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Contribution of 3′T and 3′TT overhangs to the thermodynamic stability of model siRNA duplexes

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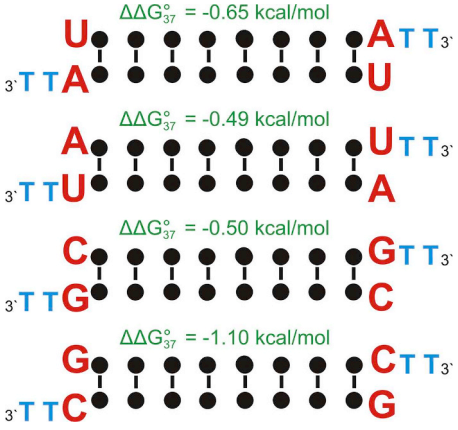
HIGHLIGHTS

- The energetic contribution of 3′T and 3′TT dangling ends within RNA duplexes has been determined
- The thermodynamic effect of 3′T overhangs strongly depends on the type and orientation of the adjacent base pair
- Further extension of the 3′-dangling end length, by a second T residue, results in additional stabilization
- The effect of 3′-dangling TT on RNA differs from the effect of 3′-dangling UU or TT on RNA or DNA, respectively

GRAPHICAL ABSTRACT



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ABSTRACT

Herein, we report comprehensive thermodynamic studies on 36 RNA/DNA duplexes designed as siRNA mimics to determine the energetic contribution of 3′T and 3′TT dangling ends. The thermodynamic effect induced by the presence of 3′T overhangs on the stability of RNA duplexes ranges from −0.28 to −0.92 kcal/mol and strongly depends on the type and orientation of the adjacent base pair. Further extension of the 3′-dangling end length, by a second T residue, results in additional stabilization of 0.14 to 0.21 kcal/mol. The results revealed that the thermodynamic contribution of 3′-dangling T and TT on RNA duplexes differs from the influence of 3′-dangling U and UU on RNA duplexes and 3′-dangling T and TT on DNA duplexes. This data suggests that using the contribution of 3′-dangling T values for RNA duplexes, instead of 3′-dangling T values for DNA duplexes or 3′-dangling U values for RNA duplexes, would improve the prediction of the stability of siRNA duplexes.

1. Introduction

The discovery of RNA interference (RNAi) by Fire et al. expanded the knowledge about post-transcriptional regulation of gene expression and opened the way for the design of new therapeutics based on siRNA molecules [1]. The strategy of using siRNA molecules is widely applied to silence the expression of selected genes [2–4].

It is likely that therapeutics based on siRNA and miRNA as

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biopharmaceuticals will be used in the future as drugs for various diseases. Clinical trials using RNAi as a treatment in humans are still ongoing. Some of the drugs based on siRNA molecules successfully passed phases I and II of clinical trials [5]. Several important features, such as pharmacodynamics, pharmacokinetics, absorption, metabolism, distribution, and excretion, have to be considered when designing functional siRNA molecules as pharmaceuticals [5]. The most popular therapeutic target towards which scientists use siRNA molecules is cancer [5], including breast cancer, melanoma, lung cancer, prostate cancer, and colon cancer [5].

There are many computer programs that help in the design of active siRNA molecules [6]. Most of these programs agree on the main features required for the design of active siRNA molecules. A well designed siRNA should have a non-self-complementary sequence [7,8], more A/U pairs at the 5'-end of the antisense strand and more G/C pairs at the 3'-end of the sense strand [9], generally weaker bonds at the 5'-end of the antisense strand [7,9,10], no internal repeats [8,9], nucleotide A at the sixth position of the antisense strand [7], nucleotide A at the third and nineteenth position of the sense strand [8], no G nucleotide at the thirteenth position, the absence of G/C nucleotides at the nineteenth position of the sense strand [8,10], and the presence of U in the ninth position of the sense strand of the siRNA duplex [8,10]. However, the use of Ui-Tei rules (part of the rules described above: more A/U pairs at the 5'-end of the antisense strand and more G/C pairs at the 3'-end of the sense strand, generally weaker bonds at the 5'-end of the antisense strand, no internal repeats) [9] for the design of a number of siRNA molecules resulted in 98% of the siRNA molecules silencing below 33%, suggesting that the rules need to be improved. Many of the parameters used in the programs for siRNA design are based on the difference in thermodynamic stability of the siRNA duplex ends [11,12]. Overhangs, both single and double nucleotides, affect thermodynamic stability of RNA and may be another feature to consider when designing siRNA molecules.

