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An Atomic Mutation Cycle for Exploring RNA's 2′-Hydroxyl Group

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The 2′-hydroxyl group plays an integral role in RNA structure and catalysis. This multifunctional component of the RNA backbone influences ribose conformation and helix geometry,[1] coordinates metal ions,[2] provides a scaffold for protein or solvent interactions,[3] and mediates tertiary interactions and catalysis via hydrogen bonding.[4−7] 2′-Deoxyribonucleotide substitution reveals the energetic importance of hydroxyl groups within RNA[4,7,8] but provides little information about the specific chemical basis for their functional contribution. In this work, we show that an atomic mutation cycle reveals whether a hydroxyl group imparts a functional contribution via hydrogen bond donation.

The cycle describes the energetic effects from three atomic mutations (2′-OCH_3, 2′-NH_2, and 2′-$NHCH_3$) relative to the ribonucleotide (2′-OH) on a RNA-mediated process (Figure 1). This approach bears some similarity to double-mutant cycles used in protein analysis,[9−11] except that mutations occur at the level of atoms rather than residues: the −O− and −H atoms of the 2′-hydroxyl group are mutated to −NH− and $-CH_3$, respectively. The left vertical of the cycle replaces the hydrogen atom of the hydroxyl group with a methyl group. A 2′-methoxynucleotide may induce deleterious effects either from removal of the hydrogen atom or from introduction of the relatively bulky, hydrophobic methyl group (eq 1a), obscuring whether the 2′-hydroxyl group acts a hydrogen bond donor. Illustrating the latter effect, 2′-methoxynucleotides impose deleterious consequences on RNA folding when the tertiary structure lacks sufficient space to accommodate the $-CH_3$ group.[12]

To resolve the effect of hydrogen atom removal from that of methyl group installation, we use the right vertical of the cycle, in which a methyl group replaces one of the hydrogen atoms on an amino group. The 2′-methylaminonucleotide imposes the consequences of the bulky methyl group but, unlike the 2′-methoxynucleotide, also retains a heteroatom-bound hydrogen atom. Therefore, the energetic cost of the 2′-methylamino mutation relative to the 2′-amino mutation provides an independent measure of the effect of methyl group installation (eq 1b).[13] Energetic differences between the vertical perturbations ($\Delta\Delta G_{OH\rightarrow OCH_3}$ and $\Delta\Delta G_{NH_2\rightarrow NHCH_3}$) provide an operational estimate for $\Delta G_{H\ removal}$ (eq 1c), and thereby may implicate the 2′-hydroxyl as an important hydrogen bond donor.

$$\Delta\Delta G_{OH\rightarrow OCH_3} = \Delta G_{H\ removal} + \Delta G_{CH_3\ installation} \quad (1a)$$

$$\Delta G_{CH_3\ installation} \approx \Delta\Delta G_{NH_2\rightarrow NHCH_3} \quad (1b)$$

$$\Delta G_{H\ removal} \approx \Delta\Delta G_{OH\rightarrow OCH_3} - \Delta\Delta G_{NH_2\rightarrow NHCH_3} \quad (1c)$$

These analogues in combination revealed the effect of methyl group installation at the cleavage site of a group II intron reaction.[14] However, whether the cycle faithfully exposes hydroxyl groups engaged in hydrogen bond donation remains unknown. Here we address this question using the *Tetrahymena* group I ribozyme reaction (Figure 2).[4,15,16] This ribozyme catalyzes nucleotidyl transfer between an oligonucleotide substrate and an exogenous guanosine nucleophile (eq 2). The cleavage site 2′-hydroxyl group participates in a hydrogen-bonding network via A207[17] and stimulates reaction chemistry by donating a hydrogen bond to the 3′-oxygen leaving group in the transition state.[4−6]

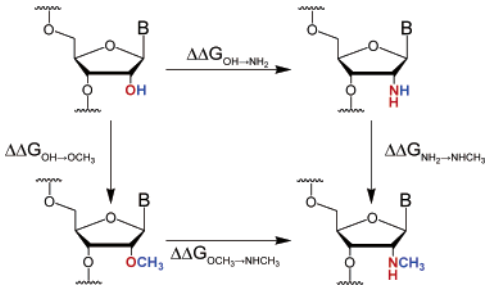

Figure 1. Atomic mutation cycle for exploring RNA's 2′-hydroxyl group.

