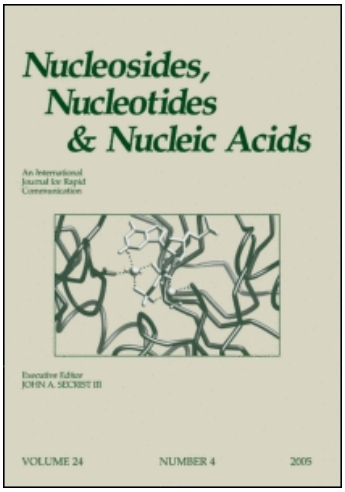

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Ribonuclease Activity of Cationic Structures Conjugated to Lipophilic Groups

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ABSTRACT

Cationic compounds containing benzene ring substituted with the bis-quaternary salt of diazabicyclo[2.2.2]octane (DABCO) bearing a polymethylene fragment at the bridge positions display ribonuclease activity. Efficacy of the catalysis is affected by geometry of the cationic structures and the size of the attached aliphatic fragment. The cleavage occurs primarily within CA sequences. The compounds do not possess tradition groups participating in the transesterification step of RNA cleavage reaction, therefore a speculative mechanism of cleavage could be inducing a conformational stress on the RNA sugar phosphate backbone providing fragility to phosphodiester bonds.

Key Words: Artificial ribonuclease; RNA; DABCO; Conformational stress.

INTRODUCTION

Recently we synthesized and tested chemical ribonucleases, conjugates of histidine and 1,4-diazabicyclo[2.2.2]octane containing a tetradecamethylene residue at the bridge position.[1,2] In control experiments with model compounds mimicking domains of the synthetic ribonucleases, we observed that some cationic molecules conjugated to

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hydrophobic structures displayed some ribonuclease activity. In the present study we investigated ribonuclease activity of the newly designed compounds, conjugates of positively charged molecule DABCO, substituted at the bridge position with aliphatic fragments of different length, and connected via a rigid linker, the benzene ring. We have found that the ribonuclease activity of these compounds is strongly affected by their geometry and the length of aliphatic substitutents, supporting the conclusion about conformational stress based mechanism of RNA cleavage.

EXPERIMENTAL

RNase T1 was purchased from Boehringer Mannheim, T4 polynucleotide kinase was purchased from Fermentas, γ-[^{32}P]-ATP was from Biosan (Russia). Other chemicals were of chemical purity grade. All solutions were prepared with MilliQ (Millipore) purified water. Detailed synthetic procedure and characterization of the compounds will be described elsewhere. Oligonucleotide ON21 was synthesized by Dr. M. Repkova (this Institute) 5′-[^{32}P]-ON21 was labeled using γ-[^{32}P]-ATP and T4 polynucleotide kinase according to published protocol.[3] Before the use, 5′-[^{32}P]-ON21 was diluted with unlabeled ON21 to concentration of $5 \cdot 10^{-5}$ M and specific activity $6 \cdot 10^{3}$ cpm/pmol. Reaction mixtures (10 μl) containing $5 \cdot 10^{-6}$ M 5′-[^{32}P]-ON21 RNA, one of the compounds at concentration ranged from 10^{-6} M to 10^{-3} M, 50 mM Tris-HCl pH 7.2, 0.2 M KCl and 0.1 mM EDTA were incubated at 37°C for 4 h or 24 h in the absence or in the presence of imidazole (ranged in concentration from 1 mM to 50 mM). Reactions were quenched by precipitation of ON21 and the cleavage products with 100 μl of 2% lithium perchlorate solution in acetone. The cleavage products were analyzed by electrophoresis in 15% PAAG/8 M urea gel. After electrophoresis the gel was dried and autoradiographed. The cleavage sites were assigned by comparison of the electrophoretic mobilities of cleavage products with these of ON21 cleavage products with RNase T1 and 2 M imidazole buffer (pH 7.0).[1] Quantitative data were obtained using Phosphoimager System (Molucular Imager, Bio-RAD).

