Research Article

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Increasing the activity of DNAzyme based on the telomeric sequence: 2'-OMe-RNA and LNA modifications

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Abstract: 2'-OMe-RNA analogues and LNA point modifications of DNA oligonucleotides were applied for the modulation of the G-quadruplex topology and enhancement of peroxidase activity of the resulting DNAzymes. The effect of the 2'-OMe-RNA analogue was studied for full length modified oligonucleotides with various sequences. In the case of LNA-point modification, we have chosen a telomeric DNA sequence and investigated various numbers of modifications. Our main goal was to prove that the application of these modifications can influence the activity of DNAzyme, especially those, which normally form poor DNAzymes. As an example, we have chosen the telomeric HT22 sequence which is known to form DNAzyme characterized by low activity. In all cases, the DNAzymes formed by a telomeric sequence with the application of the 2'-OMe-RNA analogue as well as LNA-point modification, showed significantly higher peroxidase activity. We were also able to shift the formation of hybrid or antiparallel topology to parallel topology. These results are important for the development of probes for biological applications as well as for the design of probes based on DNA sequences that normally form DNAzymes with low activity. This paper also provides information on how the application of nucleotide analogues can transform the topology of G-quadruplexes.

Keywords: DNAzyme; 2'-OMe-RNA; locked nucleic acid; G-quadruplex; peroxidase activity.

1 Introduction

Research on the design of DNA probes for bioanalytical application in recent years has entered into the field of nucleic acids analogues. Such an approach was also applied in the designing of the probes based on peroxidase-mimicking DNAzymes. The use of DNA analogues allows the formation of structures that are more stable and resistant to the action of DNases. Additionally, the introduction of such a modification can induce a change in the G-quadruplex topology, which may translate to the increase of DNAzyme activity. Artificial analogues of naturally occurring nucleic acids can be divided into two groups: i) backbone analogues and ii) base analogues. The first group contains synthetic nucleic acids formed by canonical bases and modified sugar-phosphate chain. These modifications may involve sugar group (2'-OMe-RNA, LNA – locked nucleic acid, UNA – unlocked nucleic acid), phosphoric acid residue (PS-DNA) or the replacement of both groups by amino acids (PNA – protein nucleic acid). Extensive research on the influence of these modifications on G-quadruplex structures and stability has been undertaken in recent years [1-5]. It has been observed that substitution of guanine nucleotides with 2'-OMe-RNA analogues in different positions may contribute to the increase of stability or conversely, may cause corruption of the G-quadruplex structure [6,7]. Similar results were also obtained using LNA analogues [8-12]. In general terms, substitution of DNA analogues in *anti*-positions result in a more stable G-quadruplex. In contrast, the replacement of nucleotide in *syn* positions in most cases leads to the disruption of G4 structure. The DNA analogues like 2'-OMe-RNA and LNA favour *anti* -conformation. Dominic and Jarstfer proved that the replacement of single DNA nucleotides by LNA analogues led to the change of the G-quadruplex topology from antiparallel to parallel [13]. The second group of modified DNA nucleotides includes nitrogen bases analogues, which are applied to change G-quadruplex structure and functionality. The most

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commonly used for this study are guanine analogues including xanthine, isoguanine and 8-oxoguanine [1,14-16]. An interesting application of these analogues is inversion of polarity by incorporation of xanthine and 8-oxoguanine into external G-tetrad of telomeric sequence [17]. All of these modifications resulted in G4 structure alteration that might improve G4 ability to bind hemin molecules and hence should change DNAzyme activity.

Recently, Li et al. studied the impact of various modifications on the peroxidase activity of DNAzyme [18]. They examined the influence of four DNA modifications: L-DNA (mirror image of natural D-DNA), PS-DNA, LNA and 2'-OMe-RNA. They examined 3 DNA sequences: PS2.M, c-Myc and EAD2 applying modifications to the entire DNA strands. The authors demonstrated that DNAzyme based on 2'-OMe-RNA oligonucleotide exhibited the highest activity for all three sequences. On the other hand, the use of LNA modification led to a significant decrease of enzymatic activity. The focus of the paper was put on the DNAzymes, that usually possess high activity. No research was conducted for the DNA sequence that forms DNAzymes characterized by lower activity. The two sequences that are often studied and are characterized by low activity of DNAzymes are the telomeric sequence and thrombin-binding aptamer (TBA). Previous reports showed, however, that modifications of TBA using LNA and 2'-OMe-RNA analogues can lead to the disruption of the G-quadruplex structure [6,7]. That's why we decided to focus on modification of the telomeric sequence. Using knowledge of modifications effect on the G-quadruplex structure we decided to carry out in-depth study on ribozymes formed by 2'-OMe-RNA analogues. In the second part of the study we examined the effect of point LNA modifications on the G-quadruplex/hemin structure and activity. The chemical structures of studied modifications are presented in Scheme 1.

