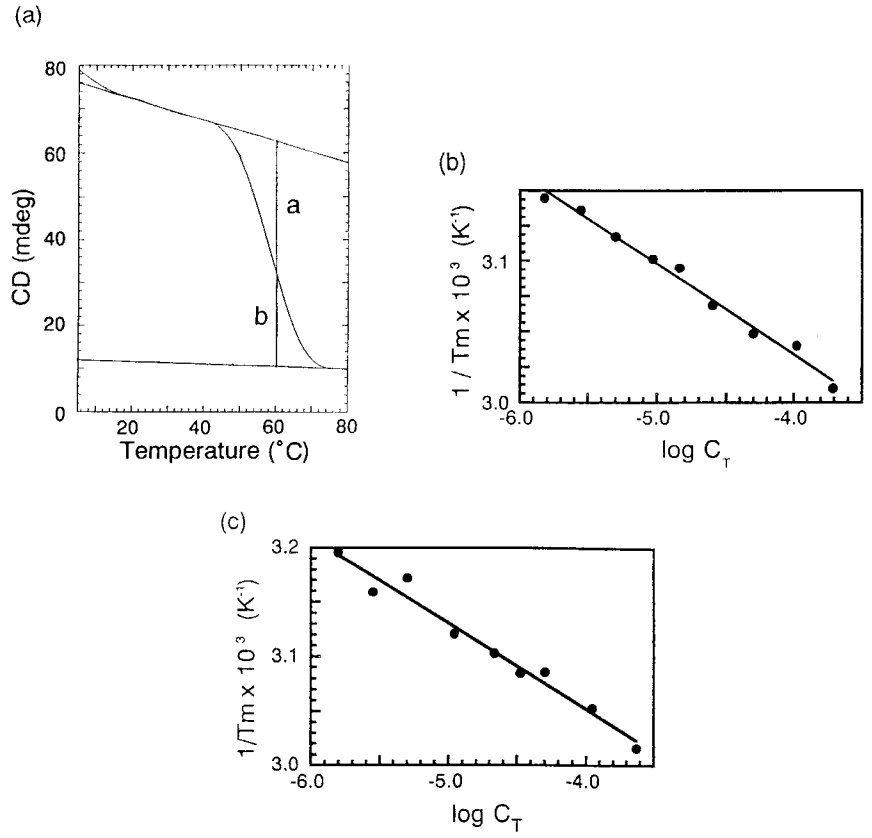


FIG. 2. (a) The CD melting curve of A:G-RNA at 262 nm at the strand concentration of 100 $\mu$M. Sloping base lines and the definitions of a and b are indicated. (b) The plots of reciprocal melting temperature ($Tm^{-1}$) versus logarithm of the strand concentration ($logC_T$) for A:G-RNA. (c) The same plots for A:G-DNA.

## RESULTS

CD spectra of A:G-RNA, A:G-DNA and d(GGACGACATC):d(GATGGAGTCC), all of which have been demonstrated to form the sheared A:G base pairs,[14,31] are shown in FIG. 1(a). CD spectra of A:U-RNA, A:T-DNA and d(GGACGCATC):d(GATGAGTCC), all of which do not form the sheared A:G base pairs,[30] are shown in FIG. 1(b). It is noted that three spectra in FIG. 1(a) are very similar.

Thermodynamic parameters for duplex formation, $\Delta H^0$ and $\Delta S^0$, were determined by two methods as described in MATERIALS AND METHODS. An example of CD melting curves is shown in FIG. 2(a). Sloping base lines, and a and b, which are used in the method (1), are also shown. Examples of the plots of reciprocal melting temperature ($Tm^{-1}$) versus the logarithm of the strand concentration ($\log C_T$) for A:G-RNA and A:G-DNA are shown in FIG. 2 (b) and (c). The strand concentrations are varied over 100-fold range (ca. 1.5 $\mu$M-230 $\mu$M). In the case of A:T-DNA, data were collected for the strand concentrations higher than 15 $\mu$M, because it was found that this oligomer forms a hairpin structure at low strand concentrations. This will be discussed later. The thermodynamic parameters determined by the two methods agreed within about 10%.

