# Base opening in RNA and DNA duplexes: Implication for RNA stability

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The energetics of a low-energy single base opening in several RNA duplex crystal structures has been calculated and compared to DNA duplexes. Base opening in RNA appears to have an overall preference towards the major groove, similar to results previously reported for B-DNA. Movement of each of the adenine, uracil, and cytosine bases into the minor groove is blocked by a high-energy barrier due to severe close contact with neighboring bases. Guanine bases are able to open towards both grooves because of the unique orientation of the base that avoids steric clash along the opening pathway. RNA bases are found to have a substantially smaller major groove opening extent than that of their B-DNA counterparts. A comparison with base opening behavior of A-DNA duplexes suggests that this difference results from helix constraint associated with A-form backbone conformation. The reduced opening extent correlates with the RNA duplex stability and is consistent with observed slower imino proton exchange rates in RNA duplexes.

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## I. INTRODUCTION

RNA plays important biological roles and is considered as a promising target for the design of novel therapeutic drugs. As a predominantly single-stranded molecule, RNA is known to fold into specific tertiary conformations that contains a variety of secondary structural motifs such as duplex stem, hairpin, internal loop and bulge [1]. The duplex regions are crucial in stabilizing the tertiary structure of RNA. An understanding of how base pairs are stabilized in these regions may shed light on the dynamic behavior of RNA folding process and facilitate the design of RNA binding drugs.

The stability of RNA duplexes has been a subject of numerous studies. Melting experiments on both homopurine-homopyrimidine polymers [2–4] and mixed sequence nucleic acids [5–7] showed that the thermodynamic stability of RNA duplexes are higher than B-DNA duplex. Proton exchange studies also indicated that, under the same buffer conditions, the exchange rates in RNA duplex is substantially lower than that in B-DNA, particularly at higher temperatures [8]. The higher stability in RNA has been found to be enthalpy driven [9]. However the underlying microscopic mechanism remains elusive. It is unclear whether the higher stability is due to A-form related backbone constraints, or it results from differences in base-base interactions, or both.

Insight into duplex stability may be obtained by investigating base pair opening processes. Imino proton exchange studies indicate that, at premelting temperatures, base pair opens one at a time [10]. A recent molecular dynamics simulation study also indicated that the base pair opening process is a localized event [11]. Therefore, investigation of low energy single base opening events is a useful step towards the understanding of the microscopic mechanism of duplex stability.

A number of modeling studies have been carried out to probe bond motions involved in base opening. The flexible nature of $\zeta$ [C3′-O3′-P-O5′] torsion and the predominant role it might play in base opening process have been implicated in the early modeling studies [12,13]. These studies also indicated that the relevant backbone motions are localized within a dinucleotide segment. $\zeta$ has been shown to be the most flexible backbone torsion angle based on normal-mode analysis of DNA duplexes [14]. This torsion angle has been established as the principal torsion and its motion as a constructive mode in promoting base opening [15]. The low-energy opening pathway has been shown to involve the displacement of the $\zeta$ torsion along with the variation of a small number of other local torsion angles without distortion in bond length and bond angle and without perturbation of the rest of the duplex. The relevance of this pathway with observed base pair opening processes have also been documented [16]. For unusual equilibrium $\zeta$ values, abnormal opening behavior have been found over a wide range of B-DNA duplex sequences in a comprehensive study of available crystal structures [17].

In this paper we examine a low-energy base opening in RNA duplex crystal structures and compare the results with that of B-DNA and A-DNA duplexes with similar sequences. The difference in the opening behavior of bases in these duplexes have been analyzed and the correlation of this difference with the observed higher stability in RNA will be discussed. The effect of backbone constraint on the opening behavior will also be described.

## II. METHODS

### A. Structure

The crystallographic coordinates of RNA and DNA duplexes studied in this work have been obtained from the nucleic acids database (NDB) [18]. The sequence ID and the corresponding references [9,19–24] are listed in Table I. The DNA duplexes have been selected because a significant portion or entire sequence matches that of the respective RNA counterpart.