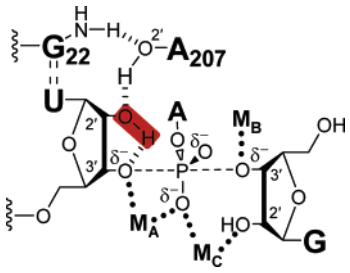

Figure 2. Transition-state model for nucleotidyl transfer catalyzed by the *Tetrahymena* group I ribozyme (adapted from ref 18). M_A, M_B, and M_C represent the known catalytic metal ions in the active site. The red oval highlights the substrate 2′-hydroxyl group under investigation. Hatched lines indicate putative hydrogen bonds, and dots symbolize metal ion coordination.

$$d(C_3UC)U_{2'X}d(A_5) + G \rightarrow d(C_3UC)U_{2'X} + Gd(A_5) \quad (2)$$
$$X = OH,\ OCH_3,\ NH_2,\ NHCH_3$$

To conduct the analysis, we used substrates containing deoxynucleotides at all positions except for the cleavage site and carried out reactions in the presence of saturating ribozyme and guanosine. These constraints ensure that all reactions start from the same ground state so that the observed rate constants monitor the same reaction steps (see ref 19 and Supporting Information). The substrate containing 2′-methoxyuridine at the cleavage site ($S_{2'OCH_3}$) reacts 143,000 ± 37,000-fold slower than does the substrate containing uridine ($S_{2'OH}$) (Figure 3), reflecting a decrease in transition-state stabilization of 7.1 ± 0.1 kcal/mol ($\Delta\Delta G^{\ddagger}_{OH\rightarrow OCH_3}$), consistent with previous reports.[4] In contrast, the substrate containing 2′-methylaminouridine ($S_{2'NHCH_3}$) reacts only 2200 ± 460-fold slower than does the substrate containing 2′-aminouridine ($S_{2'NH_2}$; $\Delta\Delta G^{\ddagger}_{NH_2\rightarrow NHCH_3}$ = 4.6 ± 0.1 kcal/mol).[20] As the 2′-hydroxyl to 2′-methoxy mutation

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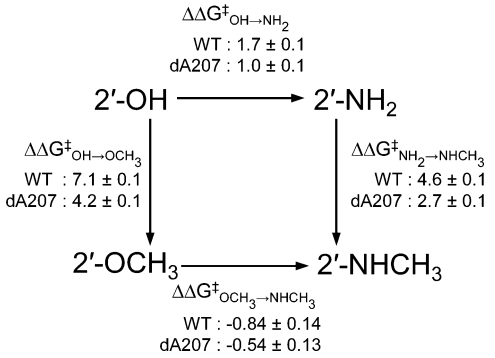

Figure 3. Atomic mutation cycles for the wild-type (WT) and mutant (dA207) *Tetrahymena* ribozymes. The $\Delta\Delta G^{\ddagger}_{X\rightarrow Y}$ values reflect differences in transition-state stabilization observed for $S_{2'X}$ relative to $S_{2'Y}$; $\Delta\Delta G^{\ddagger}_{X\rightarrow Y} = RT \ln(k_X/k_Y)$, where k_X and k_Y are the observed cleavage rates for substrates containing X and Y groups at the 2′-position, respectively. Reactions were performed at 30 °C; $\Delta\Delta G^{\ddagger}_{X\rightarrow Y}$ is reported in kcal/mol.