The thermodynamic parameters determined by the method (1) and (2) for the four duplexes are listed on TABLE 1 and 2, respectively. On the basis of these parameters, the free energy changes at 37 °C, $\Delta G^0_{37}$, are calculated and listed on TABLE 1 and 2. The melting temperatures, Tm, at the strand concentrations of 100 $\mu$M and 17 $\mu$M are calculated by the equation-2 using the determined thermodynamic parameters on Table 2 and listed on TABLE 3. The melting temperatures obtained experimentally at the strand concentration of 100 $\mu$M are also listed on TABLE 3. Additionally, the melting temperatures of the corresponding Lane's duplexes, $r(CCACGAGUGG)_2$, $d(CCACGAGTGG)_2$, $r(CCACUAGUGG)_2$ and $d(CCACTAGTGG)_2$, at 17 $\mu$M[37,38] are listed on TABLE 3 for reference. This will be discussed later.



## DISCUSSIONS

### Comparison of the CD spectra between the duplexes containing the sheared A:G base pairs and those which do not contain the sheared A:G base pairs.

FIG. 1(a) shows CD spectra of the RNA and DNA duplexes which contain the sheared A:G base pairs. It should be noted that the structures of the A:G mismatches at the central two base pairs of these duplexes are established to be the sheared type by the NMR studies[14,31] prior to the CD analyses. FIG. 1(b) shows CD spectra of the RNA and DNA duplexes which do not contain the sheared A:G base pair. Two duplexes, $r(GGACUAGUCC)_2$ and $d(GGACTAGTCC)_2$, for FIG. 1(b) are the Watson-Crick versions of $r(GGACGAGUCC)_2$ and $d(GGACGAGTCC)_2$ for FIG. 1(a), respective-

TABLE 1. Thermodynamic parameters derived from the method (1) for duplex formation of the four oligonucleotides

| | $\Delta H^\circ$(kcal • mol$^{-1}$) | $\Delta S^\circ$ (cal • mol$^{-1}$ • K$^{-1}$) | $\Delta G^\circ_{37}$(kcal • mol$^{-1}$) |
|---|---|---|---|
| A:G-RNA | -77.4 | -216 | -10.4 |
| A:G-DNA | -58.6 | -162 | -8.38 |
| A:U-RNA | -120 | -337 | -15.5 |
| A:T-DNA | -76.6 | -215 | -9.95 |

TABLE 2. Thermodynamic parameters derived from the method (2) for duplex formation of the four oligonucleotides

| | $\Delta H^\circ$(kcal • mol$^{-1}$) | $\Delta S^\circ$ (cal • mol$^{-1}$ • K$^{-1}$) | $\Delta G^\circ_{37}$(kcal • mol$^{-1}$) |
|---|---|---|---|
| A:G-RNA | -89.0 | -251 | -11.2 |
| A:G-DNA | -58.3 | -160 | -8.70 |
| A:U-RNA | -128 | -359 | -16.7 |
| A:T-DNA | -77.2 | -218 | -9.62 |



TABLE 3. Experimental (exp.) and calculated (calc.) melting temperatures (Tm) for the four duplexes

| | Tm (°C) | | | |
|---|---|---|---|---|
| | 100 $\mu$M (exp.) | 100 $\mu$M (calc.) | 17 $\mu$M (calc.) | 17 $\mu$M [a] (calc.) |
| A:G-RNA | 56.6 | 57.5 | 53.2 | 49 [b] |
| A:G-DNA | 54.3 | 54.0 | 47.6 | 52 [c] |
| A:U-RNA | 65.5 | 66.3 | 63.1 | 61 [b] |
| A:T-DNA | 54.6 | 53.7 | 48.9 | 50 [c] |

[a] The melting temperatures of the corresponding duplexes reported by Ebel et al.

[b] Calculated from thermodynamic parameters in reference 38.

[c] Experimental values taken from reference 37.

ly. The third duplex for FIG. 1(a) contains two consecutive A:G mismatches, while the third duplex for FIG. 1(b) contains a single A:G mismatch, which results in formation of a non-sheared A:G base pair, as revealed by NMR.[31]

It is remarkable that the duplexes containing the sheared A:G base pairs exhibit very similar CD spectra. Maxima at around 280 nm and 262-272 nm, and minimum at around 243 nm are their common features. This is in striking contrast to the fact that the three spectra in FIG. 1(b) do not resemble each other. It is suggested that some structural character of the duplexes containing the sheared A:G base pairs is reflected on the CD spectra. Thus CD spectra give an initial clue to the structures of A:G mismatches, although it is dangerous to deduce the structure from the CD spectra alone.