### B. Modeling of single base opening

The opening of a base is accomplished through simultaneous rotation of local backbone and glycosidic bond torsion angles without distortion in bond length and bond angle. We

 

TABLE I. RNA and DNA duplexes studied in the present paper.

| Duplex Sequence | NDB ID | Reference |
|---|---|---|
| $r(CCCCGGGG)_2$ | arh074 | [9] |
| $r(UAAGGAGGUGAU)_2$ | arl062 | [19] |
| $r(UUAUAUAUAUAUAA)_2$ | arn035 | [20] |
| A-form $d(CCCCGGGG)_2$ | adh012 | [21] |
| B-form $d(ACCGCCGGCGCC)_2$ | bdj036 | [22] |
| B-form $d(ACCGGCGCACA)_2$ | bdl035 | [23] |
| B-form $d(CGATATATCG)_2$ | bdl018 | [24] |

have found that a low-energy opening is possible by rotation of only six torsion angles related to the rotated base, while the rest of the helix is held rigid [15,16].

In order for a base to move freely out of the stacked helix, its effective rotation axis must be parallel to the helix axis. Our analysis on both single and multiple torsion angle displacements indicated that only $\zeta$ and $\delta$ [C5'-C4'-C3'-O3'] torsion angles satisfy this criterion. However, unless the sugar pucker changes significantly, the rotation of $\delta$ causes bond angle distortion in C5'-C4'-O4' or O3'-C3'-C2'. Hence $\zeta$ is the only energetically feasible torsion angle to promote base opening.

The $n$th base can be rotated towards both the major and minor grooves by the rotation of its $\zeta_n$ torsion angle. The rotation is made by fixing the lower O5' [in the $(n+1)$]th nucleotide] end while rotating the upper C3' end. Such an operation however causes the entire strand above the $n$th nucleotide to swing out of the double helix. If the strand above $(n-1)$th nucleotide is kept rigid, then the section between C3' of the $n$th nucleotide and C3' of the $(n-1)$th nucleotide gets displaced. This results in the shift of the C3' atom in the $(n-1)$th nucleotide away from its original position. Hence, the adjustment of the other backbone torsion angles along this section is necessary so as to move the C3' atom back close to its original position.

A low-energy base opening pathway that involves the adjustment of a minimum number of backbone torsions has been determined [16]. The adjusted backbone torsion angles are $\zeta_{n-1}$ (for the $n-1$ nucleotide), $\alpha_n$ [O3'-P-O5'-C5'], $\beta_n$ [P-O5'-C5'-C4'], and $\gamma_n$ (O5'-C5'-C4'-C3'). These torsion angles can be regarded as adjustable variables controlled by helix restoring force and they are determined by a search in the multidimensional torsion angle space to find a set of values that can move the C3' [of the $(n-1)$th nucleotide] back close to its original position. An angular grid of 0.5° spacing is used to describe the torsion angle search space. The set of the torsion angles that gives the minimum deviation of the C3' coordinates in the $(n-1)$th nucleotide will be adapted as the modified backbone torsion angles. The $\delta_n$ and $\epsilon_n$ [C4'-C3'-O3'-P] torsion are fixed so as to keep the sugar in its original (A or B) form. Although the use of a finite grid size gives rise to a finite displacement in the computed new position of the C3' atom, the grid size employed here seems to be optimum to yield converged results. A reduction of 50% in grid size results in $<10\%$ change in the computed opening extent.

The backbone geometric restraint sets a limit on the extent of $\zeta_n$ rotation beyond which the C3' of $(n-1)$th nucleotide deviates from its original position. This limit can be estimated based on the comparison of the deviation of the C3' position with grid tolerance [16]. The energy barrier along the rotation pathway may further limit the extent of base opening. Thus, the maximum extent of base opening is determined by both the backbone geometric restraint and the energy barrier along the grooves.

