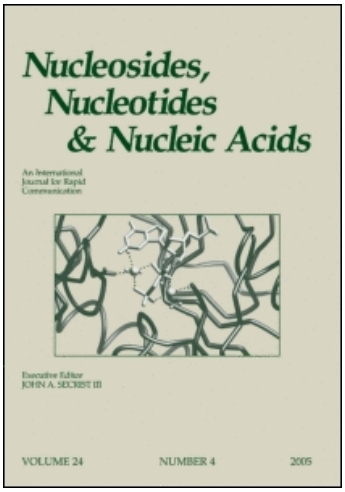


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Branchpoint Sugar Stereochemistry Determines the Hydrolytic Susceptibility of Branched RNA Fragments by the Yeast Debranching Enzyme (YDBR)

Sandra Carriero[a]; Maria M. Mangos[a]; Kazim A. Agha[a]; Anne M. Noronha[a]; Masad J. Damha[a]
[a] Department of Chemistry, McGill University, Montreal, Quebec, Canada

Online publication date: 09 August 2003

To cite this Article Carriero, Sandra , Mangos, Maria M. , Agha, Kazim A. , Noronha, Anne M. and Damha, Masad J.(2003) 'Branchpoint Sugar Stereochemistry Determines the Hydrolytic Susceptibility of Branched RNA Fragments by the Yeast Debranching Enzyme (YDBR)', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1599 — 1602
To link to this Article: DOI: 10.1081/NCN-120023043
URL: http://dx.doi.org/10.1081/NCN-120023043

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1599–1602, 2003

Branchpoint Sugar Stereochemistry Determines the Hydrolytic Susceptibility of Branched RNA Fragments by the Yeast Debranching Enzyme (YDBR)

Sandra Carriero, Maria M. Mangos, Kazim A. Agha, Anne M. Noronha, and Masad J. Damha*

Department of Chemistry, McGill University, Montreal, Quebec, Canada

ABSTRACT

A series of branched RNAs (Y-shaped) related to yeast pre-mRNA splicing intermediates were synthesized incorporating both natural (i.e., ribose) and non-natural (i.e., arabinose, xylose and acyclic nucleoside) branchpoints in order to examine the effect of sugar conformation and phosphodiester configuration on yDBR hydrolytic efficiency. The results indicate that 2′-phosphodiester scission with yDBR occurs only with a cis-arrangement of phosphate groups at the branchpoint (i.e., ribose) thereby discriminating between all other configurations.

Key Words: Lariat debranching enzyme; Branched RNA; 2′,5′-Phosphodiesterase.

The yeast lariat debranching enzyme (yDBR) is a unique metalloprotein that selectively cleaves 2′,5′-phosphodiester bonds adjacent to 3′,5′-linkages,[1] such as those observed in branched intermediates formed during nuclear pre-mRNA splicing

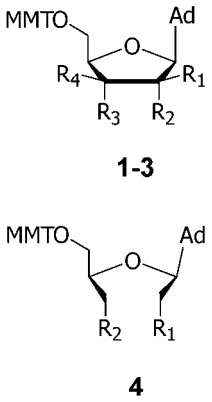
*Correspondence: Masad J. Damha, Department of Chemistry, McGill University, Otto Maass Chemistry Building, 801 Sherbrooke St. W., H3A 2K6 Montreal, Quebec, Canada; Fax: +1 514 398 3797; E-mail: Masad.damha@mcgill.ca.

DOI: 10.1081/NCN-120023043
1525-7770 (Print); 1532-2335 (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

(lariat RNA introns).[2] The atypical architecture of these oligonucleotides renders them resilient to general endo- or exonuclease activity and as such, recognition and degradation by DBR is central to intron turnover and nucleoside regeneration.[3] At the present time, the minimal substrate requirements of DBR have been investigated,[4] however little is known about the influence of branchpoint sugar conformation on enzyme selectivity. The incorporation of 'non-natural' residues as branchpoints in the synthesis of Y-shaped nucleic acids that mimic the native substrates enables us to dissect and examine the influence of sugar conformation upon the debranching process.

