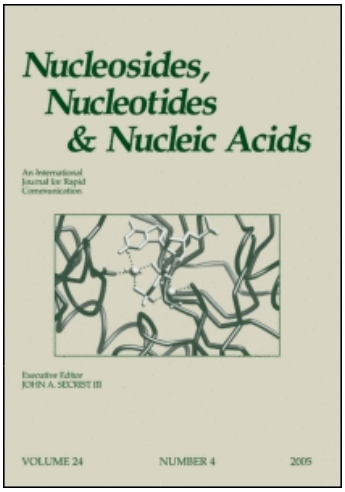

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Mechanism and Specificity of RNA Cleavage by Chemical Ribonucleases

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MECHANISM AND SPECIFICITY OF RNA CLEAVAGE BY CHEMICAL RIBONUCLEASES.

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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 $tRNA^{Phe}$ 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 $tRNA^{Phe}$ was depolymerised during 18 h incubation by D1, D2 and D3, respectively. Complete cleavage of UUCAUGUAAA, M2 RNA and $tRNA^{Phe}$ 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.

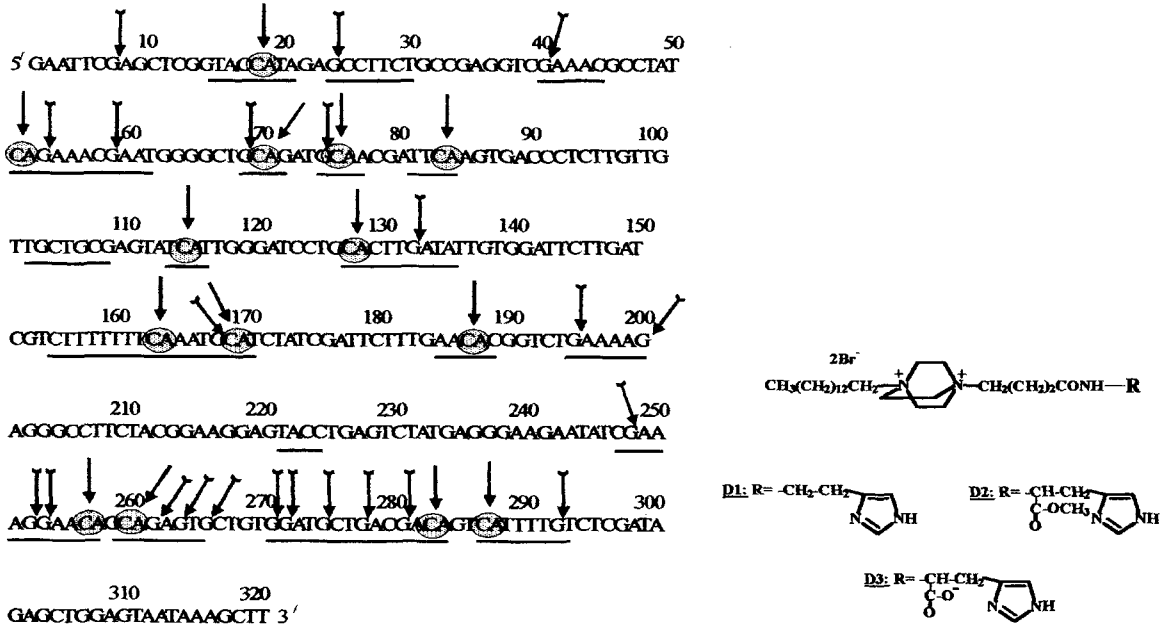

FIG.1. Cleavage of the *in vitro* transcript of full-length influenza virus M2 protein RNA by chemical ribonucleases D1 - D3 (→). Comparison with probing of the RNA structure with RNase T1 (↦). Single-stranded regions sensitive to RNase ONE are underlined .

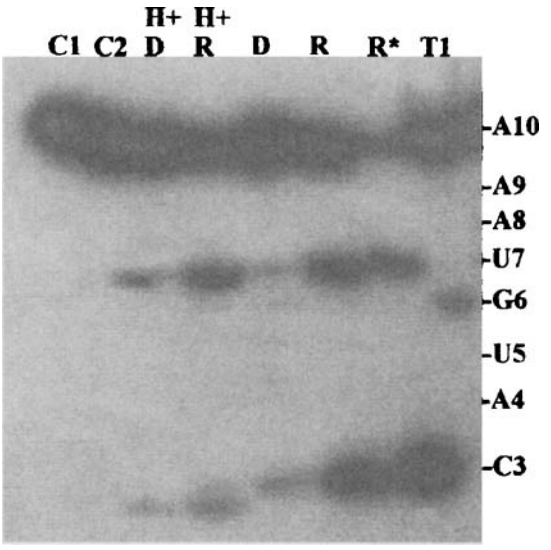

FIG.2 Cleavage of the 5'-end labelled oligoribonucleotide pUUCAUGUAAA by chemical ribonuclease D3 and by RNase A. Lanes: **C1** - control without treatment, **C2**- control treated with 0.1 M HCl; **D** $\mathbf{H^+}$ - oligonucleotide cleaved by D3 and treated with 0.1 M HCl; **R** $\mathbf{H^+}$ - 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 20^0C and 50^0C respectively; **T**1 - oligonucleotide cleaved by RNase T1.
Reaction conditions: Oligonucleotide was incubated in 50 mM imidazole pH 7.0, containing 200 mM KCl, 100μg/ml of tRNA carrier with $5\cdot10^{-4}$ M D3 for 1.5 h at 37^0C or with 10^{-6} U of RNase A for 15 min at 20^0C or for 5 min at 50^0C.

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

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