Thermodynamic data show that sequence of dangling ends and type and orientation of the duplex closing base pair affect the thermodynamic stability of RNA and DNA duplexes [13,14]. In general, it was shown that 3′-dangling ends in RNA are more stabilizing than in DNA [15,16]. In addition, there are also some significant differences in the influence of 5'- and 3'-overhangs on the thermodynamic stability of RNA and DNA. It was reported that DNA overhangs at the 5' end are more energetically favorable than the ones situated at the 3'-end, whereas for RNA dangling ends, the effect is reversed [13,17]. All thermodynamic studies performed so far focus on the contribution of RNA dangling ends on RNA duplexes or DNA overhangs on DNA duplexes. The thermodynamic parameters derived from the above studies are currently used to estimate thermodynamic stability of siRNAs that are usually designed with DNA TT 3'-dangling ends on RNA duplexes, since data is not available for DNA dangling ends on RNA duplexes. If the energetic contribution of DNA overhangs on RNA is different from DNA overhangs on DNA and RNA overhangs on RNA, then it is essential to provide detailed thermodynamic parameters for DNA overhangs on RNA duplexes.

In this paper, we report the thermodynamic analysis of 3′T and 3′TT DNA dangling ends on model siRNA duplexes and their influence on siRNA stability depending on type and orientation of the closing Watson-Crick RNA base pair, *i.e.* A-U, U-A, G-C, or C-G. This data can now be used to improve design of siRNA for therapeutic uses.

2. Materials and methods

2.1. Oligonucleotide synthesis

Oligoribonucleotides were synthesized on an automated DNA/RNA synthesizer, using β-cyanoethyl phosphoramidite chemistry [18]. The details of deprotection and purification of oligoribonucleotides were described previously [19]. The composition of all oligonucleotides was confirmed using MALDI–TOF mass spectrometry.

2.2. UV melting studies

Oligonucleotides were melted in buffer containing 100 mM NaCl, 20 mM sodium cacodylate, 0.5 mM Na_2EDTA, pH 7.0. The relatively low NaCl concentration resulted in melting temperatures in the range which allowed for melting curves with upper and lower baselines. Oligonucleotide single-strand concentrations were calculated from high temperature absorbances, and single-strand extinction coefficients were approximated by a nearest-neighbor model. Absorbance *versus* temperature melting curves were measured at 260 nm with a heating rate of 1 °C/min from 0 to 90 °C on a Beckman DU 640 spectrophotometer with a water cooled thermoprogrammer. Melting curves were analyzed, and thermodynamic parameters were calculated using a two-state model within MeltWin 3.5 software. For all sequences, the ΔH° value derived from T_m^{-1} versus ln ($C_T/4$) plots was within 15% of the ΔH° value derived from averaging the fits of individual melting curves, as expected if the melting proceeds according to two-state model (Table S1).

3. Results and discussion

Specific interactions between nucleoside residues allow for designing of oligonucleotides that are model systems for structural motifs formed within nucleic acids. Comprehensive thermodynamic analysis of such model molecules provides thermodynamic parameters of folding that can be divided into thermodynamic contribution of particular base pairs. Nearest neighbor parameters facilitate understanding of the complicity of separate base pairs in the stability of structural motifs and are irreplaceable in accurate calculations of thermodynamic stability as well as in prediction of nucleic acid secondary structures. The nearest neighbor model was described for the first time in the 1980's by Turner and co-workers [20–22]. Since then, a significant amount of thermodynamic data concerning Watson-Crick canonical base pairs, mismatches, as well as 5′- and 3′-dangling ends have been published [16,23–28]. The literature data indicate that the presence of 3′-dangling ends significantly increases thermodynamic stability of DNA and RNA duplexes [13,29–33]. Nevertheless, there are substantial differences in the contribution of DNA and RNA unpaired nucleotides on the thermodynamic stability of DNA and RNA duplexes, respectively. One of the most important differences is the magnitude of stabilization contributed by 3′-dangling ends, since such motifs stabilize RNA duplexes more than DNA duplexes. In spite of the fact that comprehensive thermodynamic analyses were already performed for 3′-unpaired nucleotides within DNA and RNA duplexes [13,29], there is still a lack of data for 3′-overhangs of DNA nucleotides on RNA duplexes, such as seen in model siRNA duplexes.