2 Methods

2.1 Materials and Instruments

DNA oligonucleotides (HPLC purified) were purchased from Genomed S.A. (Poland), 2'-OMe-RNA oligonucleotides were purchased from FutureSynthesis (Poland). LNA oligonucleotides were purchased from Eurogentec (Belgium). All oligonucleotides were used without further purification. The concentration of the oligonucleotides were quantified using UV-Vis spectroscopy at 85°C with the following extinction coefficients at 260 nm (M^{-1} , cm^{-1})

Table 1: Sequences of oligonucleotides used in this study.

Name	Sequence
PS2.M (DNA)	5'-GTG GGT AGG GCG GGT TGG-3'
CatG4 (DNA)	5'-TGG GTA GGG CGG GTT GGG AAA-3'
HT22 (DNA)	5'-AGG GTT AGG GTT AGG GTT AGG G-3'
Ref (DNA)	5'-GAG TAG TTC GTG GCC TAG-3'
ps2.m (2'-Ome-RNA)	5'-GUG GGU AGG GCG GGU UGG-3'
catg4 (2'-Ome-RNA)	5'-UGG GUA GGG CGG GUU GGG AAA-3'
ht22 (2'-Ome-RNA)	5'-AGG GUU AGG GUU AGG G-3'
Ref (2'-Ome-RNA)	5'-GAG UAG UUC GUG GCC UAG-3'
LNA1	5'-AGGGTTAGGGTTAGGGTTAG _L GG-3'
LNA2	5'-AGGGTTAG _L GGTTAGGGTTAG _L GG-3'
LNA3	5'-AG _L GGTTAG _L GGTTAGGGTTAG _L GG-3'

A = 15400, T = 8700, G = 11500, C = 7400. The sequences of used oligonucleotides are gathered in Table 1. Hemin, 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), H_2O_2 , and Triton X-100 were purchased from Sigma-Aldrich (Poland). Hemin was dissolved in DMSO and the 1×10^{-2} M stock solution was stored in the freezer. Other reagents were of analytical grade and used as received. The absorption spectra and melting profiles were recorded using a Cary 100 UV-Vis Spectrophotometer (Agilent Technologies, Australia). For the measurements of DNAzymes activity a M200 microplate reader (Tecan, Austria) was used. The CD spectra were recorded using a J-810 spectropolarimeter (Jasco, Japan). All experiments beside melting profiles, were carried out at 25°C.

2.2 CD spectroscopy

CD spectra were measured with 100 nm/min scanning speed and bandwidth of 1 nm. Spectra were recorded in quartz cells of 1 cm path length and averaged from 3 scans. All samples contained 2 μ M oligonucleotide, 10 mM Tris-HCl buffer pH=8.0 and 100 mM required cation at adequate concentration. Prior to the experiment the samples were heated at 95°C for 5 minutes and then cooled on ice for 15 minutes.

2.3 Spectrophotometric titration of hemin

Binding constants of oligonucleotide/hemin complexes were measured using spectrophotometric titration according to the procedure described by us previously [19].

2.4 pKa determination

The values of pKa of hemin in the complex with oligonucleotide were determined from changes in the UV-Vis spectra of G4/hemin complex in the 4–11 pH range. Samples containing 2 μ M DNA oligonucleotide, 10 mM Tris-HCl, 100 mM KCl, 0.0016% Triton X-100 were denatured for 5 minutes at 90°C and cooled on ice for 15 minutes. Next the hemin was added to the solution which was titrated with 1 M NaOH or 1 M HCl (4 min waiting interval after addition) while pH was monitored and UV-Vis spectra (200 – 600 nm) were recorded after the addition of titrant.

