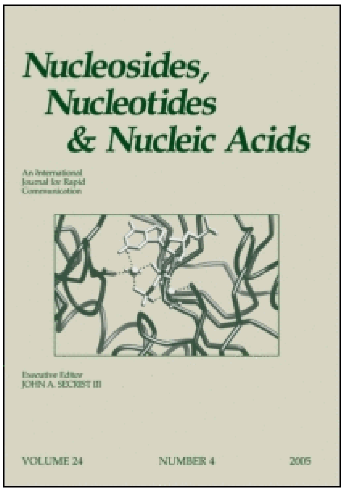

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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

Rami N. Hannoush[a]; Alexei Yu. Denisov[b]; Kalle Gehring[ab]; Masad J. Damha[ac]
[a] Departments of Chemistry and Biochemistry, McGill University, Montreal, Canada [b] Montreal Joint Centre for Structural Biology, McGill University, Montreal, Canada [c] Department of Chemistry, Otto Maass Chemistry Building, McGill University, Montreal, Quebec, Canada

To cite this Article Hannoush, Rami N. , Denisov, Alexei Yu. , Gehring, Kalle and Damha, Masad J.(2003) 'Structure of 2',5'-Linked Tetraribonucleotide Loops: A Novel RNA Motif', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1687 — 1690

To link to this Article: DOI: 10.1081/NCN-120023114

URL: http://dx.doi.org/10.1081/NCN-120023114

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1687–1690, 2003

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

**Rami N. Hannoush,[1] Alexei Yu. Denisov,[2] Kalle Gehring,[1,2]
and Masad J. Damha[1,*]**

[1]Departments of Chemistry and Biochemistry, and
[2]Montreal Joint Centre for Structural Biology,
McGill University, Montreal, Canada

ABSTRACT

We report on the three dimensional structure of an RNA hairpin containing a 2′,5′-linked tetraribonucleotide loop, namely, 5′-rGGAC($\underline{\text{UUCG}}$)GUCC-3′ (where $\underline{\text{UUCG}} = \text{U}_{2'p5'}\text{U}_{2'p5'}\text{C}_{2'p5'}\text{G}_{2'p5'}$). We show that the $\overline{2',5'}$-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.

---

*Correspondence: Masad J. Damha, Department of Chemistry, McGill University, Otto Maass Chemistry Building, 801 Sherbrooke St. W, H3A 2K6 Montreal, Quebec, Canada; Fax: +1 514 398 3797; E-mail: masad.damha@mcgill.ca.

DOI: 10.1081/NCN-120023114
1525-7770 (Print); 1532-2335 (Online)

www.dekker.com

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

We have recently shown that the oligoribonucleotide sequence 5′-$G_1G_2A_3C_4$ $(\underline{U_5}\underline{U_6}\underline{C_7}\underline{G_8})G_9U_{10}C_{11}C_{12}$-3′ folds into a hairpin structure that exhibits extra stability (r$\underline{\text{UUCG}}$ represent a 2′,5′-linked tetraloop).[1,2] We also showed that the stability imparted by $\underline{\text{UUCG}}$ is dependent on base sequence, a property that is shared with the regioisomeric 3′,5′-RNA loop (UUCG). In addition, $\underline{\text{UUCG}}$ 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($\underline{\text{UUCG}}$) gtcc ($T_m = 61.4°C$), are often superior to those with RNA tetraloops, e.g., ggac(UUCG) gtcc ($T_m = 54.6°C$).[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 ($\underline{\text{UUCG}}$) loop. These duplexes, which are not stable enough to form in an intermolecular complex, are stable at room temperature ($T_m \sim 50°C$). Thus, 2′,5′-loops have potentially important implications in the study of nucleic acid complexes where structural data is not yet available.