($S_{2'OH}$ vs $S_{2'OCH_3}$) incurs a significantly greater energetic penalty than does the 2′-amino to 2′-methylamino mutation, we infer that the hydrogen atom of the cleavage site 2′-hydroxyl group has a functionally significant role, presumably due to donation of a hydrogen bond in the transition state.[4−6,17] From the difference in these energetic penalties, the catalytic cost of removing this hydrogen atom is estimated as 2.5 ± 0.1 kcal/mol (eq 1c).[21] By definition, the horizontal perturbations of the cycle give the same result: the 2′-OCH_3 → 2′-$NHCH_3$ mutation rescues catalysis by 2.5 ± 0.1 kcal/mol after accounting for the effects of −O− → −NH− mutation.

To address the context dependence of $\Delta G_{H\ removal}$, we investigated how the A207-mediated network of hydrogen bonds[17] (Figure 2) influences the energetic contribution of the hydrogen bond donated by the cleavage site 2′-hydroxyl group. We decoupled the cleavage site 2′-hydroxyl group from the network by installing 2′-deoxyadenosine at residue 207 in the ribozyme, and we used this dA207 mutant ribozyme to conduct our analysis. The dA207 mutation mitigates the deleterious effect of the methyl group in comparison to the wild-type ribozyme (Figure 3); $S_{2'NHCH_3}$ reacts 84 ± 19-fold slower than does $S_{2'NH_2}$ ($\Delta\Delta G^{\ddagger}_{NH_2\rightarrow NHCH_3} = 2.7 \pm 0.1$ kcal/mol), as opposed to the 2200 ± 460-fold effect observed with the wild-type ribozyme ($\Delta\Delta G^{\ddagger}_{NH_2\rightarrow NHCH_3} = 4.6 \pm 0.1$ kcal/mol). This attenuation occurs presumably because the space vacated by the A207 2′-hydroxyl group better allows the active site to accommodate the steric bulk of the methyl group. For the dA207 ribozyme, the cycle estimates $\Delta G_{H\ removal} \approx (4.2 \pm 0.1$ kcal/mol -2.7 ± 0.1 kcal/mol) $\approx 1.5 \pm 0.1$ kcal/mol. The active-site hydrogen bond network therefore appears to enhance the catalytic role of the cleavage site 2′-hydroxyl group in hydrogen bond donation by ∼1.0 kcal/mol (1.5 kcal/mol vs 2.5 kcal/mol).[22]

In summary, we have shown that an atomic mutation cycle can reveal whether a given 2′-hydroxyl group within RNA donates a functionally significant hydrogen bond. To conduct the analysis, we determine by 2′-methoxynucleotide substitution the effect of replacing the 2′-hydroxyl hydrogen atom with a methyl group. We then use an amino background to account independently for the effect of methyl installation, replacing a hydrogen atom with a methyl group while retaining a heteroatom-bound hydrogen atom. When the cost of 2′-methoxynucleotide substitution exceeds that for methyl group installation, this analysis indicates that the hydrogen atom of the 2′-hydroxyl group contributes significantly to function, presumably by donating a hydrogen bond. In contrast, when the cost of 2′-methoxy substitution matches that measured for methyl group installation, the cycle provides no evidence that the 2′-hydroxyl group donates a functionally important hydrogen bond.[14] The ability to assign specific 2′-hydroxyl groups as important hydrogen bond donors provides a new strategy with which to explore the role of RNA's distinctive 2′-hydroxyl group.