2.5 DNAzyme activity measurement

The measurements were conducted using samples containing G4 oligonucleotide, 10 mM Tris-HCl buffer pH=8.0, 0.0016 % Triton X-100, hemin and 100 mM adequate salt (KCl, NaCl or NH₄Cl). First the samples were denatured at 95°C for 5 minutes and then cooled on ice for 15 min. After the addition of colorimetric substrate ABTS (1 mM), samples were transferred into a microplate and reaction was initiated by the addition of H₂O₂ (1 mM). The absorbance at 417 nm was monitored in 10 s intervals for 15 min.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 2'-OMe-RNA analogues of DNAzymes

3.1.1 CD spectra and melting profiles of 2'-OMe-RNA analogues of DNAzymes

The first part of our research focused on the characterization of 2'-OMe-RNA oligonucleotides. For this study we have chosen 4 sequences: PS2.M, which is one of the first developed G-quadruplex-based DNAzyme sequences, CatG4 that is recognized to form a highly active DNAzyme, HT22 telomeric sequence that possesses low peroxidase activity and Ref - reference sequence unable to form G4 DNA (Table 1). To distinguish between the analogues, the DNA oligonucleotide acronyms are written in capital letters and 2'-OMe-RNA oligonucleotide acronyms are written in lowercase letters. In the first stage we measured the CD spectra of the studied 2'-OMe-RNA and DNA oligonucleotides in order to verify formation of

Figure 1: Scheme of chemical structures of DNA nucleotide and its analogous 2'-OMe-RNA and LNA.

the G-quadruplex structure (Figure 2). The experiments were performed in the presence of potassium cation with or without the addition of hemin molecule. It is proven from the literature reports and our experience that PS2.M oligonucleotide forms in the presence of potassium cation the mixture of at least two structures [19]. This is proved by CD spectrum which showed positive bands at 260 and 295 nm (Figure 2A). The addition of hemin caused an increase in band intensity at 260 nm suggesting predominant formation of the parallel structure. On the other hand, the 2'-OMe-RNA analogue, ps2m exhibited spectrum characteristics for the parallel G4 topology in both studied conditions. Oligonucleotides based on CatG4 sequence showed CD spectra characteristics for parallel topology of G-quadruplex for all studied systems (Figure 2B). In contrast, the HT22 oligonucleotide in the presence of potassium cation formed a hybrid structure and the addition of hemin caused some increase of the band at 260 nm. Similar to the case of ps2.m, the ht22 oligonucleotide showed spectra characteristics for the parallel topology both in the absence and presence of hemin (Figure 2C). These results proved that by using a 2'-OMe-RNA analogue instead of DNA, it is possible to change the structure of the G-quadruplex into the parallel topology. This information is crucial since it was proven that a parallel topology of G4 is related to the high activity of peroxidase-mimicking DNAzymes [20]. In the next step we checked how the application of 2'-OMe-RNA analogues influence the thermal stabilities of studied systems (Figure 2 D, Table 2). For both ps2.m and catg4 we observed higher melting temperatures compared to their DNA analogues. In the case of the ps2.m G-quadruplex the stabilization was as high as 12°C. Interestingly, for the ht22 oligonucleotide, the destabilization of the G4 structure was observed. This suggests that a hybrid structure is preferred by telomeric sequences with long TTA loops (a single-nucleotide loops induce a parallel structure). However, the 2'-OMe-

Figure 2: Spectral characterization of G-quadruplex formed by studied sequences. CD spectra of ps2.m (A), catg4 (B), ht22 (C), where brown line corresponds to DNA oligonucleotide, orange line – DNA oligonucleotide + hemin, yellow line – RNA oligonucleotide, green line – RNA oligonucleotide + hemin. Panel D shows melting profiles of DNA and 2'-OMeRNA sequences obtained using UV-Vis spectrophotometry. Conditions: 10 mM Tris-HCl (pH=8.0), 100 mM KCl, 2 μ M DNA/2'-OMeRNA, 2 μ M hemin (if present).

Table 2: Melting temperatures (T_m) and hemin protonation constants (pKa) for the system containing G4 DNA and their 2'-OMeRNA analogues.