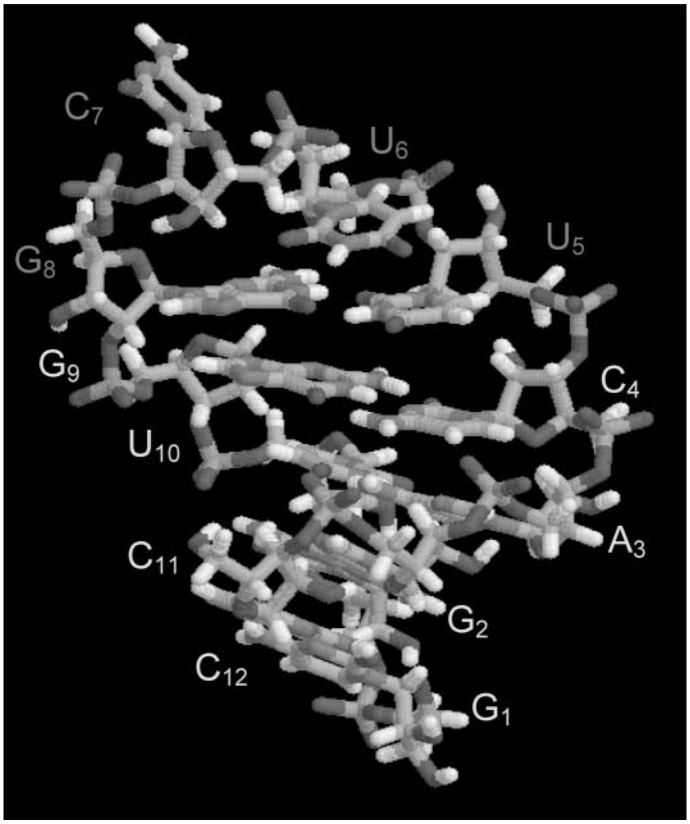

Figure 1. Energy minimized structure of an RNA hairpin containing a 2′,5′-linked tetraribonucleotide loop [5′-rGGAC($\underline{\text{UUCG}}$)GUCC-3′]. The residues of the 2′,5′-loop are U_5–G_8 while those of the 3′,5′-linked RNA stem are G_1–C_4 and G_9–C_{12}.

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 U_5:G_8 base pair, with the U_5 residue adopting an *anti* conformation, stacks on C_4:G_9 in the normal A-geometry, and thus appears as a continuation of the stem. The glycosidic bond of the G_8 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 G_8 residue adopts primarily the *anti* conformation.
(b) The loop is composed of only two nucleotides [U_6 & C_7], 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) U_5 and G_8 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) C_7 residue protrudes out into the solvent. This is in sharp contrast to the native 3′,5′-RNA case[3] where it is the U_6 residue that protrudes out and does not contribute to thermal stability.
(e) Uracil U_6 base stacks on top of U_5. 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.

ACKNOWLEDGMENTS

This research was supported by grants from NSERC Canada (MJD) and CIHR Canada (KG). RH is the recipient of NSERC and FCAR postgraduate scholarships.

REFERENCES

1. Hannoush, R.N.; Damha, M.J. Remarkable stability of hairpins containing 2′,5′-linked RNA loops. J. Am. Chem. Soc. **2001**, *123*, 12,368–12,374.
2. Hannoush, R.N.; Damha, M.J. Extra stable 2′,5′-linked RNA loops. Nucleosides & Nucleotides **2001**, *20*, 1201–1204.
3. (a) Cheong, C.; Varani, G.; Tinoco, I., Jr. Solution structure of an unusually stable RNA hairpin, 5′GGAC(UUCG)GUCC. Nature **1990**, *346*, 680–682;

(b) Varani, G.; Cheong, C.; Tinoco, I., Jr. Structure of an unusually stable RNA hairpin. Biochemistry **1991**, *30*, 3280–3289; (c) Allain, F. H.-T.; Varani, G. Structure of the P1 helix from group I self-splicing introns. J. Mol. Biol. **1995**, *250*, 333 pp.

4. (a) Premraj, B.J.; Patel, P.K.; Kandimalla, E.R.; Agrawal, S.; Hosur, R.V.; Yathindra, N. NMR structure of a 2′,5′ RNA favors a type duplex with compact C2′ endo nucleotide repeat. Biochem. Biophys. Res. Comm. **2001**, *283*, 537–543; (b) Premraj, B.J.; Yathindra, N. Stereochemistry of 2′,5′ nucleic acids and their constituents. J. Biomol. Struct. Dyn. **1998**, *16*, 313–328.