The rotation of $\zeta_n$ also induces a change in the roll angle $\rho_n$ of the $n$th base. In order to keep the base in plane, its glycosidic bond torsion angle $\chi_n$ needs to be adjusted. This can be done by an energy minimization procedure to find an angle that gives the best local minimum energy while keeping the rest of the duplex rigid.

### C. Potential energy calculation

The total potential energy barrier for base rotation is computed using the empirical functional form:

$$V=\sum_{torsion}\frac{V_n}{2}\{1+\cos[n\phi-\gamma]\}+\sum_{\mathrm{H}\ bonds}\{V_0[1-e^{-a(r-r_0]})^2-V_0\}+\sum_{nonbonded}\left[\frac{A_{ij}}{r_{ij}^{12}}-\frac{B_{ij}}{r_{ij}^{6}}+\frac{q_iq_j}{\epsilon r}\right], \quad (1)$$

where $\phi$ is a torsion angle, $V_n$,$n$, and $\gamma$ are torsion parameters; $r$ is donor-acceptor distance of a hydrogen bond, $V_0$, $a$, and $r_0$ are hydrogen bond parameters; $r_{ij}$ is the distance between two nonbonded atoms, $A_{ij}$ and $B_{ij}$ are van der Waals parameters, $q_i$ and $q_j$ are partial charges for the two atoms respectively and $\epsilon$ is the dielectric constant.

The torsion terms are included to account for the energy cost involved in the rotation of the backbone and glycosidic bond torsion angles. With the exception of the hydrogen bond terms, the potentials and their parameters are taken from the AMBER force field [25]. The stacking interactions are implicitly included in the nonbonded van der Waals and electrostatic energy terms. A distance-dependent dielectric constant [26] $\epsilon$ is used to compute electrostatic energy. The contribution of electrostatic energy to the total energy is relatively small and thus our computation is relatively insensitive to the choice of dielectric constant.

To avoid the difficulty of modeling the dynamics of hydrogen atoms, an empirical implicit hydrogen atom potential is used in this work. This potential, based on a Morse function of the donor and acceptor separation, has been shown to fit well with the potential energy obtained from *ab initio*