	DNA		2'-Ome-RNA	
	T_m	pKa	T_m	pKa
PS2.M	57.8 ± 0.1	>9.0	69.9 ± 1.0	>9.0
CatG4	78.3 ± 0.2	>9.0	> 80	>9.0
HT22	67.2 ± 0.4	8.4 ± 0.3	40.6 ± 0.3	>9.0

RNA modification prefers *anti* conformation of guanines and thus forces the oligonucleotide to adopt a parallel structure. The Ref and ref oligonucleotides did not show CD spectra characteristics for G-quadruplex and non-thermal profiles were observed for these systems, which confirmed that those oligonucleotides did not form any higher-order structures.

3.1.2 Binding constants of hemin/2'-OMe-RNA oligonucleotides

In order to verify whether the application of 2'-OMe-RNA analogues influenced the binding of hemin by G-quadruplex DNA, we performed spectrophotometric titration of hemin with the oligonucleotides studied (Table 3). Hemin possesses an intensive Soret band at ~404 nm. The intensity of this band depends on the aggregation of the monomeric hemin molecules [21]. Titration of hemin solution with DNA caused an increase in the absorbance of the Soret band since hemin in aqueous solutions forms aggregates and after binding with DNA is in a monomeric state. Based on the titration results we used a Scatchard analysis and a direct method to determine dissociation constants (Table 2). For both ps2.m and catg4, the small decrease of K_d was observed in relation to PS2.M and CatG4, respectively. For the ht22/hemin complex the decrease

Table 3: Values of dissociation constants for G-quadruplex/hemin complexes determined using Scatchard analysis and “direct” approach method (eq. in Ref. 19).

Name	K_d (Scatchard)	K_d (direct method)
ps2.m	2.8×10^{-7}	3.2×10^{-7}
catg4	1.9×10^{-7}	1.9×10^{-7}
ht22	4.7×10^{-7}	4.1×10^{-7}
PS2.M	9.0×10^{-7}	5.9×10^{-7}
CatG4	5.2×10^{-7}	2.3×10^{-7}
HT22	1.0×10^{-6}	1.4×10^{-7}

of dissociation constant was greater and the difference was 3.4 times lower. This indicates the stronger binding of hemin by 2'-OMe-RNA G-quadruplexes. Probably, the higher stability of complex is connected with the formation of the parallel topology of G4. Since the CatG4 DNA oligonucleotide already adopts parallel topology and PS2.M forms the mixture of topologies the difference of K_d for these 2'-OMe-RNA oligonucleotide was not significant. However, since HT22 forms hybrid topology in potassium presence, the use of ht22 with parallel topology caused a big change in the dissociation constant. This observation can be explained in terms of the properties of external G-tetrads in the parallel structure that are better exposed to hemin binding. This indicates that the use of 2'-OMe-RNA oligonucleotide in the place of DNA can cause the increase of hemin binding, which is significant especially for those oligonucleotides that typically binds hemin poorly (HT22, TBA).

3.1.3 pKa of hemin/oligonucleotide complexes of 2'-OMe-RNA analogues of DNAzyme

Another feature that is important in the characterization of peroxidase-mimicking DNAzymes is the pK_a of hemin in a complex with G4 (Table 3). It was proven by us and others that a high pK_a value favors high peroxidase activity of DNAzyme [19,22]. In order to determine the value of pK_a we recorded absorption spectra of hemin in the presence of oligonucleotide at varying pH. The pH was changed by the addition of HCl or NaOH solutions and the measurements were performed between pH 12 and 4. The plot in Figure 3 presents the dependences of A_{360}/A_{403} ratio on pH. The pK_a is determined by the difference in the absorbance between absorbance at 360 nm and absorbance of 403 nm which is around the Soret band of hemin spectrum. The pK_a can be precisely determined from plots that possess sigmoidal

Figure 3: Determination of pK_a of studied G-quadruplex/hemin complexes. Conditions: 10 mM Tris-HCl, 100 mM KCl, 0.0016% Triton X-100, 2 μ M DNA/2'-OMeRNA, 2 μ M hemin.

shapes [22]. For plots that did not reach plateau in the studied pH range, the pK_a was set as higher than 9. For both PS2.M and CatG4 sequences the changes in pK_a for both DNA and 2'-OMe-RNA were not observed since their pK_a exceeded values of 9. A difference was, however, observed for telomeric oligonucleotide, for which pK_a was 8.4 for HT22 whereas for ht22 the pK_a increased to more than 9. Since a higher pK_a value is required for good peroxidase activity, the result for ht22 suggests that application of 2'-OMe-RNA analogue can positively influence peroxidase activity of DNAzyme based on this sequence.

