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# Cleavage of RNA by an amphiphilic compound lacking traditional catalytic groups

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**Abstract**

Recently, in experiments with combinatorial libraries of amphiphilic compounds lacking groups, known as catalysts of transesterification reaction, we discovered novel RNA-cleaving compounds [N. Kovalev, E. Burakova, V. Silnikov, M. Zenkova, V. Vlassov, Bioorg. Chem. 34 (2006) 274–286]. In the present study, we investigate cleavage of RNA by the most active representative of these libraries, compound named Dp12. Sequence-specificity of RNA cleavage and influence of reaction conditions on cleavage rate suggested that Dp12 enormously accelerates spontaneous RNA cleavage. Light scattering experiments revealed that the RNA cleavage proceeds within multiplexes formed by assembles of RNA and Dp12 molecules, at Dp12 concentration far below critical concentration of micelle formation. Under these conditions, Dp12 is presented in the solution as individual molecules, but addition of RNA to this solution triggers formation of the multiplexes. The obtained data suggest a possible mechanism of RNA cleavage, which includes interaction of the compound with RNA sugar-phosphate backbone resulting in changing of ribose conformation. This leads to juxtaposition of the 2′-hydroxyl group and internucleotide phosphorus atom at a distance needed for the transesterification to occur.


*Keywords:* RNA cleavage; Artificial ribonucleases; Conformation effect; Hydrophobic effect; In-line conformation

## 1. Introduction

Artificial ribonucleases, synthetic compounds capable to catalyze RNA cleavage — can find important applications in molecular biology and biotechnology. A number of artificial ribonucleases capable of cleaving RNA under physiological conditions have been designed [2–19]. Most of them are built of two parts: one, which provides catalyses of transesterification and another one, which provides affinity of the compound to RNA, thus increasing local concentration of a catalyst in the vicinity of linkages to be cleaved. Catalytic part of artificial RNases (here and after aRNases) usually contains complexes of transition metals ($Zn^{2+}$ [2–4], $Eu^{3+}$ [5,6], $Pb^{2+}$ [7,8]) or organic structures bearing functionalities, known to be involved in the catalysis in the active centers of natural ribonucleases (imidazole [9,10], guanidinium, amino or carboxyl groups [11,12]). RNA-binding part of aRNases is built of cationic structures [10, 13–15], intercalating molecules [9,13,16] or antisence oligonucleotides [17–19]. The most active aRNases appeared to be the ones built of metal complexes, however recently non-metal aRNases were synthesized, which display activity comparable to that of the metal complexes [14]. These artificial ribonucleases are conjugates of 1,4-diazabicyclo [2.2.2] octane (DABCO), substituted by an aliphatic fragment at the bridge nitrogen atom and conjugated with imidazole containing structures [10,14]. In experiments with different modified DABCO-based compounds, it was found, that some truncated compounds lacking imidazole residue exhibited considerable ribonuclease activity. These compounds were built of hydrophobic and cationic structures. In experiments with combinatorial libraries of these compounds [1], we identified several efficient RNA-cleaving molecules. The most active of them (Dp12) is built of two diazabicyclo [2.2.2] octane residues,

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substituted with tetradecamethylene fragment, and connected by a rigid linker. This was a surprising finding, because the molecule does not contain groups known to participate directly in the transesterification reaction. There is no clear understanding how cationic and hydrophobic fragments conjugated together could cleave RNA. This finding indicated that hydrophobic domain possesses some features needed for the catalysis of transesterification reaction. It is known, that in general in RNA–protein complexes [20,21] and in natural ribonucleases [22–24] hydrophobic interactions play important roles, particularly, in changing conformation of RNA and e.g., in the case of RNases helping the linkage to be cleaved to adopt the most favorable conformation for transesterification [25].

In the present article we describe properties and RNA cleavage by the compound Dp12, identified as the most active among. Results of the study suggest, that this compound cleaves RNA via interactions with its sugar-phosphate backbone, that induce conformational alteration of RNA structure, favorable for transesterification to occur. We have found, that Dp12 enormously enhances rate of spontaneously RNA cleavage and efficiency of catalysis can be considerably increased by synergetic action of Dp12 and $OH^-$, or imidazole, or monovalent ions.

## 2. Materials and methods

### 2.1. Miscellaneous chemicals, enzymes, and RNAs

Chemicals for synthesis were purchased from Aldrich (USA). Chemicals for electrophoresis were purchased from Sigma (USA), [$\gamma$-$^{32}$P]-ATP was from Biosan (Russia). Solutions for RNA handling were prepared using Milli-Q water, filtered through membranes with 0.2 μm pore size (Millipore, USA) and autoclaved. T4 polynucleotide kinase was from Fermentas (Lithuania), ribonuclease T1, bovine alkaline phosphatase were from Sigma (USA). Oligonucleotide r(UCGAAUUUCCACAGAAUUCGU) (ON21) was synthesized by Dr. M. Repkova, (this institute) by standard phosphoramidite chemistry and purified using RP-HPLC.