RESULTS AND DISCUSSION

It is known that RNA molecules can be spontaneously cleaved at some specific motifs (predominantly Pyr-Pu) under physiological conditions in the absence of any catalyst. It was found that this enhanced fragility of phosphodiester bonds is observed in dinucleotide structures, where the distance between 2′-hydroxyl group and phosphorus atom is somewhat shorter and an angle 2′-O-P-5′-O is larger then average ones.[4] Therefore it could be suggested that compounds capable to induce some conformational stress in RNA sugar-phosphate backbone might facilitate spontaneous cleavage of RNA. Such molecules could be rigid structures with cationic group capable of binding to RNA backbone and bending the RNA.

In the present work we synthesized cationic compounds built of two diazobicyclo[2.2.2]-octane (DABCO) moieties, substituted at one of the bridge positions with aliphatic fragment and connected by rigid linker (benzene ring) (Fig. 1). DABCO residues bearing two positively charged quaternary nitrogen atoms were expected to efficiently

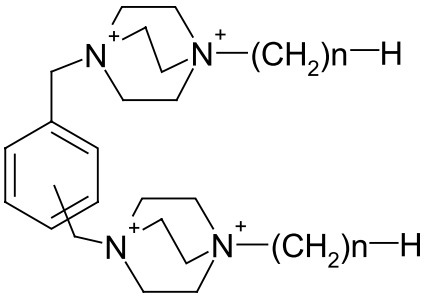

Figure 1. General structure of the compounds Dxn.

bind to RNA. The planar benzene ring determines spatial arrangement of DABCO residues. The distance between positive charges of the molecule is varied by location of DABCO residues in the benzene ring (ortho, meta, para). The compounds Dxn do not contain groups known to directly catalyze the transphosphorylation step of the RNA cleavage reaction (imidazole-, amino- or carboxylic-groups). The compounds were named Dxn, where D corresponds to DABCO, *x*—indicates location (o—ortho, m—meta or p—para) of DABCO residues in the benzene ring, and *n* shows the length of aliphatic polymethylene substitutes (n = 1, 4, 6 or 12).

Ribonuclease activity of the compounds Dxn was assayed in experiments with synthetic oligoribonucleotide ON21 (21 nucleotide long) that forms a hairpin structure (Fig. 2). The stem of this hairpin is identical to aminoacceptor stem of yeast $tRNA^{Phe}$ and the sequence of the loop corresponds to fragment 59–65 of this tRNA. Earlier we have found that sequence 59–65 of yeast $tRNA^{Phe}$ is particularly sensitive to cleavage with chemical ribonucleases.[1] The reaction was performed at 37°C in 50 mM Tris-HCl, pH 7.2, 0.2 M KCl, 0.1 mM EDTA. Concentrations of ON21 and compounds Dxn were 5 μM and 10 μM, respectively. Comparison of ribonuclease activity of the

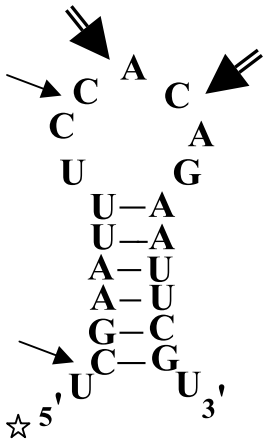

Figure 2. Secondary structure of ON21. Arrows show sites of cleavage by the compounds Dxn. Arrow sizes correspond to cleavage efficacies. The asterisk indicates the position of 5′-[^{32}P]-label.

