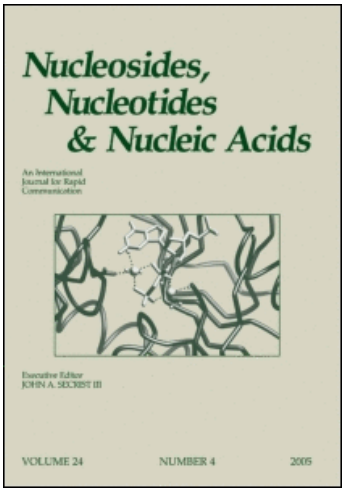

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AMINO-LINKED RIBOZYMES: POST-SYNTHETIC CONJUGATION OF HALF-RIBOZYMES

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ABSTRACT: A convergent approach, based on the reductive amination of 3'-phosphoglycaldehyde-ribozyme **3** with 5'-aminohexyl-ribozyme **1** generated an amino-linked ribozyme **4** in good yields. Catalytic activity of the cross-linked ribozyme is discussed.

INTRODUCTION

Trans-cleaving hammerhead ribozymes[1] show great promise as therapeutic agents due to their inherent catalytic activity combined with highly-specific binding to a defined target RNA.[2] Improvements in the chemical synthesis of RNA[3] have led to the site-specific introduction of various chemical modifications in ribozymes providing nuclease resistance[4] and enhanced catalytic activity.[5] As part of an ongoing effort to increase the overall yield of ribozyme synthesis, our group has designed an alternative approach[6] where two half-ribozymes are synthesized using known solid-phase methodologies, and chemically ligated through a covalent linkage, post-synthetically. A critical requirement is that the site of chemical ligation must not interfere with the ribozyme core to ensure that full catalytic activity is retained. It has been previously shown that the stem II and/or loop II of the hammerhead ribozyme are not essential for catalytic activity.[6,7] Similarly to our previous work[6], the standard loop II[5] was deleted to allow introduction of the appropriate chemical functionalities.

REDUCTIVE AMINATION COUPLING OF HALF-RIBOZYMES

The stem II/loop II region of the chemically stabilized 37-mer ribozyme RPI.3718[5,8] was modified to accomodate post-synthetic chemical coupling. We selected a

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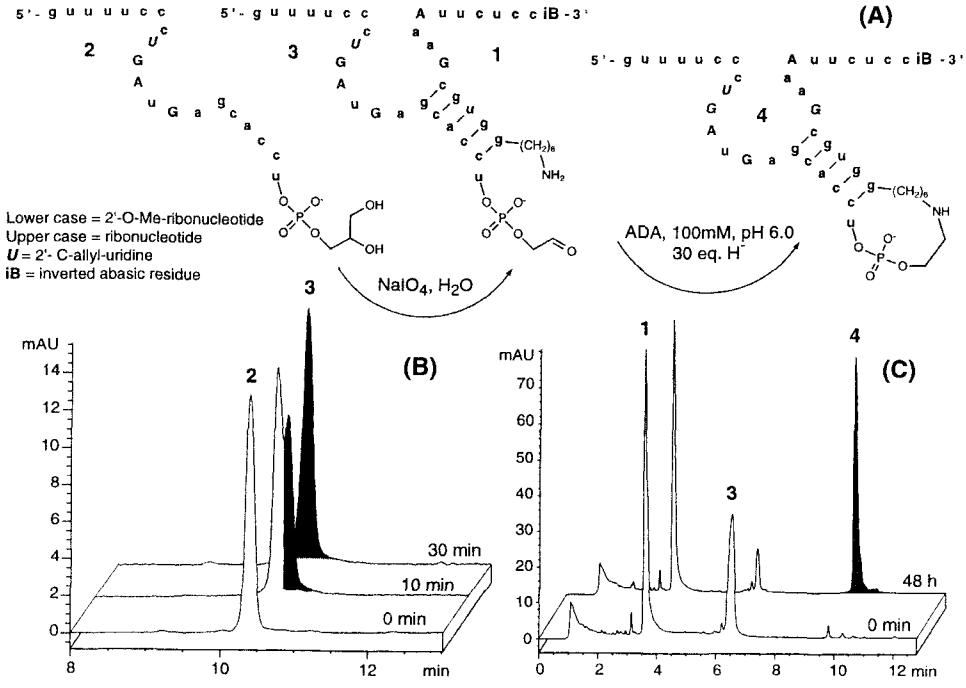

FIGURE 1. (A) Oxidative cleavage of **2** and coupling of **3** with **1** under reductive amination conditions. (B) Anion-exchange HPLC analysis of the oxidative cleavage reaction after 0, 10 and 30 min. (C) Anion-exchange HPLC analysis of the BH_3.Pyr.-mediated reductive amination of **3** with **1** after 0 and 48 h.

linear amino-linkage to covalently bridge the 5'- and 3'-half-ribozymes. Half-ribozymes **1** and **2** (FIG. 1A) derived from RPI.3718, were synthesized on a 2.5 μmol scale on glyceryl-CPG[9] and on an inverted abasic residue loaded on polystyrene,[10] respectively. Following purification according to standard methods,[3] the 3'-phosphoglyceryl-5'-half-ribozyme **2** (125 μM aqueous solution) was subjected to oxidative cleavage with 10 molar equivalents of a 100 mM aqueous solution of sodium periodate (FIG. 1A).