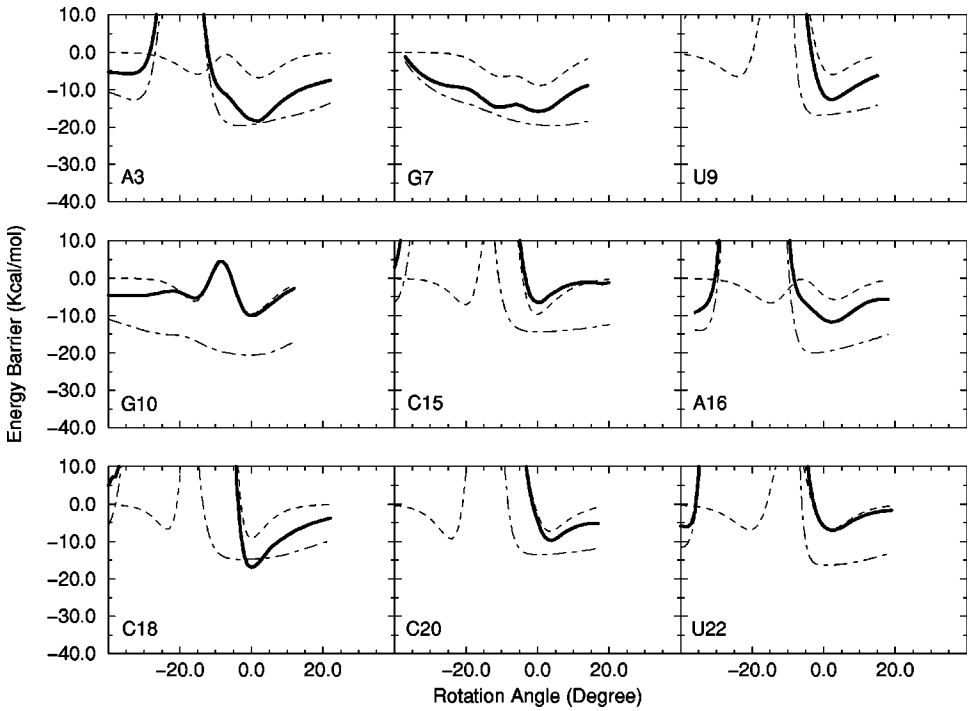


FIG. 1. The energy barrier for the opening of the randomly selected bases (identified by its residue number on each panel) along one strand of the RNA duplex $r[UAAGGAGGUGAU]_2$. The solid line is the total energy, the dash-dotted line is the van der Waals energy, and the long-dashed line is the hydrogen bond energy.

calculations [27] and to give fair account of observed premelting and melting behavior of interbase hydrogen bonds in DNA [28,29].

## III. RESULTS AND DISCUSSION

### A. Base opening profile in RNA

The main features of a single base opening in RNA can be illustrated from the profile of the opening pathway and energetics of the bases in $r[UAAGGAGGUGAU]_2$. Similar features are found in other RNA structures investigated in the present study. Figure 1 shows the energy profile for the rotation of each of the several selected bases in $r[UAAGGAGGUGAU]_2$. It can be seen that energy barriers in the minor groove of these bases are higher than those in the major groove. The rotation of each of the pyrimidine bases towards the minor groove immediately encounters a high-energy barrier due to a severe steric clash with its complementary base. The motion of each of the adenine bases towards the minor groove is restricted to $\sim 10°$ because of high-energy barrier along the groove. This leaves the major groove rotation as the only pathway for the opening of adenine, uracil, and cytosine bases. Although no large energy barrier is found along the major groove, the maximum extent of the major groove opening $-\Delta\zeta_{max}^{major}$ of each of these bases is limited to only $\sim 17°$ due to the geometrical constraint of the backbone.

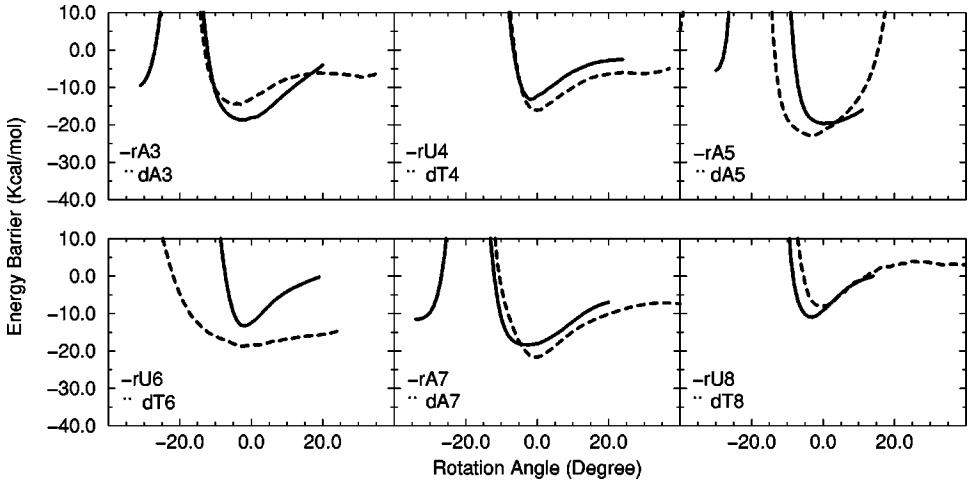


FIG. 2. Comparison of the energy barrier for the opening of the selected bases (identified by its residue number on each panel) along one strand of the RNA duplex $r[UUAUAUAUAUAUAA]_2$ and those of the B-DNA duplex $d[CGATATATCG]_2$. The solid line is the total energy for a RNA base and the dashed line for a B-DNA base.