In this study, oligonucleotides were designed to mimic siRNA duplexes, *i.e.* they consist of an RNA core with 3′T or 3′TT overhangs (Table 1). The sequences were self-complementary, which resulted in lower errors in the 3′-dangling end contribution [13]. All of the oligonucleotides were designed to eliminate the possibility of folding into competing structures, *e.g.* slipped duplexes. The thermodynamic effects of the 3′T and 3′TT dangling ends were determined based on type (A-U or G-C) and sequence context (A-U or U-A) of the adjacent Watson-Crick RNA base pair and were an average of the effects obtained for three duplexes with different core sequences but the same 3′-dangling end motif.

The energetic contribution of a 3′T dangling end was calculated based on experimental values, according to the equation:

$$\Delta\Delta G^\circ_{37}\begin{pmatrix}XT\\Y\end{pmatrix} = \frac{1}{2}\left[\Delta G^\circ_{37(\text{duplex with}\begin{pmatrix}XT\\Y\end{pmatrix})} - \Delta G^\circ_{37(\text{core duplex})}\right] \quad (1)$$

Table 1
Thermodynamic parameters of duplex formation.[a]

Duplexes (5′-3′)	T_M^{-1} *vs* log C_T plots				
	$-\Delta H^\circ$ (kcal/mol)	$-\Delta S^\circ$ (eu)	$-\Delta G^\circ_{37}$ (kcal/mol)	T_M[b] (°C)	$\Delta\Delta G^\circ_{37}$[c] (kcal/mol)
UUCUGCAGA**A**	79.5 ± 1.8	223.1 ± 5.6	10.34 ± 0.10	56.3 ± 1.9	0
UUCUGCAGA**AT**	89.9 ± 1.5	253.3 ± 4.4	11.36 ± 0.07	57.9 ± 1.4	−1.02 ± 0.12
UUCUGCAGA**ATT**	86.7 ± 5.6	242.3 ± 17.2	11.55 ± 0.30	59.6 ± 5.7	−1.21 ± 0.32
UUGUGCACA**A**	76.0 ± 2.8	210.8 ± 8.4	10.63 ± 0.16	58.6 ± 3.2	0
UUGUGCACA**AT**	85.6 ± 2.0	238.9 ± 6.0	11.49 ± 0.11	59.6 ± 2.0	−0.86 ± 0.19
UUGUGCACA**ATT**	89.6 ± 1.4	250.1 ± 4.2	11.97 ± 0.08	60.5 ± 1.4	−1.34 ± 0.17
UGUUGCAAC**A**	79.2 ± 1.5	221.4 ± 4.5	10.52 ± 0.07	57.2 ± 1.6	0
UGUUGCAAC**AT**	85.6 ± 1.6	238.7 ± 4.8	11.62 ± 0.09	60.1 ± 1.6	−1.10 ± 0.12
UGUUGCAAC**ATT**	96.8 ± 1.7	273.6 ± 5.3	11.88 ± 0.09	58.3 ± 1.5	−1.36 ± 0.12
GCUUGCAAG**C**	98.8 ± 3.6	276.5 ± 10.8	13.08 ± 0.23	62.1 ± 3.3	0
GCUUGCAAG**CT**	107.1 ± 2.3	296.9 ± 6.9	15.00 ± 0.19	66.6 ± 2.1	−1.92 ± 0.29
