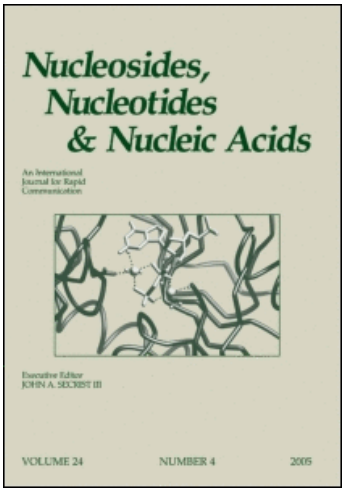


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Conformation of Lariat Structures Formed in the Splicing of Pre-mRNA by NMR Spectroscopy

A. Sandström[a]; N. Balgobin[a]; A. Nyilas[a]; G. Remaud[a]; J. -M. Vial[a]; X. -X. Zhou[a]; J. Chattopadhyaya[a]
[a] Department of Bioorganic Chemistry, Box 581, Biomedical Center, University of Uppsala, Uppsala, Sweden

To cite this Article Sandström, A. , Balgobin, N. , Nyilas, A. , Remaud, G. , Vial, J. -M. , Zhou, X. -X. and Chattopadhyaya, J.(1988) 'Conformation of Lariat Structures Formed in the Splicing of Pre-mRNA by NMR Spectroscopy', Nucleosides, Nucleotides and Nucleic Acids, 7: 5, 827 — 830
To link to this Article: DOI: 10.1080/07328318808056338
URL: http://dx.doi.org/10.1080/07328318808056338

NUCLEOSIDES & NUCLEOTIDES, 7(5&6), 827-830 (1988)

CONFORMATION OF LARIAT STRUCTURES FORMED IN THE SPLICING OF PRE-MRNA BY NMR SPECTROSCOPY

A. Sandström, N. Balgobin, A. Nyilas+, G. Remaud, J.-M. Vial, X.-X. Zhou and J. Chattopadhyaya*

Department of Bioorganic Chemistry, Box 581, Biomedical Center, University of Uppsala, S-751 23 Uppsala, Sweden.

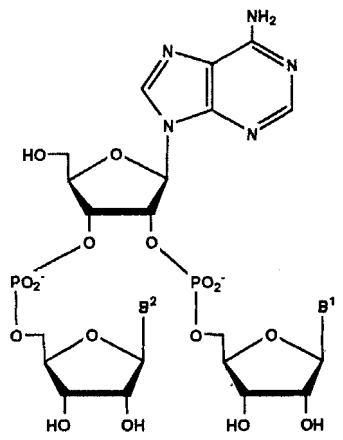
Abstract: Temperature dependent ^{1}H- and ^{31}P-NMR studies have shown that lariat (branched) trimers show a preferential 2' → 5' stacking, while the branched tetramers resemble 3'→ 5' linked linear trimers, reminiscent of a single stranded A-RNA helix.

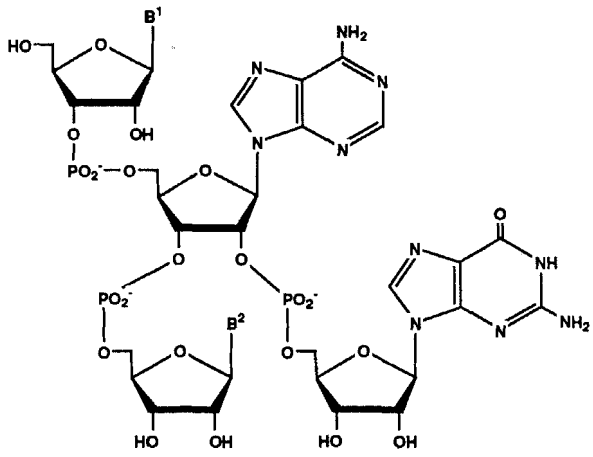
The biochemistry and mechanisms concerning the splicing reactions of eukaryotic mRNA precursors have been studied extensively during the last few years[1]. In Group II and nuclear mRNA splicing reactions, intron mRNA forms a 2'→ 5' branched structure (lariat). The branch-point adenosine is linked via a 3'→ 5' phosphodiester to either a cytidine or a uridine residue and the branch consists of a 2′→ 5′ phosphodiester linked guanosine residue. We herein review our results[2-6] on the conformational analysis of a few branched tri- and tetramers 1 - 10 by ^{1}H- and ^{31}P-NMR spectroscopy.

Conformation of branched trimers based on ^{1}H-NMR.