TABLE II. Backbone torsion angles and the opening extent of the adenine and uracil bases in RNA duplex $r[UUAUAUAUAUAUAA]_2$ and the adenine and thymine bases in B-DNA duplex $d[CGATATATCG]_2$. The rotation of the $\zeta_{T6}$ in $T_6$ causes its sugar to flip without opening the base towards either grooves. The extent of sugar rotation for this base is marked by brackets to distinguish it from the base opening extent. The negative sign for $\Delta\zeta$ is introduced so that the positive (negative) displacement correlates to major (minor) groove opening.

| Duplex | Base | Backbone torsion angles (°) | | | | | | Opening extent (°) | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\zeta$ | $\alpha$ | $\beta$ | $\gamma$ | $\delta$ | $\epsilon$ | $-\Delta\zeta_{max}^{minor}$ | $-\Delta\zeta_{max}^{major}$ |
| $r[UUAUAUAUAUAUAA]_2$ | $A_3$ | −68.6 | −74.7 | 174.4 | 300.7 | 283.7 | −162.1 | −13 | 19 |
| | $U_4$ | −86.3 | −57.2 | 189.7 | 304.0 | 276.8 | −162.4 | −8 | 23 |
| | $A_5$ | −73.1 | −70.6 | 192.1 | 290.1 | 284.1 | −141.7 | −9 | 10 |
| | $U_6$ | −66.3 | −62.8 | 179.4 | 307.5 | 279.4 | −151.7 | −8 | 18 |
| | $A_7$ | −71.4 | −77.6 | 168.7 | 299.3 | 282.5 | −158.6 | −13 | 19 |
| | $U_8$ | −80.9 | −85.5 | 164.7 | 294.0 | 274.8 | −154.9 | −9 | 13 |
| $d(CGATATATCG)_2$ | $A_3$ | −87.0 | −64.1 | 190.3 | 306.8 | 231.7 | −179.1 | −6 | 36 |
| (B-form) | $T_4$ | −106.8 | −63.7 | 188.6 | 305.3 | 251.4 | −179.6 | −7 | 37 |
| | $A_5$ | −97.2 | −54.5 | 188.8 | 309.0 | 232.9 | −162.3 | −14 | 17 |
| | $T_6$ | −189.4 | −59.2 | 188.1 | 321.3 | 218.0 | −113.4 | [−24] | (24) |
| | $A_7$ | −82.8 | −61.0 | 214.2 | 320.2 | 226.7 | −188.3 | −11 | 56 |
| | $T_8$ | −90.1 | −57.5 | 187.1 | 314.6 | 246.2 | −191.6 | −8 | 60 |

The energy barrier in the minor groove of each of the guanines is significantly lower than in the other bases. The barrier height of ~20 Kcal/mol is in the range of the observed base pair opening activation enthalpies [8,30]. Thus, it is possible for guanine bases to open towards the minor groove. The lower energy barrier in the minor groove results from a unique orientation of the O6-C6 bond in guanine that avoids close contact with neighboring bases along the minor groove opening pathway [16]. While guanine bases can be opened towards both grooves, statistically there is an overall preference for RNA bases to open towards major groove.

### B. Comparison with B-DNA

Figure 2 shows the comparison of the opening profile of several bases in the central *AUAUAUA* region of RNA duplex $r[UUAUAUAUAUAUAA]_2$ with their counterparts in the central *ATATAT* region of B-DNA duplex $d[CGATATATCG]_2$. The maximum extent of the opening along with backbone torsion angles for these two duplexes are also given in Table II. It is noted that the rotation of the $\zeta_{T6}$ in $T_6$ causes its sugar to flip without opening the base towards either grooves. This arises because $\zeta_{T6}$ has an unusual value of −189.4°. Our previous analysis on a variety of crystal structures with different sequences indicated that this is a universal behavior for bases with equilibrium $\zeta$ falling into the range of ~−200° [17].

