

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>

## Mechanism and Specificity of RNA Cleavage by Chemical Ribonucleases

N. Beloglazova<sup>a</sup>; A. Vlassov<sup>a</sup>; D. Konevets<sup>a</sup>; V. Sil'nikov<sup>a</sup>; M. Zenkova<sup>a</sup>; R. Giege<sup>b</sup>; V. Vlassov<sup>a</sup>

<sup>a</sup> Institute of Bioorganic Chemistry, Novosibirsk, Russia <sup>b</sup> Institut de Biologie Moléculaire et Cellulaire du C.N.R.S., Strasbourg, France

**To cite this Article** Beloglazova, N. , Vlassov, A. , Konevets, D. , Sil'nikov, V. , Zenkova, M. , Giege, R. and Vlassov, V.(1999) 'Mechanism and Specificity of RNA Cleavage by Chemical Ribonucleases', *Nucleosides, Nucleotides and Nucleic Acids*, 18: 6, 1463 – 1465

**To link to this Article:** DOI: 10.1080/07328319908044753

**URL:** <http://dx.doi.org/10.1080/07328319908044753>

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.

## MECHANISM AND SPECIFICITY OF RNA CLEAVAGE BY CHEMICAL RIBONUCLEASES.

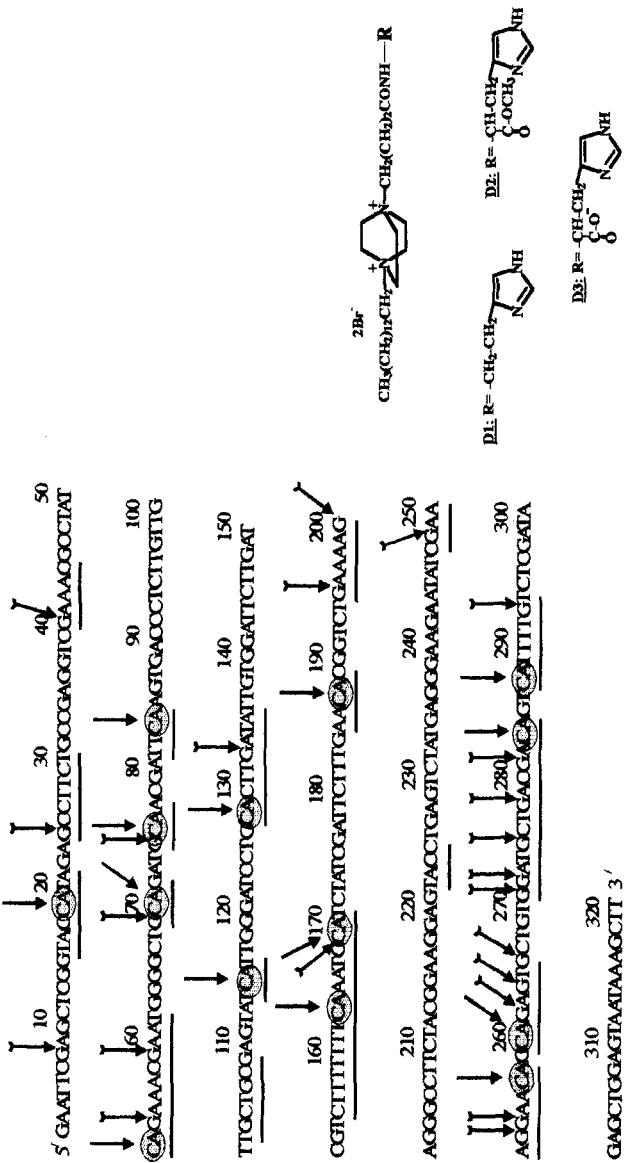
*N.Beloglazova, A.Vlassov, D.Konevetc, V.Sil'nikov, M.Zenkova, R.Giege<sup>#</sup>, V. Vlassov\**

Institute of Bioorganic Chemistry, Lavrentiev ave.8, Novosibirsk 630090, Russia

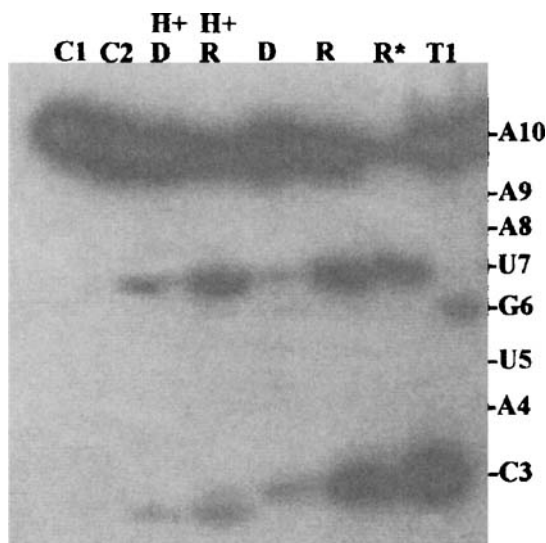
<sup>#</sup>Institut de Biologie Moléculaire et Cellulaire du C.N.R.S., Strasbourg, France

