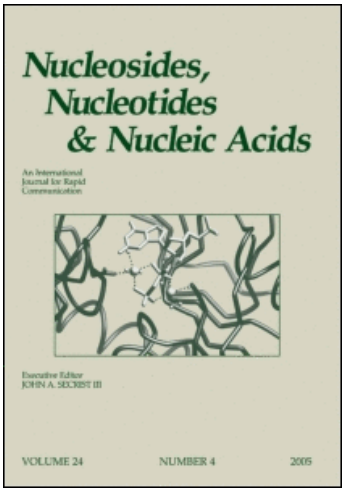

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EXTRA STABLE 2′,5′-LINKED RNA LOOPS

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ABSTRACT

We prepared hairpins that differ in the connectivity of phosphodiester linkages in the loop (RNA vs 2′, 5′-RNA). We find that the stability of the extra stable RNA hairpin 5′-rGGAC(UUCG)GUCC-3′ is the same as that observed for the hairpin containing a 2′,5′RNA loop, i.e. 5′-rGGAC(UUCG)GUCC-3′ (where UUCG = $U_{2'p5'}U_{2'p5'}$ $C_{2'p5'}G_{2'p5'}$). Also significant is the finding that when the stem is duplex DNA, duplex 2′,5′-RNA, or DNA:2′,5′-RNA, hairpins with the UUCG loop are more stable than those with UUCG loop.

RNA hairpin loops has been a subject of considerable interest in recent years (1–5). They play an important structural role by interacting with proteins and nucleic acids, serving as termination site for transcriptional processes, or stabilizing mRNA or antisense oligomers *in vivo* (1).

Certain RNA hairpins are unusually stable such as those containing the loop sequence C(UUCG)G (1,2). As part of our continuing effort to explore molecular recognition of 2′,5′-linked RNA, we wished to learn if 2′,5′-RNA, like 'normal' RNA, can fold into unusually stable loops, and if so, whether the same sequences are extra stable. To our knowledge, the work presented here is the first attempt to examine the thermodynamic stability of RNA hairpins containing 2′,5′-phosphodiester linkages.

Several features make 2′,5′-RNA loops an attractive choice for stabilization of hairpin loops. Previous reports have suggested that 2′,5′-linked oligoribonucleotides base-stack more readily than the corresponding 3′,5′-linked isomers (6,7). This

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Table 1. Thermodynamics[a] of Hairpin Formation at 0.01M Na_2HPO_4, 0.1 mM EDTA

	Sequence[b]	Designation	T_m[c] (°C)	ΔH° (*kcal/mol*)	ΔS° (*e.u.*)	ΔG°_{37} (*kcal/mol*)
1	GGA-C (uucg) G-UCC	RDR	63.4	−47.3	−140.1	−3.74
2	GGA-C(UUCG)G-UCC	RRR	71.8	−53.4	−154.8	−5.41
3	GGA-C(<u>UUCG</u>)G-UCC	R<u>R</u>R	69.3	−55.6	−162.1	−5.33
4	GGA-C(<u>UUUG</u>)G-UCC	R<u>R</u>′R	66.9	−47.3	−139.2	−4.16
5	GGA-C(<u>UUUU</u>)G-UCC	R<u>R</u>′R	60.5	−46.7	−139.9	−3.28
6	gga-c(uucg)g-tcc	DDD	56.2	−36.6	−111.1	−2.14
7	gga-c(UUCG)g-tcc	DRD	54.6	−36.0	−109.8	−1.92
8	gga-c(<u>UUCG</u>)g-tcc	D<u>R</u>D	61.4	−39.9	−119.4	−2.91
9	<u>GGA-C</u>(UUCG)g-tcc	<u>R</u>RD	48.1	−38.9	−121.1	−1.35
10	<u>GGA-C(UUCG)g</u>-tcc	<u>RR</u>D	52.8	−39.5	−121.3	−1.93
11	<u>GGA-C</u>(UUCG)<u>G-UCC</u>	<u>RRR</u>	45.2	−30.8	−98.2	−0.79
12	<u>GGA-C(UUCG)G-UCC</u>	<u>RRR</u>	54.8	−42.1	−128.5	−2.29

[a]Errors in thermodynamic parameters are within ±7% for ΔH° and ΔS°, and ± 0.20 kcal/mol for ΔG°_{37}. For a more accurate calculation, ΔG°_{37} was determined from $\Delta H^\circ$and ΔS° before rounding off. [b]Capital letters represent RNA residues; underlined letters are 2′,5′-RNA residues (*e.g.* <u>CU</u> = $C_{2'p5'}U_{2'p}$); DNA residues are represented by small letters. [c]The melting curves show a single cooperative and completely reversible transition that is independent of oligonucleotide concentration over a 30 fold range.

would give 2′,5′-RNA an 'advantage', since base stacking interactions within the loop are known to contribute significantly to the stability of RNA hairpin loops. We also reasoned that the potentially more rigid 2′,5′-RNA core (or loop) would reduce the overall degrees of freedom, and hopefully, permit some adjustment to conform to the helical geometry of the adjoining stem (or duplex). In addition, 2′,5′-RNA would be less susceptible than 3′,5′-RNA to attack by nucleases in biological media (6).