Substantial difference is found in $-\Delta\zeta_{max}^{major}$ between bases in RNA and B-DNA. Both adenine and uracil bases in the RNA duplex have substantially smaller $-\Delta\zeta_{max}^{major}$ values than the corresponding bases in the B-DNA duplex. This behavior is not only limited to AU pairs. As can be seen in Table III, which gives the comparison between RNA duplex $r[CCCCGGGG]_2$ and B-DNA duplex $d(ACCGCCGGCGCC)_2$ and $d[ACCGGCGCACA]_2$, the $-\Delta\zeta_{max}^{major}$ of both guanine and cytosine bases are consistently smaller than those in the B-DNA duplexes.

In contrast, the profile of the minor groove rotation of the RNA bases is largely similar to B-DNA. The minor groove opening extent $-\Delta\zeta_{max}^{minor}$ of uracil and cytosine bases is limited to ~8° due to steric clash with its complementary base. The $-\Delta\zeta_{max}^{minor}$ for adenine is ~12° due to high-energy barrier in the minor groove. Similar opening extent was found for adenine, thymine and cytosine bases in B-DNA [16]. On the other hand, the energy barrier of guanines along minor groove is relatively small. As a result these bases are able to open towards the minor groove to a maximum extent of ~40° which is comparable to that of guanine bases in B-DNA [16].

A substantially smaller opening extent in three out of four types of bases does not seem to hinder the possibility for imino proton exchange in RNA duplexes. From Tables II and III, one finds the maximum translational displacement of adenine, uracil and cytosine bases are ~2 Å. A geometric analysis shows that such a displacement creates an open space on the minor groove side, which is sufficient to allow a water molecule to pass through to the site of imino proton on either uracil or guanine.

Some insight into the cause of the difference in $-\Delta\zeta_{max}^{major}$ between RNA and B-DNA can be gained from the comparison of their equilibrium backbone torsion angles listed in Tables II and III. With the exception of $\delta$, substantial difference is found between a torsion angle in RNA and its counterpart in B-DNA. The variation of backbone torsion angles in RNA is relatively small and insensitive of the base type, which gives rise to a relatively uniform major groove opening extent. On the other hand, the torsion angles in B-DNA vary substantially, resulting in rather large variation in the major groove opening extent. Our analysis indicates a close correlation between $-\Delta\zeta_{max}^{major}$ and $\epsilon$. In general, relatively large $-\Delta\zeta_{max}^{major}$ occurs if $\epsilon < -170°$, which is the case for most bases in B-DNA. In contrast, $\epsilon$ of most bases in RNA falls in the range of −150°, thus giving rise to a smaller $-\Delta\zeta_{max}^{major}$.

The torsion angle $\epsilon$ is not the only factor for $-\Delta\zeta_{max}^{major}$. For instance, $-\Delta\zeta_{max}^{major}$ of the $G_7$ base in A-form $d[CCCCGGGG]_2$ is 18° even though its $\epsilon = -172°$. This

TABLE III. Backbone torsion angles and the opening extent of the guanine and cytosine bases in RNA duplex $r[CCCCGGGG]_2$, in B-DNA duplexes $d[ACCGCCGGCGCC]_2$ $d(ACCGGCGCACA)_2$, and in A-DNA duplex $d[CCCCGGGG]_2$.