GCUUGCAAG**CTT**	109.2 ± 3.3	302.8 ± 9.8	15.26 ± 0.26	66.9 ± 3.0	−2.18 ± 0.35
GUCUGCAGA**C**	100.3 ± 3.1	279.5 ± 9.2	13.58 ± 0.21	63.5 ± 2.8	0
GUCUGCAGA**CT**	105.6 ± 2.9	291.1 ± 8.6	15.33 ± 0.24	68.2 ± 2.8	−1.75 ± 0.32
GUCUGCAGA**CTT**	110.7 ± 4.0	306.1 ± 11.7	15.75 ± 0.32	68.1 ± 3.6	−2.17 ± 0.38
GGUUGCAAC**C**	91.4 ± 4.3	253.6 ± 13.0	12.74 ± 0.27	63.0 ± 4.4	0
GGUUGCAAC**CT**	105.2 ± 4.0	292.2 ± 11.7	14.61 ± 0.32	65.8 ± 3.6	−1.87 ± 0.42
GGUUGCAAC**CTT**	105.0 ± 5.2	290.2 ± 15.5	14.96 ± 0.42	67.1 ± 4.9	−2.22 ± 0.50
CAUUGCAAU**G**	89.3 ± 2.2	259.7 ± 6.8	8.79 ± 0.05	48.2 ± 1.7	0
CAUUGCAAU**GT**	89.7 ± 2.5	258.8 ± 7.7	9.43 ± 0.08	50.6 ± 2.1	−0.64 ± 0.09
CAUUGCAAU**GTT**	91.6 ± 1.4	264.2 ± 4.2	9.67 ± 0.04	51.1 ± 1.1	−0.88 ± 0.07
CAAUGCAUU**G**	81.4 ± 4.5	234.3 ± 14.1	8.76 ± 0.12	49.2 ± 4.0	0
CAAUGCAUU**GT**	89.8 ± 2.8	258.8 ± 8.8	9.51 ± 0.10	50.8 ± 2.4	−0.75 ± 0.15
CAAUGCAUU**GTT**	92.6 ± 1.4	267.4 ± 4.2	9.67 ± 0.04	51.0 ± 1.1	−0.91 ± 0.13
CGUUGCAAC**G**	91.0 ± 1.7	254.2 ± 5.2	12.15 ± 0.11	60.8 ± 1.7	0
CGUUGCAAC**GT**	98.4 ± 4.9	275.7 ± 14.6	12.89 ± 0.34	61.6 ± 4.5	−0.74 ± 0.35
CGUUGCAAC**GTT**	101.2 ± 2.0	283.4 ± 5.9	13.35 ± 0.13	62.4 ± 1.8	−1.20 ± 0.17
ACUUGCAAG**U**	85.7 ± 1.0	243.6 ± 3.2	10.11 ± 0.04	53.9 ± 1.0	0
ACUUGCAAG**UT**	92.8 ± 1.2	264.4 ± 3.8	10.75 ± 0.05	55.0 ± 1.1	−0.64 ± 0.07
ACUUGCAAG**UTT**	89.9 ± 2.3	254.2 ± 7.1	11.08 ± 0.11	56.8 ± 2.1	−0.97 ± 0.12
ACGUGCACG**U**	85.9 ± 1.5	234.6 ± 4.6	13.13 ± 0.11	66.5 ± 1.7	0
ACGUGCACG**UT**	92.9 ± 2.5	255.4 ± 7.4	13.72 ± 0.20	66.4 ± 2.6	−0.59 ± 0.23
ACGUGCACG**UTT**	91.4 ± 5.0	249.5 ± 15.2	14.06 ± 0.34	68.3 ± 5.6	−0.93 ± 0.36
ACCUGCAGG**U**	97.9 ± 2.7	268.2 ± 8.0	14.74 ± 0.22	68.6 ± 2.8	0
ACCUGCAGG**UT**	101.9 ± 3.4	279.7 ± 10.1	15.17 ± 0.29	68.9 ± 3.4	−0.43 ± 0.36
ACCUGCAGG**UTT**	100.0 ± 4.9	271.5 ± 14.4	15.77 ± 0.43	71.8 ± 5.2	−1.03 ± 0.48

[a] Measured in 100 mM NaCl, 20 mM sodium cacodylate, 0.5 mM Na_2EDTA, pH 7
[b] Calculated for 10^{-4} M oligomer concentration.
[c] Calculated according to 2 * Eq. (1) or 2 * Eq. (2), $\Delta\Delta G^\circ_{37}$ values > 0.5 kcal/mol are considered a significant difference [35].