Sequences of the oligonucleotides prepared for study are indicated in Table 1, and include the extra-stable RNA hairpin rGGAC(UUCG)GUCC as reference (2). As can be seen from Table 1, the oligomers share the same stem and loop sequence, but differ in the sugar composition and/or the connectivity of phosphodiester bonds. Table 1 and Fig. 1 list the thermodynamic parameters for hairpin formation at 0.01M Na_2HPO_4, 0.1 mM Na_2EDTA (pH 7.0) as determined by averaging the fitted thermodynamic values from at least six independent melting profiles of each hairpin. The calculated ΔG°_{37} and T_m values for the extra stable RNA hairpin 2 are very similar to what has been obtained previously (2).

The key observations can be summarized as follows:

(a) The stability of the extra stable RNA hairpin rGGAC(UUCG)GUCC-3′ is the same as that observed for the hairpin containing a 2′,5′-<u>UUCG</u> tetra-loop, *i.e.* GGAC($U_{2'p5'}U_{2'p5'}C_{2'p5'}G_{2'p5'}$)GUCC (compare entries **2** and **3**). Thus, when the stem is duplex RNA, both UUCG and <u>UUCG</u> are extra stable and contribute equally to the overall hairpin stability.

(b) When the stem is either duplex DNA, duplex 2′,5′-RNA, or hybrid 2′,5′-RNA:DNA, the degree of stabilization provided by the <u>UUCG</u> loop is

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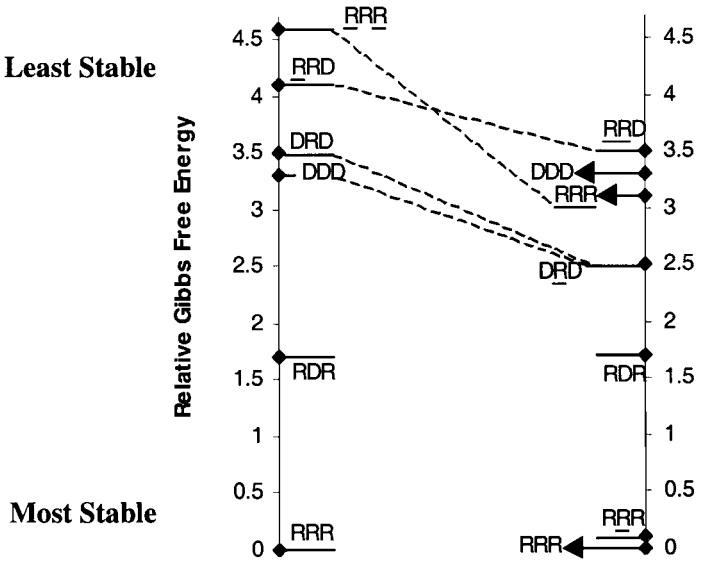

Figure 1. Relative Gibbs free energies of hairpin formation ($\Delta\Delta G^{\circ}_{37}$) in kcal/mol. All values are referenced relative to the RRR hairpin exhibiting the lowest (most negative) free energy value.

greater than that of the UUCG loop. This is evident from Fig. 1, which compares the $\Delta\Delta G_{37}{}^{\circ}$ values of the various hairpins. Thus, dashed lines with a negative slop represent cases where stabilization of a 2′,5′-tetra-loop is greater relative to a 3′,5′-linked tetra-loop. For example, replacing the UUCG loop with UUCG leads to a gain of 10°C in the melting temperature of the 2′,5′RNA:2′,5′-RNA stem, or a $\Delta\Delta G^{\circ}_{37} \approx 1.5$ kcal/mol in free energy (compare entries **11 & 12**). We therefore conclude that while the stability imparted by UUCG is virtually independent of the stem composition (e.g., DNA:DNA, RNA:RNA, 2′,5′-RNA:2′,5′-RNA), the 3′,5′-UUCG tetraloop exerts extra stability only when the stem is RNA:RNA.

(c) It has been possible to observe the formation of a stable 2′,5′-RNA:DNA hybrid by linking the hybrid's strands to the UUCG loop. These 2′,5′-RNA:DNA duplexes, which are not stable enough to form in an intermolecular complex (8), were stable at room temperature ($T_m > 50\,^{\circ}$C; see entry **10**, Table 1) and analyzed by spectroscopic methods for the first time (data not shown).

(d) The stability imparted by the UUCG tetra-loop is dependent on the loop base sequence (entries **3** versus **4 & 5**), a property that is shared with the regioisomeric 3′-5′-RNA loop (2).

We are presently studying the effect of sugar substitutions at the loop closing base pairs (e.g. deoxy versus ribose, versus 2′,5′-linked ribose), and conducting NMR experiments to determine the structure of 2′,5′-RNA/RNA and 2′,5′-RNA/DNA double helices.

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