A pseudorotational analysis[7], based on the coupling constants[8,9] between H1' and H2', shows that the branch-point sugars in the natural trimers[2], 1 and 2, prefer the S conformation at 5 - 80 °C [~60% S at ~5 °C]. The 2′→ 5′ linked guanosine sugar moiety follows the same trend [45-48% N at 5 °C and ~35% N at 80 °C] while the 3′→ 5′ linked pyrimidine sugar moiety is virtually unaffected by temperature changes [~49% N for 3′→ 5′-linked uridine and ~55% N for 3′→ 5′-cytidine]. The unnatural branched trimers[3,4] 3, 4, 5 and 6 also prefer the S conformation for their branch-point sugars, especially if a pyrimidine nucleoside is linked to the 2′-phosphate. The 2′→ 5′ sugar moieties have a strong preference for the N-type conformation for compounds 3, 4 and 6, and they also show relatively large conformational changes upon increasing temperature. In compound 5, on the other hand, the branch-point sugar conformation is temperature-independent. The

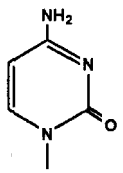
3′→ 5′ sugar moieties show a small preference for the S conformation which is also independent of any temperature change. It has been shown that the stacking between the nucleobases can be qualitatively understood in an oligonucleotide by studying the temperature dependence of the anomeric and the aromatic proton chemical shifts[10]. In the stacked form, the H2A, H5U and H5C absorb more upfield than in the destacked form due to the diamagnetic effect of the neighbouring bases. The chemical shifts of H8A, H8G, H6U and H6C, on the other hand, show a dependence also of the glycosidic bond torsion angle. A comparison of the chemical shift changes of the aromatic and anomeric protons for the branched trimers 1 - 6 and the dimers A(2′→ 5′)G, A(2′→ 5′)C, A(2′→ 5′)U, A(2′→ 5′)A, A(3′→ 5′)A, A(3′→ 5′)C and A(3′→ 5′)U show that the branched trimers 1 - 6 prefer a 2′→ 5′ stacking[2-4].



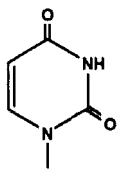


1: B^1= G, B^2= U
2: B^1= G, B^2= C
3: B^1= U, B^2= G
4: B^1= C, B^2= G
5: B^1= B^2= G
6: B^1= B^2= A

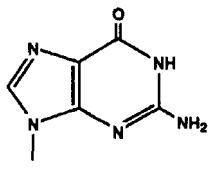
7: B^1= U, B^2= U
8: B^1= U, B^2= C
9: B^1= A, B^2= U
10: B^1= A, B^2= C



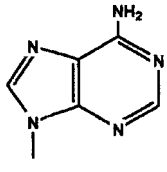
C



U



G



A

Conformation of branched tetramers based on ^1H-NMR.

The temperature dependencies of the anomeric and aromatic proton chemical shifts of the four branched tetramers 7 - 10 are only slightly different[5] from those of the branched trimers. The observed differences, however, indicate a stacking behaviour resembling the linear 3′→ 5′ linked trimers such as AAPy and PyAPy[11]. An analysis of the equilibria of the pseudorotamer populations at different temperatures show[5] that the branch-point adenosine sugar moiety exists in 62 - 66% N form at 7 °C in 9 and 10, this preference is, however, stronger [69 - 74% N] in 7 and 8. At 80 °C, all four tetramers prefer the S-type conformations [34 - 41% N]. This conformational feature is basically similar to those of the linear (3′→ 5′) oligoribonucleotides[12] where a decrease of temperature promotes an increase of N pseudorotamer population. These results in conjunction with NOE-data[5] (not shown) suggest that the branched tetramers adopt the conformation of a distorted single stranded A-RNA[12]. The structures of the branched tetranucleotides 7 - 10 are therefore distinctly different from those of the branched trimers 1 - 6 ; there is no 2′→ 5′ stacking between A and G, but the 3′-terminal pyrimidine is stacked with the branch-point adenosine, which in turn stacks partly with the 5′-terminal nucleobase. NOE-data have also shown partial stacking between the 5′-terminal nucleobase and the 2′-terminal guanosine, consistent with our observations[5] of the temperature-dependent ^1H chemical shifts.

Conformation of branched trimers and tetramers based on ^{31}P-NMR.