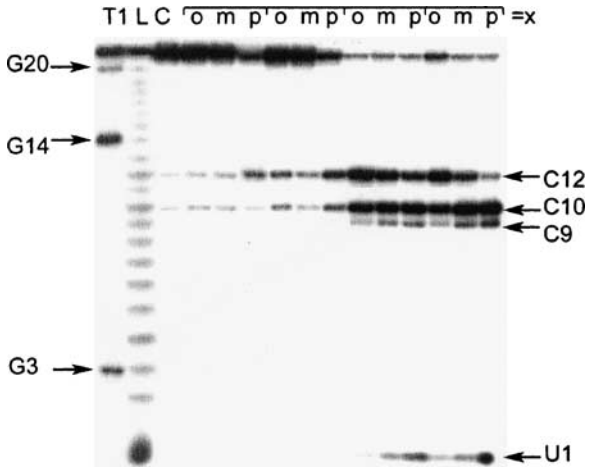

Figure 3. Cleavage of 5′-[^{32}P]-ON21 (UCGAAUUUCCACAGAAUUCGU) with compounds Dxn. Autoradiograph of 15% denaturing PAAG. Lanes: C—incubation control, L and T1—partial hydrolysis in 2 M imidazole buffer, pH 7.0 and by RNase T1 under denaturing conditions, respectively. Reaction conditions: 50 mM Tris-HCl, pH 7.2, 0.2 M KCl, 0.1 mM EDTA, 37°C, 24 h. Concentration of ON21 is 5 μM. Concentration of the Dxn compounds was 1 mM for the compounds Dx1, Dx4, Dx6 and 10 μM for the compounds Dx12.

compounds Dxn is shown in Fig. 3. All the compounds display similar specificity: major cleavages occur at the two adjacent C–A phosphodiester bonds located in the hairpin loop; C–C and U–C phosphodiester bonds are cleaved less efficiently.

RNA cleavage activity of the compounds Dxn depends on the length of their aliphatic residues and it is considerably affected by their geometry (Fig. 4). Para-isomers having the largest distance between DABCO residues and linear arrangement of positive charges catalyze phosphodiester bond cleavage display the highest ribonuclease activity. Ortho-isomers having the smallest distance between DABCO residues display the lowest cleavage rate. All the Dx1 compounds are inactive and the

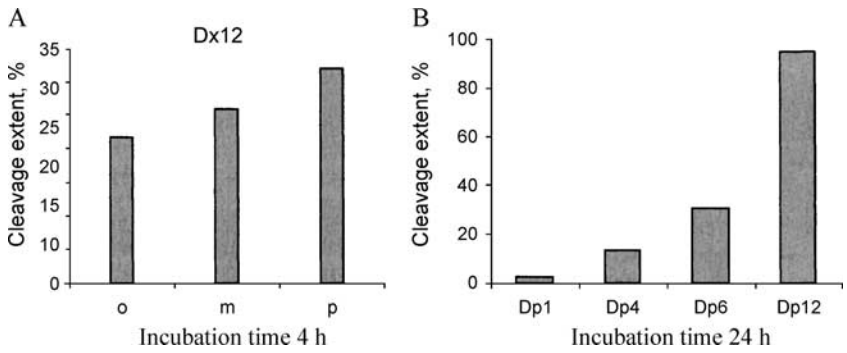

Figure 4. Effect of geometry (A) and the length of polymethylene fragment (B) on the ribonuclease activity of the compounds Dxn. Cleavage conditions are described in experimental section. Data are presented for concentration of the compounds Dxn 10 μM.

compounds Dx4 exhibit only trace ribonuclease activity. The compounds with lipophilic tail with 6 methylene groups and longer are considerably more active and maximal activity was displayed by compounds Dx12.

The distances between the two nearest quaternized N^+ atoms of the compounds Dxn were calculated using Hyper Chem 6.0 software to be 5.72 Å, 6.42 Å, and 7.57 Å for the compounds Don, Dmn, and Dpn, respectively. The distance between two adjacent internucleotide phosphate groups in RNA is (7.68 ± 0.05 Å). The quaternary nitrogen atoms of Dpn can be expected to efficiently bind to negatively charged oxygens of the phosphate. This is in agreement with the idea about induction of a conformational stress in the sugar-phosphate backbone of RNA due to the compounds binding. The specificity of the cleavages is in accordance with the expected mechanism—the main cleavages occur at CA motifs that are the most fragile.[5]

As compared with previously described compound ABL4C3[1] containing one quaternized DABCO residue, tetradecamethylene fragment, and histidine residue, compound Dp12 under identical conditions displays 3-times higher ribonuclease activity (data not shown). This is an evidence that cationic and aliphatic domains of artificial ribonucleases strongly contribute to their cleavage activity. One can expect that two adjacent phosphate residues interact with two DABCO moieties of compound Dp12, which bend the ribose-phosphate backbone and facilitate intramolecular transesterification reaction. The role of aliphatic fragment in this process remains to be investigated.

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