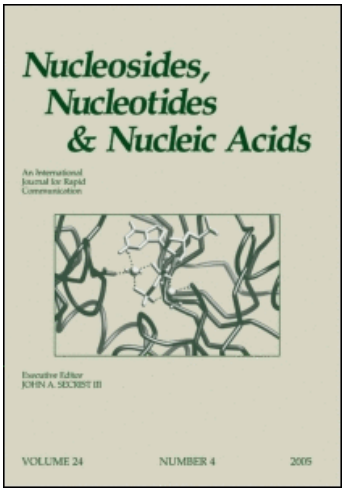

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Structural Requirements at the 15.1-16.1 Position of the Hammerhead Ribozyme

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STRUCTURAL REQUIREMENTS AT THE 15.1-16.1 POSITION OF THE HAMMERHEAD RIBOZYME

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ABSTRACT: Apart from the $A^{15.1}$•$U^{16.1}$ -> $I^{15.1}$•$C^{16.1}$ change which reverses the polarity of an important H-bond in the hammerhead structure no other functional group changes at the 15.1 purine residue seem to be compatible with the requirements of efficient catalysis. The $I^{15.1}$ and $A^{15.1}$ ribozymes are equally suitable for practical applications because there are only minor differences in the acceptance of stabilising residues.

3D structure based rational engineering of the hammerhead ribozyme enabled the design of a molecule which cleaves efficiently at new non-natural NCH sites[1]. The availability of inosine$^{15.1}$ ribozymes which cleave the 12 NCH triplets enables the specific targeting of several oncogene mutations and improves hammerhead ribozyme based gene inactivation strategies by allowing targeting at previously inaccessible mRNA sites.

To complete previous kinetic comparisons[1] the acceptance of various core substitution patterns was compared with NUH/$A^{15.1}$ and NCH/$I^{15.1}$ systems. Short substrates containing GCH and GUH (H=non G) triplets were compared using 3 different modified ribozymes (Table I)[2,3,4].

Table 1 Comparison of single turnover cleavage rates. Conditions: pH 6.0, 250 nM substrate , 2.5 µM ribozyme, 10 mM Mg^{++}

GCH/GUH k_{obs} min^{-1}	all-ribo	G^5, A^6, G^8, G^{12}, $I^{15.1}/A^{15.1}$ ribonucleotides 2′-O-allyl enviroment		
		U^4= ribo U	U^4= 2′-NH_2 U	U^4 = 2′-O-alkylU
GCA / GUA	0.38 / 0.12	0.10 / 0.06	0.08 / 0.04	0.02 / 0.01
GCC / GUC	0.18 / 0.13	0.03 / 0.015	0.01 / 0.014	0.003 / 0.001
GCU / GUU	0.028 / 0.04	0.025 / 0.031	0.013 / 0.012	0.002 / 0.008

U^4= 2′-O-alkyl, U^4 =NH_2dU and U^4 =riboU substituted ribozymes show similar activity with the NCH and with the natural NUH triplets.

Comparison of multiple turnover parameters at pH 7.4, 10 mM Mg^{++}, 37 °C gave K_m = 100 nM and k_{cat} =6.5 min^{-1} for GCA vs K_m=30 nM and k_{cat} =2.0 min^{-1} for GUA cleaving all ribo ribozymes. To gain more insight into the structural requirements at the 15.1-16.1 position of the hammerhead ribozyme we synthesised several variants of the active $I^{15.1}$•$C^{16.1}$ structure and tested these ribozyme analogues with their complementary substrates. The results are summarised in Fig 1.

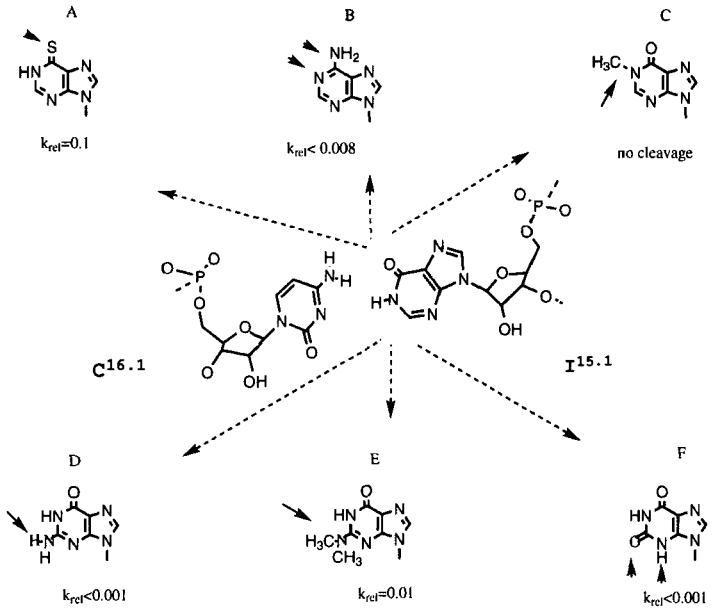

Fig.1 Middle line shows the relative orientation of the bases of the $I^{15.1}$•$C^{16.1}$ base pair, k_{rel} values describe the single turnover cleavage rates relative to $I^{15.1}$

At the purine$^{15.1}$ N1 and/or C6 site no modifications were tolerated which influence the H-bond interaction with $C^{16.1}$, except 6-thio-inosine substitution which reduced the activity 10-fold. At the purine$^{15.1}$ C2 and/or N3 site , modifications which allow the formation of a regular base pair with the 16.1 pyrimidine and changes at the N3 position were not tolerated.

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