whereas, thermodynamic effect of double 3′TT dangling ends was calculated according to:

$$\Delta\Delta G^\circ_{37}\left(\begin{matrix}\mathrm{XTT}\\ \mathrm{Y}\end{matrix}\right) = \frac{1}{2}\left[\Delta G^\circ_{37(\text{duplex with}\left(\begin{matrix}\mathrm{XTT}\\ \mathrm{Y}\end{matrix}\right))} - \Delta G^\circ_{37(\text{core duplex})}\right] \quad (2)$$

The X and Y correspond to any RNA residues that form a closing Watson-Crick base pair, the value of 1/2 is due to the use of self-complementary duplexes.

According to the data in Table 1, the presence of 3′T dangling ends always stabilizes RNA duplexes. The most significant increase in thermodynamic stability is observed for a 3′T unpaired nucleoside adjacent to a CG closing base pair in the $\begin{matrix}5'\mathrm{CT}3'\\ \mathrm{G}\end{matrix}$ context. The presence of an unpaired T residue adjacent to a CG pair at both 3′ ends of an RNA duplex results in thermodynamic stabilization ranging from −1.75 to −1.92 kcal/mol, with the average of −0.92 kcal/mol per each 3′T dangling end (Tables 1 and 2). On the contrary, direct proximity of the other pyrimidine residue, *i.e.* uridine, results in a significantly lower contribution of 3′T. In this case, the increase of thermodynamic stability varies from −0.43 to −0.64 kcal/mol per two 3′T residues (one 3′T residue on each end of the duplex) with an average $\Delta\Delta G^\circ_{37}$ of −0.28 kcal/mol per each 3′T overhang. A similar thermodynamic effect was also observed for a GC closing base pair. The unpaired T residue at both 3′ ends of an RNA duplex in the proximity of G changed RNA duplex thermodynamic stability by −0.64 to −0.75 kcal/mol, with an average of -0.36 kcal/mol per each 3′T dangling end. The presence of a 3′T residue adjacent to adenosine forming the last type of closing base pair, *i.e.* AU, on each end of the duplex induced an increase in RNA

Table 2
Thermodynamic parameters for 3′T and 3′TT dangling ends on RNA duplexes.[a]

$\begin{matrix}5'\mathrm{XT}3'\\ \mathrm{Y}\end{matrix}$	Average free energy contribution[b] [kcal/mol]	$\begin{matrix}5'\mathrm{XTT}3'\\ \mathrm{Y}\end{matrix}$	Average free energy contribution[c] [kcal/mol]	Difference[d] [kcal/mol]
$\begin{matrix}\mathrm{AT}\\ \mathrm{U}\end{matrix}$	−0.50 ± 0.04	$\begin{matrix}\mathrm{ATT}\\ \mathrm{U}\end{matrix}$	−0.65 ± 0.06	−0.15
$\begin{matrix}\mathrm{CT}\\ \mathrm{G}\end{matrix}$	−0.92 ± 0.10	$\begin{matrix}\mathrm{CTT}\\ \mathrm{G}\end{matrix}$	−1.10 ± 0.12	−0.18
$\begin{matrix}\mathrm{GT}\\ \mathrm{C}\end{matrix}$	−0.36 ± 0.07	$\begin{matrix}\mathrm{GTT}\\ \mathrm{C}\end{matrix}$	−0.50 ± 0.04	−0.14
$\begin{matrix}\mathrm{UT}\\ \mathrm{A}\end{matrix}$	−0.28 ± 0.07	$\begin{matrix}\mathrm{UTT}\\ \mathrm{A}\end{matrix}$	−0.49 ± 0.10	−0.21

[a] Measured in 100 mM NaCl, 20 mM sodium cacodylate, 0.5 mM Na_2EDTA, pH 7.0.
[b] An average value calculated according to Eq. (1) for three model duplexes containing a 3′-dangling T with the same adjacent pair.
[c] An average value calculated according to Eq. (2) for three model duplexes containing a 3′-dangling TT with the same adjacent pair.
[d] Energetic contribution of the second 3′-dangling T, calculated by subtraction of $\Delta\Delta G^\circ_{37}$ value determined for 3′T from $\Delta\Delta G^\circ_{37}$ value determined for 3′TT.