Complete conversion of **2** into the 3'-phosphoglycaldehyde-5'-half-ribozyme **3** could be observed within 30 min (FIG. 1B). Half-ribozymes **3** (125 μM) and **1** (500 μM) were then reacted with 30 molar equivalents of reducing agent (TABLE 1) in sodium N_2-acetamido-2-imino-diacetate (ADA) buffer (100 mM) at pH 6.0 for 48 hours.

The borane.pyridine complex produced a high yield (TABLE 1) of the amino-linked ribozyme **4** (FIG. 1C). After HPLC purification, **4** was identified as the desired amino-linked ribozyme on the basis of ES-MS analysis (calcd 11928.6, found 11929.0).

TABLE 1. Synthesis of the amino-linked ribozyme **4**. Yields are calculated based on the disappearance of the limiting reagent **3**. [a] 500 mM in H_2O, 7 days, [b] 100 mM in H_2O, [c] 2.5 mmol eq. $BH_4^-.g^{-1}$ of resin, [d] 80 mM in EtOH. NR: no reaction after 48 h.

Reagent	$NaBH_3CN$ a	$NaBH(OAc)_3$ b	Amberlyst A-26 BH_4 c	$NaBH_4$ b	BH_3.Pyr d	BH_3.$HNMe_2$ b
Yield of **4**	50.2%	NR	NR	NR	81.2%	4.4%

TABLE 2. Cleavage rate of a RNA short substrate by the "active" ribozyme **4**, its "inactive" analog and RPI.3718.[5] Over 82%, 55% and ~0% of the RNA short substrate was cleaved over one hour with ribozymes RPI.3718, **4** and inactive **4** respectively. [a] [RNA substrate] ~ 1nM, [Rz] = 500 nM, 50 nM tris.HCl pH 8.0, 37 °C, 40 mM Mg^{2+}.

Ribozyme	"active" **4**	"inactive"	RPI.3718
$k_{obs}.(min^{-1})$ [a]	0.012	<0.0001	0.144

CATALYTIC ACTIVITY OF THE AMINO-LINKED RIBOZYMES

It was critical to ascertain the effect of the amino-linkage on the rate of catalytic cleavage. "Active" **4**, its "inactive" counterpart containing two mutations in the catalytic core that abolish cleavage activity,[5,8] as well as the control RPI.3718, were assayed[5] at ribozyme saturation for their cleavage rate on short RNA substrate (TABLE 2).

Although the amino-linked ribozyme **4** was approximately ten times slower than RPI.3718 in the early time points, the extent of cleavage over one hour was 53% and 82%, respectively (TABLE 2), confirming that one can extensively modify the loop II/stem II region without dramatically effecting cleavage efficiency. As expected, the inactive amino-linked ribozyme completely lacked detectable cleavage activity.

CONCLUSION

Our results demonstrate that post-synthetically amino-linked ribozymes possess the necessary catalytic activity required to be considered as alternatives to solid-phase synthesized ribozymes. A large-scale quantitative comparison of this approach with solid-phse synthesis is under investigation and will be reported elsewhere.

REFERENCES

(1) Cech, T. R. *Curr. Opin. Struc. Biol.* **1993**, *2*, 605-609.

(2) Christoffersen, R. E.; Marr, J. J. *J. Med. Chem.* **1995**, *38*, 2023-2037.

(3) Wincott, F.; DiRenzo, A.; Shaffer, C.; Grimm, S.; Tracz, D.; Workman, C.; Sweedler, D.; Gonzalez, C.; Scaringe, S.; Usman, N. *Nucleic Acids Res.* **1995**, *23*, 2677-2684.

(4) Usman, N.; Cedergren, R. J. *Trends in Biochem. Sci.* **1992**, *17*, 334-339.

(5) Beigelman, L.; McSwiggen, J.; Draper, K.; Gonzalez, C.; Jensen, K.; Karpeisky, A.; Modak, A.; Matulic-Adamic, J.; DiRenzo, A.; Haeberli, P.; Sweedler, D.; Tracz, D.; Grimm, S.; Wincott, F.; Usman, N. *J. Biol. Chem.* **1995**, *270*, 25702-25708.

(6) Bellon, L.; Workman, C.; Sherrer, J.; Usman, N.; Wincott, F. *J. Am. Chem. Soc.* **1996**, *118*, 3771-3772.

(7) Tuschl, T.; Eckstein, F. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 6991-6994. Benseler, F.; Fu, D. J.; Ludwig, J.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1993**, *115*, 8483-8484.

(8) Jarvis, T. C.; Alby, L. J.; Beaudry, A. A.; Wincott, F. E.; Beigelman, L.; McSwiggen, J. A.; Usman, N.; Stinchcomb, D. T. *RNA.* **1996**, *2*, 419-428.

(9) Urata, H.; Akagi, M. *Tetrahedron Lett.* **1993**, *34*, 4015-4018.

(10) Ortigao, J. F. R.; Rosch, H.; Selter, H.; Frohlich, A.; Lorenz, A.; Montenarh, M.; Seliger, H. *Antisense Res. Dev.* **1992**, *2*, 149-146.