| Duplex | Residue | Backbone dihedral angles (°) | | | | | | Opening extent (°) | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\zeta$ | $\alpha$ | $\beta$ | $\gamma$ | $\delta$ | $\epsilon$ | $-\Delta\zeta_{max}^{minor}$ | $-\Delta\zeta_{max}^{major}$ |
| $r[CCCCGGGG]_2$ | $C_2$ | −71.3 | −73.5 | 189.1 | 299.5 | 284.3 | −150.8 | −5 | 12 |
| | $C_3$ | −70.8 | −71.5 | 182.8 | 301.8 | 281.7 | −147.6 | −5 | 15 |
| | $C_4$ | −72.7 | −64.7 | 191.7 | 304.4 | 282.5 | −155.4 | −6 | 18 |
| | $G_5$ | −71.3 | −67.5 | 180.8 | 306.8 | 284.6 | −149.1 | −29 | 16 |
| | $G_6$ | −71.4 | −61.2 | 196.8 | 301.2 | 286.6 | −152.4 | −35 | 19 |
| | $G_7$ | −69.7 | −70.7 | 190.3 | 300.6 | 285.8 | −159.3 | −35 | 17 |
| $d[ACCGCCGGCGCC]_2$ | $C_5$ | −90.0 | −32.3 | 191.1 | 317.0 | 226.6 | −176.6 | −11 | 43 |
| (B-form) | $C_6$ | −144.3 | −55.8 | 171.6 | 316.9 | 218.0 | −156.4 | −9 | 23 |
| | $G_7$ | −125.3 | −47.9 | 212.0 | 309.2 | 217.9 | −172.0 | −52 | 18 |
| | $G_8$ | −110.1 | −54.5 | 194.9 | 307.1 | 219.7 | −161.7 | −35 | 24 |
| $d(ACCGGCGCACA)_2$ | $C_2$ | −93.8 | −136.6 | 170.6 | 232.5 | 252.0 | −162.3 | −8 | 5 |
| (B-form) | $C_3$ | −34.7 | −29.4 | 205.2 | 337.7 | 274.5 | −211.1 | −29 | 60 |
| | $G_4$ | −129.6 | −67.6 | 145.6 | 332.6 | 227.8 | −196.8 | −60 | 60 |
| | $G_5$ | −104.5 | −313.7 | 215.8 | 36.5 | 195.5 | −182.6 | −14 | 43 |
| $d(CCCCGGGG)_2$ | $C_2$ | −68.2 | −51.4 | 204.6 | 310.9 | 278.1 | −160.8 | −6 | 22 |
| (A-form) | $C_3$ | −60.2 | −74.6 | 177.9 | 296.2 | 275.2 | −163.8 | −7 | 24 |
| | $C_4$ | −75.4 | −77.3 | 184.4 | 300.3 | 279.0 | −161.0 | −5 | 15 |
| | $G_5$ | −63.3 | −213.9 | 180.5 | 167.4 | 269.8 | −157.3 | −25 | 39 |
| | $G_6$ | −74.8 | −95.1 | 179.1 | 286.2 | 278.8 | −139.8 | −32 | 8 |
| | $G_7$ | −66.7 | −75.9 | 192.4 | 280.6 | 281.3 | −171.9 | −23 | 18 |

results from an increase of its $\beta$ torsion angle from normal value of 190° to 212°. Therefore, $-\Delta\zeta_{max}^{major}$ can be changed by deviation of $\beta$ from its normal range. Similarly, large variation in other torsion angles, such as $\alpha$ and $\gamma$, can also affect $-\Delta\zeta_{max}^{major}$. An example is the $G_5$ base in A-form $d[CCCCGGGG]_2$ which will be discussed in the next section.

The maximum extent of base opening in all the duplexes examined in this work is primarily determined by backbone geometric restraint. This restraint is imposed by the structural features of the backbone. As a result there is close correlation between the equilibrium backbone torsion angles and base opening extent. This inter-relationship can be further demonstrated from a comparative study of RNA and A-DNA duplexes.

### C. Comparison with A-DNA

RNA and A-DNA duplexes share similar characteristics of an A-form backbone with only moderate differences in the backbone torsion angles. It is therefore of interest to compare the base opening behavior in these duplexes. The comparison between corresponding bases in RNA duplex $r[CCCCGGGG]_2$ and A-DNA duplex $d[CCCCGGGG]_2$ is presented in Fig. 3. The opening extent along with back-