Table 3
Thermodynamic parameters for 3′T and 3′U dangling ends within DNA and RNA duplexes.[a]

DNA[b]		RNA[c]	
$^{5'}\mathrm{XT}^{3'}_{\mathrm{Y}}$	Free energy contribution [kcal/mol]	$^{5'}\mathrm{XU}^{3'}_{\mathrm{Y}}$	Free energy contribution [kcal/mol]
$^{\mathrm{AT}}_{\mathrm{T}}$	+0.13	$^{\mathrm{AU}}_{\mathrm{U}}$	−0.6
$^{\mathrm{CT}}_{\mathrm{G}}$	−0.52	$^{\mathrm{CU}}_{\mathrm{G}}$	−1.2
$^{\mathrm{GT}}_{\mathrm{C}}$	−0.35	$^{\mathrm{GU}}_{\mathrm{C}}$	−0.6
$^{\mathrm{TT}}_{\mathrm{A}}$	−0.29	$^{\mathrm{UU}}_{\mathrm{A}}$	−0.1

[a] 1 M NaCl buffer.
[b] The average error in the free energy contribution values is 0.07 kcal/mol, see original reference for statistical analysis of the values [13].
[c] See original reference for statistical analysis of the values [29].

duplex thermodynamic stability of −0.86 to −1.10 kcal/mol. The average stabilization per each 3′T adjacent to A was −0.50 kcal/mol. Analysis of thermodynamic effects caused by a single, unpaired 3′T residue on the stability of an RNA duplex indicates that stabilization depends on both type and orientation of the closing base pair, which is consistent with previous reports concerning thermodynamics of RNA 3′-dangling ends within RNA and DNA 3′-dangling ends within DNA duplexes [13,29].

Literature data suggest that the thermodynamic effect of any dangling end is a result of stacking interactions between the terminal unpaired nucleoside and one or both of the bases in the adjacent base pair [13]. The sequence dependence of stacking is probably an outcome of solvation and dipole effects [13,34]. In our studies, similar magnitude of stabilization is observed for 3′T overhangs that are adjacent to purine residues, *i.e.* −0.36 *vs.* −0.50 kcal/mol for G and A, respectively (Table 2). The 3′-unpaired T in the proximity of U stabilizes an RNA duplex similar to a 3′-unpaired T adjacent to a purine ($\Delta\Delta G^{\circ}_{37}$ = −0.28 kcal/mol). Nevertheless, the direct neighborhood of 3′T with cytidine causes an increase in stability which differs > 0.4 kcal/mol from the other three residues ($\Delta\Delta G^{\circ}_{37}$ = −0.92 kcal/mol). This suggests that stacking interactions might not be the only factor that contributes to an increase of RNA duplex thermodynamic stability by a 3′T dangling end.