Synthesis of lariat intron mimics involves the coupling of a 2′,3′-O-bis-adenosine phosphoramidite to the free hydroxyl termini of two adjacent support bound linear RNA molecules followed by chain extension in the conventional 3′-5′ direction to yield the Y-shaped RNAs.[5] Bis-phosphoramidites containing the wild-type ribose (**1**), its corresponding 2′ and 3′-epimers, arabinose (**2**) and xylose (**3**), and its 2′,3′-acyclic analog, "*seco*"-ribose (**4**) were synthesized from their appropriately protected nucleosides as described previously.[6] The branching synthons were characterized by FAB-MS (NBA matrix) and ^{31}P-NMR (see Fig. 1). The introduction of phosphityl groups at the 2′ and 3′-furanose oxygens generates two new chiral centers and gives rise to 4 diastereomers, and an expected 8 signals in the proton decoupled ^{31}P-NMR spectrum. However, an unforeseen 16 peaks were seen for **1**, indicating the presence of either a through-bond or through-space P-P interaction.[6] The precise origin for this observed splitting effect is discerned by spectral analysis of **3**. In this case, no splitting of the signals was observed, thus ruling out the existence of $^{5}J_{P\text{-}P}$ coupling. Presumably, **3** exists exclusively in a C3′-*endo* sugar pucker, resulting in a *trans*-diaxial arrangement of the 2′ and 3′-phosphite groups which places the electron lone pairs sufficiently far from each other and abolishes any through space effects. This further establishes an O4′-*endo* and/or C3′-*endo* conformation for **2**, as these are



	Compound	# of peaks ^{31}P NMR	# of coupled nuclei	Major Conformer
1	**Bis-ribo-A** R_1,R_4=H R_2,R_3=OP(O(CH$_2$)$_2$CN)N*i*Pr$_2$	16	8	C2′-endo
2	**Bis-arabino-A** R_2,R_4=H R_1,R_3=OP(O(CH$_2$)$_2$CN)N*i*Pr$_2$	16	8	O4′-endo C3′-endo
3	**Bis-xylo-A** R_1,R_3=H R_2,R_4=OP(O(CH$_2$)$_2$CN)N*i*Pr$_2$	7	0	C3′-endo
4	**Bis-seco-A** R_1,R_2=OP(O(CH$_2$)$_2$CN)N*i*Pr$_2$	6	0	N/A

Figure 1. Proton-decoupled ^{31}P-COSY-NMR splitting characteristics of 2′,3′-O-bis-adenosine phosphoramidites used as branching synthons in Y-RNA synthesis. Spectra were obtained on a Mercury 400 spectrometer using CD_3CN as the solvent. All signals were centred in the 149–153 ppm range.

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

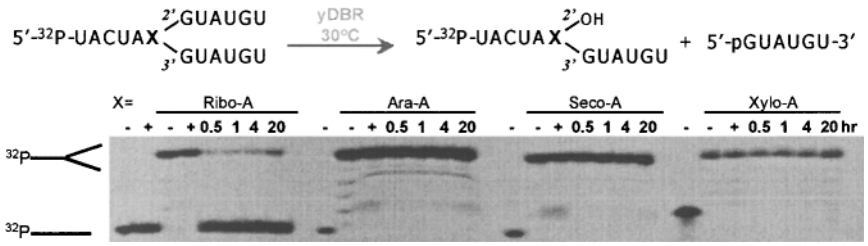


Figure 2. yDBR digestion assays of branched RNA analogs. Reactions contained 5′-^{32}P-labelled branched substrates (500 fmol) dissolved in 125 mM KCl, 0.5 mM $MgCl_2$, 1 mM DTT, 10% glycerol and 20 mM HEPES at pH 7.6. Reactions were initiated by the addition of yDBR (50U). Reaction products were resolved by 16% polyacrylamide sequencing gel containing 7 M urea and visualized by autoradiography.

the only conformations that support splitting of the phosphorus chemical shifts via electron lone pairs.