Temperature-dependent ^{31}P-NMR is a powerful tool for studying conformations of nucleic acids since ^{31}P chemical shifts are sensitive to temperature-dependent changes in O-P-O bond angles and R-O-P-O torsion angles which in turn reflects the conformational flexibilities across the phosphate backbone[13-15]. The temperature dependencies of the ^{31}P shifts can not give quantitative thermodynamic informations regarding stack ⇌ destack equilibrium[16], it can, however, show the relative conformational differences of the phosphate backbone in a qualitative manner. An examination[6] of the $\Delta\delta\ ^{31}P$ [$\delta\ ^{31}P$ at 81°C - $\delta\ ^{31}P$ at 10° C] for the trimers 1 - 6 has shown that the 2′→ 5′ phosphate has a more constrained conformation than the 3′→ 5′ phosphate, probably due to more stacking. The relative magnitude of the $\Delta\delta\ ^{31}P$ also show[6] that adenosine(2′→ 5′)pyrimidine stacking is a more dominant conformational feature than adenosine(2′→ 5′)purine stacking in the branched trimers 1 - 6. The 3′→ 5′ phosphate linkages in the branched trimers show relatively small $\Delta\delta$, suggesting that the 3′→ 5′ phosphate conformation is less constrained. The branch-point sugar in 3 and 4 has a high degree of temperature-independent S conformation (90% S) producing a $\Delta\delta\ ^{31}P$ of ~0.50 ppm for its 3′→ 5′phosphate which suggests that it has a constrained backbone, this is also true for the compounds 6, 1 and 2

with ~75% - ~65% S-conformation giving the $\Delta\delta$ ^{31}P for their 3′→ 5′phosphates of 0.40, 0.31 and 0.32 ppm respectively. In the ^{31}P NMR studies of the branched tetramers, 7 - 10, the chemical shift changes suggest that the branch-point adenosine (A*) stacks more strongly to a 5′-terminal A than to a 5′-terminal U, as evident from the $\Delta\delta$ of ~0.68 and ~0.62 ppm respectively. The A* also stacks better to a 3′-terminal C than to a 3′-terminal U [$\Delta\delta$ ~0.34 and ~0.31 ppm respectively]. The 3′-terminal nucleobase also affects the stacking in the A*(2′→ 5′)G moiety, with a 3′-terminal U the $\Delta\delta$ for the 2′→ 5′ phosphate is ~0.50 ppm and with a 3′-terminal C the $\Delta\delta$ is ~0.41 ppm, suggesting that a 3′-terminal U makes the A*(2′→ 5′)G phosphate conformation more constrained[6].

Acknowledgements: Authors gratefully acknowledge research grants from the Swedish Board for Technical Development (STU), the Swedish Natural Research Council, and Wallenbergstiftelsen.

+Center for Agricultural Biotechnology, 2101 Gödöllö, Hungary

REFERENCES

1. a). R. A. Padgett et al., Annu. Rev. Biochem., 55, 1119 (1986),
   b). T. R. Cech and B. L. Bass, Annu. Rev. Biochem., 55, 599 (1986).
2. G. Remaud, J.-M. Vial, A. Nyilas, N. Balgobin and J. Chattopadhyaya, Tetrahedron, 43, 947 (1987).
3. J.-M. Vial, G. Remaud, N. Balgobin and J. Chattopadhyaya, Tetrahedron, 43, 3997 (1987).
4. G. Remaud, X.-X. Zhou, B. Öberg and J. Chattopadhyaya, (in press), in "Reviews of Heteroatom Chemistry", ed. by S. Oae, MYU publishing Inc., Tokyo, 1987.
5. X.-X. Zhou, A. Nyilas, G. Remaud and J. Chattopadhyaya, Tetrahedron, 44, 571 (1988).
6. A. Sandström, G. Remaud, J.-M. Vial, X-X. Zhou, A. Nyilas, N. Balgobin and J. Chattopadhyaya, J. Chem. Soc. Chem. Commun., (in press).
7. C. Altona and M. Sundaralingam, J. Am. Chem. Soc., 94, 8205 (1972), and ibid, 95, 2333 (1973).
8. C. A. G. Haasnoot, F. A. A. M. de Leeuw and C. Altona, Tetrahedron, 36, 2783 (1980).
9. F. A. A. M. de Leeuw and C. Altona, J. Chem. Soc. Perkin II, 375 (1982).
10. C. Giessner-Prettre and B. Pullman, Quaterly Reviews of Biophysics, 20, 113 (1987).
11. C.-H. Lee and I. Tinoco Jr., Biophys. Chem., 11, 283 (1980).
12. C. Altona, Recl. Trav. Chim. Pays-Bas, 101, 413 (1982).
13. D. G. Gorenstein and D. Kar, Biochem. Biophys. Res. Commun., 65, 1073 (1973).
14. D. G. Gorenstein, J. Am. Chem. Soc., 97, 898 (1975).
15. D. G. Gorenstein and D. Kar, J. Am. Chem. Soc., 99, 672 (1977).
16. C. A. G. Haasnoot and C. Altona, Nucl. Acid Res., 6, 1135 (1979).