The effect of one DNA 3′-overhang on DNA duplexes and one RNA 3′-overhang on RNA duplexes have been studied previously (Table 3). The influence of a dangling T at the 3′ end of a DNA duplex in 1 M NaCl was +0.13 kcal/mol for $^{5'}\mathrm{AT}^{3'}_{\mathrm{T}}$, −0.52 kcal/mol for $^{5'}\mathrm{CT}^{3'}_{\mathrm{G}}$, −0.35 kcal/mol for $^{5'}\mathrm{GT}^{3'}_{\mathrm{G}}$, and −0.29 kcal/mol for $^{5'}\mathrm{TT}^{3'}_{\mathrm{A}}$ [17]. For dangling U on an RNA duplex, in 1 M NaCl, the change of Gibbs free energy was −0.6 kcal/mol for $^{5'}\mathrm{AU}^{3'}_{\mathrm{U}}$, −1.2 kcal/mol for $^{5'}\mathrm{CU}^{3'}_{\mathrm{G}}$, −0.6 kcal/mol for $^{5'}\mathrm{GU}^{3'}_{\mathrm{C}}$, and − 0.1 kcal/mol for $^{5'}\mathrm{UU}^{3'}_{\mathrm{A}}$ [33]. Comparison of the results described herein with literature data concerning the influence of U or T 3′-dangling ends within RNA or DNA duplexes, respectively, shows that the tendency of changing thermodynamic stability of RNA duplexes by the presence of a 3′T dangling end is quite different in reference to 3′-dangling T on DNA duplexes but similar to RNA duplexes containing a 3′U dangling end. The data analysis shows that the presence of a 3′-unpaired T on a DNA duplex causes the most favorable change of Gibbs free energy when 3′T is adjacent to cytidine and the most energetically unfavorable when it is adjacent to adenosine, with the following order of stabilization based on the nucleotide adjacent to the 3′T: dC > dG > dT > dA. On the contrary, the magnitude of RNA duplex stabilization caused by the presence of a 3′-dangling U can be arranged in the order rC > rG = rA > rU, where C is the most favorable and U is the least favorable neighborhood for 3′-dangling U. Our results show that the contribution of 3′-unpaired T within RNA duplexes is more like 3′U within RNA than 3′T within DNA and is the most favorable adjacent to C and the least favorable when is placed close to U or G according to the following direction: C > A > G ≥ U.

The direct comparison of the magnitude of thermodynamic effects presented herein with recently published data is difficult since various salt concentrations were used in the studies. However, it is clear that the magnitude of thermodynamic contribution will be higher in 1 M NaCl in comparison to 100 mM NaCl. Analysis of Tables 2 and 3 shows that even in 100 mM NaCl, the presence of a 3′-dangling T on RNA duplexes is more stabilizing in case of $^{5'}\mathrm{AT}^{3'}_{\mathrm{U}}$ and $^{5'}\mathrm{CT}^{3'}_{\mathrm{G}}$ than 3′-dangling T on DNA duplexes and comparable in case of $^{5'}\mathrm{GT}^{3'}_{\mathrm{C}}$ and $^{5'}\mathrm{UT}^{3'}_{\mathrm{A}}$. Nevertheless, in 1 M NaCl, the magnitude of thermodynamic stabilization caused by 3′-unpaired T on RNA is expected to be more stabilizing in reference to DNA duplexes. Therefore, we can hypothesize that, in general, the presence of 3′ unpaired T on RNA duplexes in 1 M NaCl would be more stabilizing than a 3′ dangling T on a DNA duplex. On the contrary, the majority of thermodynamic effects caused by the presence of 3′-dangling T nucleotides on RNA duplexes are slightly less stabilizing in 100 mM NaCl in comparison to the effects caused by 3′-unpaired U on RNA duplexes measured in 1 M NaCl. The only exception is 3′T neighboring with U which stabilizes an RNA duplex more than a 3′U neighboring with U. However, also here, we can hypothesize that a 3′T overhang would stabilize RNA duplexes more than a 3′ unpaired U in 1 M NaCl, since the difference between $\Delta\Delta G^{\circ}_{37}$ values in both salt conditions is small.

The effect of adding a second 3′T overhang, resulting in formation of 3′TT on an RNA duplex is not as remarkable as a single 3′-unpaired T nucleoside. An additional T residue always increases the thermodynamic stability, but the value of $\Delta\Delta\Delta G^{\circ}_{37}$ oscillates in the range − 0.14 - −0.21 kcal/mol (Table 2). Our results are in agreement with other studies concerning 3′UU overhangs within RNA duplexes [33]. Previously published data indicate that addition of the second dangling U residue when the first dangling nucleotide is U results in change of thermodynamic stability only by −0.1 kcal/mol, and the effect seems to be sequence independent.

The results described herein show that thermodynamic contribution of 3′-dangling T and 3′-dangling TT on RNA duplexes differs from published data for 3′ dangling U and 3′-dangling UU on RNA duplexes and 3′-dangling T on DNA duplexes. This data suggests that using the contribution of 3′-dangling T values for RNA duplexes (instead of 3′-dangling T values for DNA duplexes or 3′-dangling U values for RNA duplexes) would improve the prediction of the stability of siRNA duplexes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpc.2018.12.006.

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