**Reliability of the determined thermodynamic parameters.**

The thermodynamic parameters derived from the two methods for the four duplexes agreed within about 10%. This guarantees that the bimolecular, two state model is a reasonable approximation for the transition of the duplexes studied. Otherwise, the discrepancy in the parameters derived by the two methods should be found.[39]

As shown in TABLE 3, the melting temperatures calculated by the equation-2 using the determined thermodynamic parameters on TABLE 2 at the strand concentration of 100 $\mu$M agree well with the observed experimental melting temperatures within 0.9 °C. This is also an indication that the determined parameters are reliable.

Very often, only the melting temperatures at a certain strand concentration are published in literatures, instead of the thermodynamic parameters. Thus such a calculation of the melting temperature at a certain strand concentration is required in order to compare our data with others'.

**The formation of a hairpin structure of A:T-DNA at low strand concentrations.**

It was found exceptionally for A:T-DNA that the melting temperatures become independent of the strand concentrations in the low concentration range (< 4 $\mu$M). This indicates that a hairpin structure is dominantly formed for A:T-DNA at low strand concentrations. Therefore CD melting curves recorded at strand concentrations higher than 15 $\mu$M were used to determine the thermodynamic parameters for duplex formation of

A:T-DNA. Under such strand concentrations, plots of the reciprocal melting temperature versus the logarithm of the strand concentration can fit to a straight line, and the parameters derived from the two methods agreed within about 10%. This guarantees that the determined parameters are those for duplex formation of A:T-DNA.

**RNA duplexes are more stable than the corresponding DNA duplexes.**

As mentioned above, the thermodynamic parameters derived from the two methods agreed within about 10%. Therefore the values on TABLE 2 and 3 which were derived from the method (2) will be cited in the following discussions for simplification. It should be noted that the following discussions are completely valid even if the values on TABLE 1 derived from the method (1) are used.

A:U-RNA is more stable than the corresponding A:T-DNA by 7.1 kcal·mol$^{-1}$ in terms of $\Delta G^0_{37}$ (TABLE 2), and by 14.2 °C in terms of the calculated melting temperature at the strand concentration of 17 $\mu$M (TABLE 3). The situation is the same when the central two Watson-Crick A:U(T) base pairs are replaced by the sheared A:G base pairs. That is, A:G-RNA is more stable than the corresponding A:G-DNA by 2.5 kcal·mol$^{-1}$ and 5.6 °C, respectively.

The RNA duplexes are stabilized more than the corresponding DNA duplexes regarding the $\Delta H^0$ term (-128 v.s. -77.2 kcal·mol$^{-1}$ for the A:U(T) species and -89.0 v.s. -58.3 kcal·mol$^{-1}$ for the A:G species). This could be due to the better stacking interaction achieved generally in RNA of the A form than in DNA of the B form.[38] The extra hydrogen bonding involving 2'-OH in RNA might also be responsible. On the other hand, the RNA duplexes are destabilized more than the corresponding DNA duplexes regarding the $\Delta S^0$ term (-359 v.s. -218 cal·mol$^{-1}$·K$^{-1}$ for the A:U(T) species and -251 v.s. -160 cal·mol$^{-1}$·K$^{-1}$ for the A:G species). The restriction of the conformational freedoms of sugar moieties imposed by 2'-OH in the RNA duplexes could explain this.[38] After all, the greater stability of the RNA duplexes is brought by the $\Delta H^0$ terms surpassing the $\Delta S^0$ terms.

**Difference in the decline in the thermodynamic stability caused by the replacement of the Watson-Crick A:U(T) base pairs by the sheared A:G base pairs between RNA and DNA.**

When the central two Watson-Crick base pairs of A:U-RNA are replaced by the sheared A:G base pairs, resulting in A:G-RNA, the thermo-

dynamic stability of the duplex declines by 5.5 kcal·mol$^{-1}$ and 9.9 °C in terms of $\Delta G^0_{37}$ and the calculated melting temperature at the strand concentration of 17 $\mu$M, respectively (TABLES 2 and 3). By the replacement, the RNA is destabilized regarding the $\Delta H^0$ term by 39 kcal·mol$^{-1}$ (-89.0-(-128)). On the other hand, the RNA is stabilized regarding the $\Delta S^0$ term by 108 cal·mol$^{-1}$·K$^{-1}$ (-251-(-359)). Totally, the RNA is destabilized due to the destabilizing $\Delta H^0$ term surpassing the stabilizing $\Delta S^0$ term by the replacement.