**ABSTRACT:** Cleaving of model RNA substrates by chemical ribonucleases constructed by conjugation of 1,4 diazabicyclo[2,2,2]octane with histamine and histidine was investigated. Similarly to RNase A, the chemical RNases produce fragments with 5' hydroxy-group and 3'-cyclophosphate. The cleavage occurs as the catalytic reaction: more than 150 phosphodiester bonds in RNA can be cleaved by one molecule of RNase mimic.

Reagents capable of cleaving RNA under physiological conditions hold promise for probing RNA structure in solution and as reactive groups for antisense oligonucleotide conjugates [1]. We have synthesized conjugates of 1,4-diazabicyclo[2,2,2]octane with imidazole (histamine) group (D1) and imidazole and carboxylic groups (histidine) (D2 and D3) to imitate typical structures found in active centers of ribonucleases [2]. Ribonuclease activity of the compounds D1 - D3 was studied using oligoribonucleotide (10 mer), yeast tRNA<sup>Phe</sup> and influenza virus M2 protein RNA (M2 RNA) as model substrates. The reaction was performed at 37°C in 50 mM Imidazole buffer pH 7.0. It was found that all the compounds demonstrate substantial ribonuclease activity: 60, 90 and 100% of tRNA<sup>Phe</sup> was depolymerised during 18 h incubation by D1, D2 and D3, respectively. Complete cleavage of UUCAUGUAAA, M2 RNA and tRNA<sup>Phe</sup> by D3 required 4 h, 3 h and 18 h incubation, respectively, indicating that the rate of RNA hydrolysis was strongly affected by the RNA structure stability. The chemical RNases attack preferentially single-stranded regions of RNA (Fig.1). The rate of phosphodiester bonds hydrolysis by the compounds decreases in the order CA> UG >> CG, UG>>UC, CC, UU, AA, GG.



**FIG.1.** Cleavage of the *in vitro* transcript of full-length influenza virus M2 protein RNA by chemical ribonucleases D1 - D3 (→). Comparison with probing of the RNA structure with RNase T1 (↔). Single-stranded regions sensitive to RNase ONE are underlined.



**FIG.2** Cleavage of the 5'-end labelled oligoribonucleotide pUUCAUGUAAA by chemical ribonuclease D3 and by RNase A. Lanes: C1 - control without treatment, C2 - control treated with 0.1 M HCl; D  $H^+$  - oligonucleotide cleaved by D3 and treated with 0.1 M HCl; R  $H^+$  - oligonucleotide cleaved by RNase A and treated with 0.1 M HCl; D - oligonucleotide cleaved by D3; R and R\* - oligonucleotide cleaved by RNase A at 20°C and 50°C respectively; T1 - oligonucleotide cleaved by RNase T1.

Reaction conditions: Oligonucleotide was incubated in 50 mM imidazole pH 7.0, containing 200 mM KCl, 100 µg/ml of tRNA carrier with  $5 \cdot 10^{-4}$  M D3 for 1.5 h at 37°C or with  $10^{-6}$  U of RNase A for 15 min at 20°C or for 5 min at 50°C.

Similarly to RNase A, chemical constructs D1-D3 cleave RNA to fragments with 5'-hydroxyl group and 3'-cyclophosphate as it follows from electrophoretic mobilities of the fragments (Fig.2). Concentration dependences of the reaction show that the process is catalytic: more than 150 phosphodiester bonds can be cleaved by one molecule of D3.

#### ACKNOWLEDGEMENT

This work was supported by the grants INTAS 96-1418 and RFBR 96-03-32361a.

#### REFERENCES

1. Vlassov, V.; Sil'nikov, V.; Zenkova, M. *Molecular Biology* (Moscow) **1998**, 32, 50-57.
2. Konevets, D.; Beck, I.; Beloglazova, N.; Sulimenkov, I.; Sil'nikov, V.; Zenkova, M.; Shishkin, G.; Vlassov, V. *Tetrahedron*, **1998**, accepted.