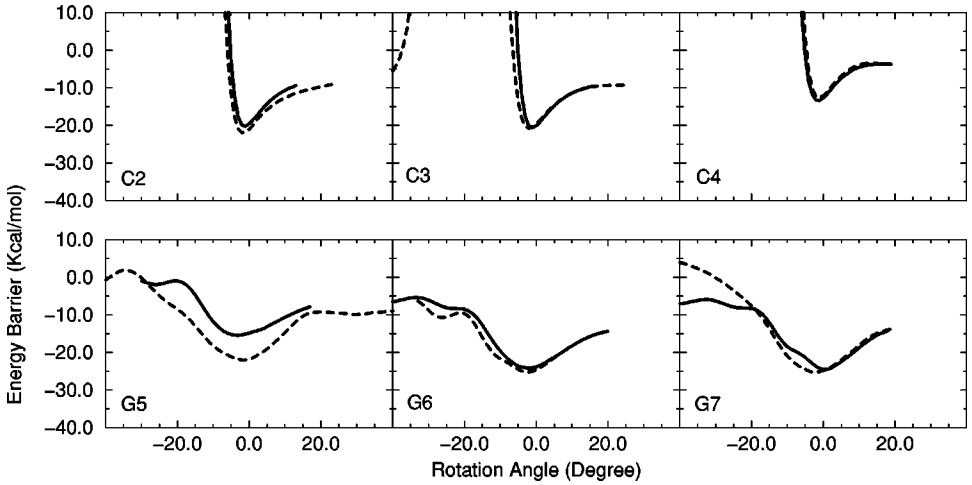


FIG. 3. Comparison of the energy barrier for the opening of the selected bases (identified by its residue number on each panel) along one strand of the RNA duplex $r[CCCCGGGG]_2$ and those of the A-DNA duplex $d[CCCCGGGG]_2$. The solid line is the total energy for a RNA base and the dashed line for a A-DNA base.

bone torsion angles are also included in Table III. One finds that the energy barrier for base opening is indeed very similar, which results from similarity in base pair orientation and stacking arrangement within the A-conformation family. However a slightly varied opening extent $-\Delta\zeta_{max}^{major}$ is found for the bases in the A-DNA duplex. Both $C_2$ and $C_3$ have slightly larger $-\Delta\zeta_{max}^{major}$ than their RNA counterpart. A smaller $-\Delta\zeta_{max}^{major}$ is found for the A-DNA $G_6$ base. The variation arises from the deviation in the $\alpha$ and $\beta$ angles.

The $-\Delta\zeta_{max}^{major}$ for the $G_5$ in the A-DNA duplex $d[CCCCGGGG]_2$ is 39°, which seems to be abnormally large as compared with the value for each of the other bases. This arises because its backbone torsion angles $\alpha$ and $\gamma$ are significantly different from the normal range adopted by other bases in either A-DNA or RNA duplexes listed in Table III.

## IV. CONCLUDING REMARKS

With a similar opening behavior towards the minor groove, and with the minor groove pathway blocked for three out of four types of bases, the difference in major groove opening behavior has direct implication on the difference in the stability between RNA and B-DNA duplexes. Our finding of the smaller major groove opening extent in RNA duplexes may provide an explanation to the observed higher thermal stability in RNA duplexes. Melting experiments consistently showed that the thermodynamic stability of RNA duplexes are higher than B-DNA duplex [2–7]. This higher stability was found to be enthalpy driven [9]. The difference in the enthalpy between RNA and B-DNA duplex is ~4 Kcal/mol per base pair. This may account for the extra energy needed to displace additional backbone torsions in the neighboring nucleotides to allow for the same scope of opening (~30° rotation) as that in B-DNA duplexes.

The smaller opening extent is also consistent with the observed slower proton exchange rate in RNA duplexes as compared with that in B-DNA duplexes under the same buffer conditions [8]. Although a ~2 Å base displacement towards the major groove is sufficient to allow the solvent water to access the imino protons, the smaller cavity created in the minor groove limits the freedom of the water movement considerably. As a result a slower exchange rate is expected.

Our study suggests that tighter constraint on the motion of backbone bonds may contribute to the higher stability in RNA duplexes. More insights into duplex stability can be explored by investigation of multi-base opening processes. Motions along the opening pathway may be further examined by other methods such as normal mode analysis and molecular dynamics. Studies along these lines are in progress.


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