The degree of decline in the thermodynamic stability caused by the replacement of the two Watson-Crick A:T base pairs by the sheared A:G base pairs is much less for the DNA duplex than for the RNA duplex. That is, A:G-DNA is less stable than A:T-DNA just by 0.92 kcal·mol$^{-1}$ and 1.3 °C in terms of $\Delta G^0_{37}$ and the calculated melting temperature at the strand concentration of 17 $\mu$M, respectively. The trend of the effect of the replacement is similar to that observed in the RNA. By the replacement, the DNA is destabilized regarding the $\Delta H^0$ term by 18.9 kcal·mol$^{-1}$ (-58.3-(-77.2)), and stabilized regarding the $\Delta S^0$ term by 58 cal·mol$^{-1}$·K$^{-1}$ (-160-(-218)). The destabilization caused by the replacement concerning the $\Delta H^0$ term is less in the DNA than in RNA (18.9 v.s. 39 kcal·mol$^{-1}$), which is the reason why the decline of the thermodynamic stability caused by the replacement is much less in the DNA duplex.

The difference in the degree of decline in the thermodynamic stability caused by the replacement between RNA and DNA can be well rationalized on the basis of our previous structural studies of A:G-RNA and A:G-DNA.[14,31] The same sheared A:G base pairs are formed in both cases. However, the stacking interactions involving the sheared A:G base pairs are different. It is poor for A:G-RNA, while for A:G-DNA it is as good as that observed in the standard B form DNA with Watson-Crick base pairs. Thus the larger decline in the stability for the RNA may be due to the poorer stacking interaction brought by the replacement.

In this section, the large decline of the thermodynamic stability by the replacement for the RNA has been stressed. However, it should also be noted that A:G-RNA is still more stable than A:T-DNA and A:G-DNA.

The ratio $\Delta H^0/\Delta S^0$ is nearly identical for the four duplexes studied (359±5K). Such enthalpy-entropy compensation has been observed

and has recently been interpreted in terms of the components of the thermodynamic parameters.[38,41] The phenomena seem to be general.

**Comparison with other studies.**

This is the first study where the stability of the sheared A:G base pair is evaluated systematically in terms of the thermodynamic parameters. Comparison is made between RNA and DNA, and between the sheared A:G base pairs and the Watson-Crick A:U(T) base pairs. It is important that the structures of the A:G base pairs in A:G-RNA and A:G-DNA have already been established by NMR studies. Very often thermodynamic studies of the A:G mismatches were carried out without a knowledge on the structures of the base pairs.

Available thermodynamic data on the sheared A:G base pairs are not so systematic in this context.[15,24,39,40,42] The only systematic data which can be compared with our data to some extent are reported by Lane's group. The thermodynamic parameters for $r(CCACGAGUGG)_2$ and $r(CCACUAGUGG)_2$,[38] and the experimental melting temperatures for d(CCACGAGTGG)$_2$ and $d(CCACTAGTGG)_2$ at strand concentration of 17 $\mu$M and salt concentration of 0.1 M[37] are published. And it is established that the sheared A:G base pairs are formed in $r(CCACGAGUGG)_2$[38]. Their sequences are very similar to ours. The sequences of the two residues at both ends are interchanged. In order to make comparison, the melting temperatures at the strand concentration of 17 $\mu$M are calculated on the basis of the published parameters[38] and listed on TABLE 3.

When the melting temperatures are compared between our duplexes and Lane's duplexes, common features are found. A:U-RNA is the most stable among the four duplexes, and its stability decreases when the A:U base pairs are replaced by the sheared A:G base pairs. The stability of the A:T-DNA, on the other hand, does not change a lot by the replacement. Thermodynamic parameters of A:G-DNA and A:T-DNA are not determined for their duplexes. Therefore detailed analysis and discussion concerning the background of the behaviour of the melting temperatures are not possible in their cases.

**Possible formation of the sheared A:G base pairs in RNA such as ribozymes.**

As noted above, in spite of the decline in the thermodynamic stability caused by the replacement of the Watson-Crick A:U base pairs by the sheared A:G base pairs, A:G-RNA is still more stable than A:T-DNA

and A:G-DNA. This suggests that the sheared A:G base pairs could be formed stably in RNA such as ribozymes, and could play some biological role.

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