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Supporting Information Available: Experimental details of substrate and ribozyme preparation, ribozyme reaction conditions, characterization of the protonation state of $S_{2'NH_2}$ and $S_{2'NHCH_3}$, and table of observed cleavage rates. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Saenger, W. In *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; p 556.
(2) (a) Smith, D.; Pace, N. R. *Biochemistry* **1993**, *32*, 5273−5281. (b) Shan, S. O.; Herschlag, D. *Biochemistry* **1999**, *38*, 10958−10975. (c) Gordon, P. M.; Sontheimer, E. J.; Piccirilli, J. A. *Biochemistry* **2000**, *39*, 12939−12952.
(3) (a) Egli, M.; Portmann, S.; Usman, N. *Biochemistry* **1996**, *35*, 8489−8494. (b) Hermann, T.; Patel, D. J. *J. Mol. Biol.* **1999**, *294*, 829−849. (c) Dertinger, D.; Dale, T.; Uhlenbeck, O. C. *J. Mol. Biol.* **2001**, *314*, 649−654. (d) Elliott, M. B.; Gottlieb, P. A.; Gollnick, P. *RNA* **2001**, *7*, 85−93. (e) Hou, Y. M.; Zhang, X. L.; Holland, J. A.; Davis, D. R. *Nucleic Acids Res.* **2001**, *29*, 976−985. (f) Juneau, K.; Podell, E.; Harrington, D. J.; Cech, T. R. *Structure* **2001**, *9*, 221−231.
(4) Herschlag, D.; Eckstein, F.; Cech, T. R. *Biochemistry* **1993**, *32*, 8312−8321.
(5) Knitt, D. S.; Narlikar, G. J.; Herschlag, D. *Biochemistry* **1994**, *33*, 13864−13879.
(6) Yoshida, A.; Shan, S.; Herschlag, D.; Piccirilli, J. A. *Chem. Biol.* **2000**, *7*, 85−96.
(7) (a) Silverman, S. K.; Cech, T. R. *Biochemistry* **1999**, *38*, 8691−8702. (b) Klostermeier, D.; Millar, D. P. *Biochemistry* **2002**, *41*, 14095−14102.
(8) (a) Abramovitz, D. L.; Friedman, R. A.; Pyle, A. M. *Science* **1996**, *271*, 1410−1413. (b) Schwans, J. P.; Cortez, C. N.; Olvera, J. M.; Piccirilli, J. A. *J. Am. Chem. Soc.* **2003**, *125*, 10012−10018.
(9) Horovitz, A.; Fersht, A. R. *J. Mol. Biol.* **1990**, *214*, 613−617.
(10) Horovitz, A. *Folding Des.* **1996**, *1*, R121−R126.
(11) Fersht, A. *Structure and Mechanism in Protein Science*; W. H. Freeman and Co.: New York, 1999; p 131.
(12) Schwans, J. P. Doctoral thesis, University of Chicago, 2003.
(13) This analysis suffers from the same caveats as the double-mutant cycles used for protein analysis:[9] mutations that introduce changes in structure or favorable, non-native interactions obscure the value of $\Delta G_{H\ removal}$.
(14) Gordon, P. M.; Fong, R.; Deb, S. K.; Li, N. S.; Schwans, J. P.; Ye, J. D.; Piccirilli, J. A. *Chem. Biol.* **2004**, *11*, 237−246.
(15) Zaug, A. J.; Grosshans, C. A.; Cech, T. R. *Biochemistry* **1988**, *27*, 8924−8931.
(16) Herschlag, D. *Biochemistry* **1992**, *31*, 1386−1399.
(17) Strobel, S. A.; Ortoleva-Donnelly, L. *Chem. Biol.* **1999**, *6*, 153−165.
(18) Shan, S. O.; Kravchuk, A. V.; Piccirilli, J. A.; Herschlag, D. *Biochemistry* **2001**, *40*, 5161−5171.
(19) Shan, S. O.; Herschlag, D. *RNA* **2000**, *6*, 795−813.
(20) The cleavage rates for $S_{2'NH_2}$ and $S_{2'NHCH_3}$ represent the reactions of the neutral states of the amine and methylamine, respectively (see Supporting Information).
(21) If the presence of the methyl group deleteriously affects the ability of the 2′-methylamino group to donate a hydrogen bond relative to the 2′-amino group (due to steric, conformational, or other factors), then the 2′-amino/2′-methylaminonucloside comparison may overestimate the cost of methyl group installation, thereby underestimating the energetic cost of hydrogen atom removal.
(22) Consistent with this conclusion, 2′-deoxynucleotide substitution at the cleavage site diminishes wild-type and dA207 mutant ribozyme activity by ∼1000-fold and ∼100-fold, respectively.[17]

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