Transcripts of yeast tRNA$^{Phe}$, fragments of MDR1 mRNA (190-mer and 670-mer) and fragment of HIV1 RNA (96-mer) were synthesized *in vitro* using linearized plasmids from ICBFM collection: dYF90 (*Bst2UI*), pBluescriptMDR670 (DraI, Sma1 for 190-mer, and 668-mer, respectively), pHIV2 (FokI), respectively, and T7 RNA polymerase as described earlier [26]. Total tRNA from *Escherichia coli*, used as a carrier to supplement labeled RNAs, was from Vector, Russia.

Dp12 was synthesized by Ms E. Burakova (this institute) as described recently [1].

### 2.2. Critical micelle concentration of Dp12

Critical micelle concentration (CMC) of Dp12 was determined in Tris–HCl buffer at pH 7.0, containing 0.2 M KCl, 0.1 mM EDTA by static light scattering using a VA Instruments Co., Ltd. LS-01 apparatus (Saint-Petersburg, Russia) calibrated with a dust-free benzene ($R_{90} = 11.84 \times 10^{-6}\text{cm}^{-1}$). The intensity of light scattering ($I_{90}$) was measured using the vertically polarized light (633 nm) at angle $\theta = 90°$. The CMC was determined by the linear least-squares fitting of the light scattering intensity ratio ($I_{90}/I_0$) that is the intensity of light scattering normalized to the intensity of the incident light. A sharp increase in the intensity ratio is observed upon a micelle formation.

### 2.3. Kinetics of the RNA/Dp12 interactions

The kinetics of the RNA/Dp12 interaction was studied by measuring static light scattering of RNA/Dp12 solution. Because the light scattering intensity ratio ($I_{90}/I_0$) is directly proportional to the molecular mass and size of particles present in solution, the changes of the intensity of light scattering ($I_{90}$) versus time were measured at angle $\theta = 90°$.

The weighted average molecular mass, $M_w$, the radius of gyration, $R_G$, and the second virial coefficient, $A_2$, of the complexes Dp12 with RNA (96-mer and 668-mer) were determined by laser multiangle static light-scattering in a Tris–HCl buffer at pH 7.0, containing 0.2 M KCl, 0.1 mM EDTA, Dp12 at concentration $1 \times 10^{-5}$ M, and RNA (96-mer or 668-mer) at concentration 0.1 mg/ml. The Rayleigh ratio $R_\theta$ was measured using the vertically polarized light (633 nm) at angles in the range $40° \leqslant \theta \leqslant 140°$ (13 angles) using a VA Instruments Co., Ltd. LS-01 apparatus calibrated as described above. The data were used to plot the angular and concentration dependencies of the ratio $HC/\Delta R_\theta$ according to the Zimm method [27]. Here, $C$ is the particle concentration (g mL$^{-1}$), $\Delta R_\theta$ is the excess light scattering over that of the solvent at angle $\theta$, and $H$ is an instrumental optical constant equal to $4\pi^2 n^2 v^2 / N_A \lambda^4$, where $N_A$ is the Avogadro's number, $\lambda$ is the wavelength of incident light in vacuum, $n$ is the refractive index of the solvent, and $v$ is the refractive index increment of the protein. Values of the weight-average molar mass, $M_w$, were estimated as averages from the intercepts of both the concentration dependence of $HC/\Delta R_\theta$ as $\theta \to 0$ (the extrapolation was performed on 13 angles) and the angular dependence of $HC/\Delta R_\theta$ as $C \to 0$ (the extrapolation was performed on 5–8 concentrations by dilution of standard reaction mixture by a factor of 1.1–1.5). Values of the radius of gyration, $R_G$, were estimated from the slope of the angular dependence of $HC/\Delta R_\theta$ as $C \to 0$. Values of the second virial coefficient, $A_{pr-pr}$, were estimated from the slope of the concentration dependence of $HC/\Delta R_\theta$ as $\theta \to 0$. The second virial coefficient characterizes primarily the thermodynamic affinity of the particles (complexes Dp12 with RNA) to the solvent (a Tris–HCl buffer in our case): it is poor, if $A_{pr-pr} < 0$, or, by contrast, it is good if $A_{pr-pr} > 0$, and it is ideal if $A_{pr-pr} = 0$) [28], i.e. provide the circumstantial evidence for the complexes surface hydrophilicity/hydrophobicity.