

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

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

J. Ludwig<sup>a</sup>; M. Blaschke<sup>a</sup>; M. Dunkel<sup>a</sup>; B. Beijer<sup>a</sup>; K. Ross<sup>a</sup>; B. S. Sproat<sup>a</sup>

<sup>a</sup> Innovir GmbH, Rosdorf, Germany

**To cite this Article** Ludwig, J. , Blaschke, M. , Dunkel, M. , Beijer, B. , Ross, K. and Sproat, B. S.(1999) 'Structural Requirements at the 15.1-16.1 Position of the Hammerhead Ribozyme', *Nucleosides, Nucleotides and Nucleic Acids*, 18: 6, 1519 – 1520

**To link to this Article:** DOI: 10.1080/07328319908044774

**URL:** <http://dx.doi.org/10.1080/07328319908044774>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

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

J.Ludwig, M.Blaschke, M.Dunkel, B.Beijer, K.Ross and B.S.Sproat

Innovir GmbH, Olenhuser Landstrasse 20b, D-37124 Rosdorf, Germany

**ABSTRACT:** Apart from the  $A^{15.1} \cdot U^{16.1} \rightarrow I^{15.1} \cdot C^{16.1}$  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<sup>1</sup>. The availability of inosine<sup>15.1</sup> 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<sup>1</sup> 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)<sup>2,3,4</sup>.

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

| GCH/GUH<br>$k_{obs}$ min <sup>-1</sup> | all-ribo     | G <sup>5</sup> , A <sup>6</sup> , G <sup>8</sup> , G <sup>12</sup> , I <sup>15.1</sup> /A <sup>15.1</sup> ribonucleotides<br>2'-O-allyl environment |                                       |                              |
|--|--------------|---|---------------------------------------|------------------------------|
|  |              | U <sup>4</sup> = ribo U   | U <sup>4</sup> = 2'-NH <sub>2</sub> U | U <sup>4</sup> = 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<sup>4</sup>= 2'-O-alkyl, U<sup>4</sup>=NH<sub>2</sub>dU and U<sup>4</sup>=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  $k_{cat} = 6.5 \text{ min}^{-1}$  for GCA vs  $K_m = 30$  nM and  $k_{cat} = 2.0 \text{ min}^{-1}$  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  $I^{15.1} \cdot C^{16.1}$  structure and tested these ribozyme analogues with their complementary substrates. The results are summarised in Fig 1.

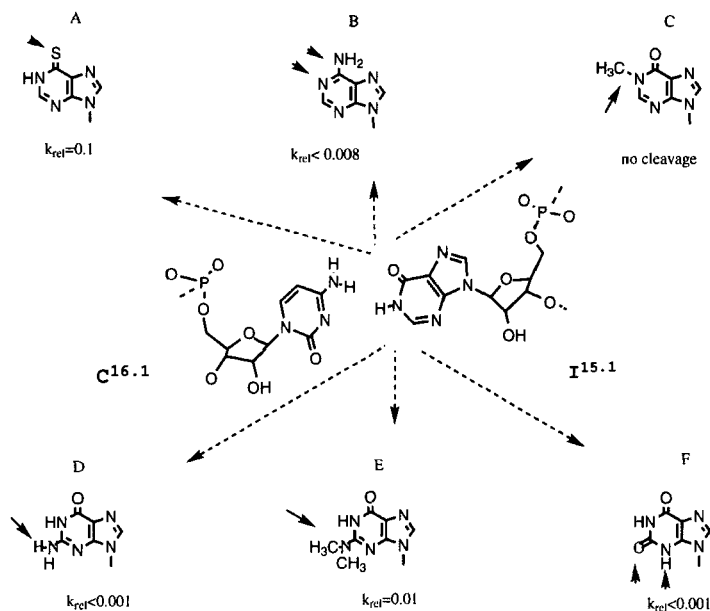


Fig.1 Middle line shows the relative orientation of the bases of the  $I^{15.1} \cdot C^{16.1}$  base pair,  $k_{rel}$  values describe the single turnover cleavage rates relative to  $I^{15.1}$

At the purine<sup>15.1</sup> 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<sup>15.1</sup> 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.

#### REFERENCES

1. J. Ludwig, M. Blaschke and B. S. Sproat, *Nucleic Acids Res* **26**, 2279-85 (1998).
2. W. A. Picken, D. B. Olsen, F. Benseler, H. Aurup and F. Eckstein, *Science* **253**, 314-7 (1991).
3. G. Paoletta, B. S. Sproat and A. I. Lamond, *EMBO J* **11**, 1913-9 (1992).
4. S. P. Lyngstadaas, S. Risnes, B. S. Sproat, P. S. Thrane and H. P. Prydz, *Embo J* **14**, 5224-9 (1995).