The incorporation of branching synthons **1-4** into Y-RNA molecules related to the intronic portion of yeast actin pre-mRNA, allowed us to examine the effect of sugar conformation on branchpoint recognition and the efficiency of 2′-bond scission by yDBR (Fig. 2).[7] Selective hydrolysis of the 2′,5′-phosphodiester bond by yDBR was evident only in the wild-type ribo-A branched RNA, and was deemed nearly complete after only 30 min. On the other hand, the branchpoint analogs were resistant to enzyme hydrolysis even after a 20 h incubation period, suggesting that the local orientation of vicinal phosphate groups at the branching sugar dramatically affects debranching efficacy (i.e., *cis*-arrangement). Moreover, preliminary results have indicated a concentration dependent inhibition of wild-type Y-RNA debranching in the presence of ara-A branched RNA, such that a 50% reduction in debranching efficiency was observed at a concentration of 0.45 μM of inhibitor (data not shown). Such an inhibitor would signify a potential choice for enzyme co-crystallization and X-ray analysis of the yDBR active site structure.

Our results indicate that only ribose as a branchpoint directs optimal debranching activity, suggesting that the configuration of vicinal phosphate groups and/or sugar pucker of the branchpoint to be a crucial element for optimal enzyme recognition and hydrolytic efficacy. Further studies would include the use of conformationally locked ribo-branchpoints and other branchpoints containing a *cis*-arrangement of vicinal phosphate groups (e.g., L-ribo-A). Such a study would allow us to elucidate the particular nature of the structural effects that collectively contribute to enzymatic recognition and subsequent processing events.

ACKNOWLEDGMENTS

We wish to thank NSERC (Canada) for financial support and NSERC (K.A.A) and FCAR (K.A.A. and S.C) post-graduate funding. We are grateful to

Dr. Jef D. Boeke (Johns Hopkins University) for his generous donation of purified yDBR.

REFERENCES

1. Chapman, K.B.; Boeke, J.D. Isolation and characterization of the gene encoding yeast debranching enzyme. Cell **1991**, *65*, 483–492.
2. Wallace, J.C.; Edmonds, M. Polyadenylated nuclear RNA contains branches. Proc. Natl. Acad. Sci. USA. **1983**, *80*, 950–954.
3. Nam, K.; Lee, G.; Trambley, J.; Devine, S.E.; Boeke, J.D. Severe growth defect in a *schizosaccharomyces pombe* mutant defective in intron lariat degradation. Mol. Cell. Biol. **1997**, *17* (2), 809–818.
4. Nam, K.; Hudson, R.H.E.; Chapman, K.B.; Ganeshan, K.; Damha, M.J.; Boeke, J.D. Yeast lariat debranching enzyme: substrate and sequence specificity. J. Biol. Chem. **1994**, *269* (32), 20,613–20,621.
5. (a) Damha, M.J.; Ogilvie, K.K.; Conformational Properties of Branched RNA Fragments in aqueous solution. Biochemistry **1988**, *27*, 6403–6416; (b) Damha, M.J.; Zabarylo, S. Automated solid-phase synthesis of branched oligonucleotides. Tetrahedron Lett. **1989**, *30* (46), 6295–6298.
6. Damha, M.J.; Ogilvie, K.K. Synthesis and spectroscopic analysis of branched RNA fragments: messenger RNA splicing intermediates. J. Org. Chem. **1988**, *53*, 3710–3722.
7. Ooi, S.L.; Dann, III, C.; Nam, K.; Leahy, D.J.; Damha, M.J.; Boeke, J.D. RNA lariat debranching enzyme. Methods Enzymol. **2001**, *342*, 233–250.

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