

Structure of 2',5'-Linked Tetraribonucleotide Loops: A Novel RNA Motif

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ABSTRACT

We report on the three dimensional structure of an RNA hairpin containing a 2',5'-linked tetraribonucleotide loop, namely, 5'-rGGAC(UUCG)GUCC-3' (where UUCG = U_{2'p5'}U_{2'p5'}C_{2'p5'}G_{2'p5'}). We show that the 2',5'-linked RNA loop adopts a conformation that is quite different from that previously observed for the native 3',5'-linked RNA loop. The 2',5'- RNA loop is stabilized by (a) U:G wobble base pairing, with both bases in the *anti* conformation, (b) extensive base stacking, and (c) sugar–base contacts, all of which contribute to the extra stability of this hairpin structure.

Key Words: Hairpin; RNA tetraloop; 2'-5'-Linked RNA; NMR.

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We have recently shown that the oligoribonucleotide sequence 5'-G₁G₂A₃C₄(U₅U₆C₇G₈)G₉U₁₀C₁₁C₁₂-3' folds into a hairpin structure that exhibits extra stability (rUUCG represent a 2',5'-linked tetraloop).^[1,2] We also showed that the stability imparted by UUCG is dependent on base sequence, a property that is shared with the regioisomeric 3',5'-RNA loop (UUCG). In addition, UUCG exerts higher stability in hairpins with stem constructs such as DNA:DNA, 2',5'-RNA:2',5'-RNA, DNA:2',5'-RNA, and RNA:2',5'-RNA compared to the 3',5'-linked counterpart (UUCG). As a result, the relative stabilities of hairpins with a 2',5'-linked tetraloop, e.g., ggac(UUCG) gtcc ($T_m = 61.4^\circ\text{C}$), are often superior to those with RNA tetraloops, e.g., ggac(UUCG) gtcc ($T_m = 54.6^\circ\text{C}$).^[1] In fact, it has been possible to observe the formation of a 2',5'-RNA:DNA hybrid duplex by linking the hybrid's strands to a (UUCG) loop. These duplexes, which are not stable enough to form in an intermolecular complex, are stable at room temperature ($T_m \sim 50^\circ\text{C}$). Thus, 2',5'-loops have potentially important implications in the study of nucleic acid complexes where structural data is not yet available.

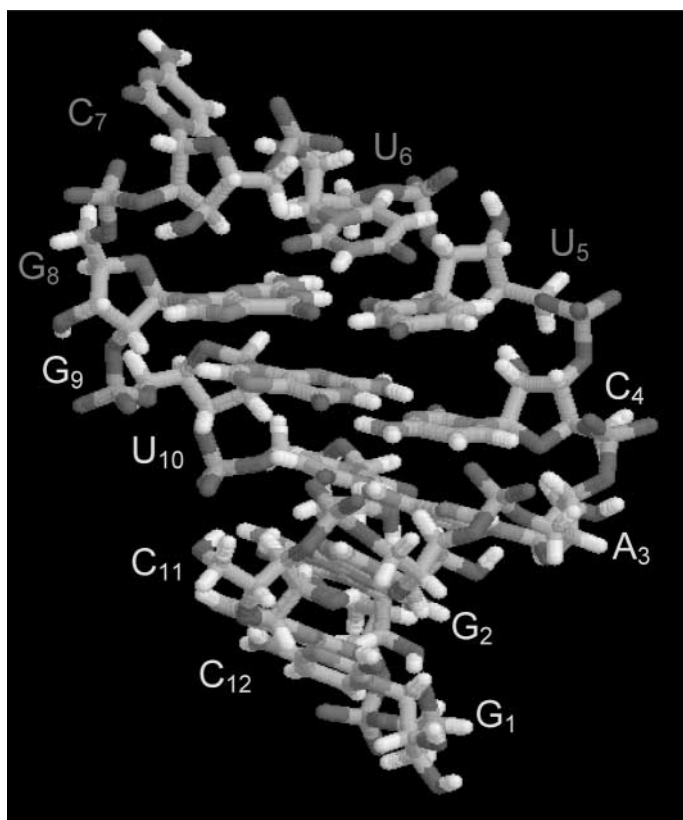


Figure 1. Energy minimized structure of an RNA hairpin containing a 2',5'-linked tetranucleotide loop [5'-rGGAC(UUCG)GUCC-3']. The residues of the 2',5'-loop are U₅-G₈ while those of the 3',5'-linked RNA stem are G₁-C₄ and G₉-C₁₂.

To gain further insight into the molecular basis behind the unusual stability of the 2',5'-linked tetraloop, we investigated its structure by high-resolution NMR spectroscopy. The three dimensional structure was derived from interproton distances and scalar couplings. Our data show that the 2',5'-linked tetraloop adopts a uniquely folded structure (Fig. 1) that is different from that of the native 3',5'-linked loop.^[3] The different sugar pucker and intrinsic bonding interactions within the 2',5'-linked ribonucleotides help explain the unusual stability and conformational properties displayed by the 2',5'-linked tetraloop. These are summarized as follows (Fig. 1):

- (a) A mismatch U₅:G₈ base pair, with the U₅ residue adopting an *anti* conformation, stacks on C₄:G₉ in the normal A-geometry, and thus appears as a continuation of the stem. The glycosidic bond of the G₈ residue is mobile and adopts both *syn* and *anti* conformations. On the other hand, when the stem of the hairpin is switched to DNA (duplex DNA), the G₈ residue adopts primarily the *anti* conformation.
- (b) The loop is composed of only two nucleotides [U₆ & C₇], both in the C-2' *endo* pucker form. Since the internucleotide phosphodiester bond is 2',5'-linked, then the C-2' *endo* pucker is in a compact form^[4] rather than extended form as would have been the case for 3',5'-linked RNA.
- (c) U₅ and G₈ sugars adopt C-3' *endo* conformation [extended form for 2',5'-RNA]^[4]. This helps extend the backbone in order to bridge the stem.
- (d) C₇ residue protrudes out into the solvent. This is in sharp contrast to the native 3',5'-RNA case^[3] where it is the U₆ residue that protrudes out and does not contribute to thermal stability.
- (e) Uracil U₆ base stacks on top of U₅. This is evident from the aromatic to sugar H1' NOE which is typical of helical strands (data not shown).

These unusual specific interactions contribute to the unusual thermodynamic stability of 2',5'-RNA loops.

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