3.1.4 Activity of 2'-OMe-RNA ribozymes

The last part of the study on 2'-OMe-RNA analogues included verification of their catalytic activity using the indicator reaction with ABTS (Figure 4). We observed that for DNAzymes based on ps2.m and ht22 oligonucleotides, the increase of activity was significant in comparison with their DNA analogues. In contrast, for DNAzyme based on catg4 oligonucleotides we observed a decrease of the catalytic activity. The enhancement effect can be explained by the fact that for both ps2.m and ht22, transition of conformation to the parallel topology takes place, which is the most advantageous structure for the formation of G-quadruplex/hemin complex and hence an increase in the catalytic activity is observed. The increase of activity in the case of ht22 oligonucleotide is, however, much higher than in the case of ps2m oligonucleotide. The enhancement effect is the most spectacular for ht22 (5 times increase), for which the pK_a value was increased noticeably. PS2.M forms quite good DNAzyme so the increase of the activity is not so spectacular like in the

Figure 4: Activity of DNAzymes and 2'-OMe-ribozymes in the oxidation reaction of ABTS in the presence of K^+ . Conditions: 10 mM Tris-HCl (pH=8.0), 100 mM KCl, 0.0016% Triton X-100, 0.5 μ M DNA/2'OMeRNA, 0.5 μ M hemin, 1 mM ABTS, 1 mM H_2O_2 .

case of ht22 oligonucleotide where change in the topology allows on the better hemin binding and higher pKa of hemin/oligonucleotide complex. In the case of catg4 sequence, the DNA oligonucleotide CatG4 already formed advantageous parallel topology and possessed pKa value high enough. One can assume that the application of 2'-OMe-RNA causes formation of a too stiff structure (very high T_m), which may influence DNAzyme activity. Nonetheless, the activity of catg4 is still high. One should point out another advantage connected with the usage of 2'-OMe-RNA analogues that is related to the applications of DNAzymes in living cells. These artificial DNA analogues are resistant to restriction enzymes.

3.2 LNA analogues of DNAzymes

3.2.1 CD spectra and melting profiles of LNA-modified oligonucleotides

In the second stage of the presented research we decided to examine the influence of LNA analogues on DNAzyme activity. Yang and co-workers studied the catalytic activity of LNA oligonucleotides (entire molecule modified) and concluded that these DNAzymes didn't possess peroxidase activity, which they explained by a too stiff structure and lack of the G-quadruplex formation [18]. Acknowledging that modification of all nucleotides in a strand could lead to a too stiff structure we assumed that the application of point modification should lead to the alteration of the G-quadruplex topology. In the G-quadruplex structure the glycosidic bond can adopt two conformations: *syn* and *anti*. In parallel G4 all nucleosides in the particular G-quartet

adopts an *anti* or *syn* conformation (in nature most of them forms *anti* conformation). On the other hand, in an antiparallel G-quadruplex two nucleosides adopt an *anti* and two *syn* conformation but in a hybrid G4 the ratio is 3+1. For this stage of research we decided to choose a HT22 oligonucleotide, which in the presence of potassium cation forms hybrid topology of the G-quadruplex and in the presence of sodium cation forms an antiparallel structure. Expecting alteration of the G4 topology, we designed three oligonucleotides varying in the number of point LNA modifications (1, 2 and 3). To the modifications we subjected external G-tetrad from the 5' end of the oligonucleotide (oligonucleotides sequences gathered in the Table 1).

In order to verify how LNA point-modifications affect the G-quadruplex topology we measured CD spectra in potassium, sodium or ammonium cation presence (Figure 6). As it was mentioned earlier, HT22 oligonucleotide formed hybrid topology in the presence of K^+ and antiparallel topology in the presence of Na^+ . In the presence of NH_4^+ the CD spectrum also showed that antiparallel topology was formed. As seen in Figure 6B, even a single LNA modification (LNA1) led to the switch of the G-quadruplex topology to the parallel topology in the presence of potassium cation and the mixture of topologies in the presence of sodium or ammonium cations. Similar changes in CD spectra were registered for the LNA2 oligonucleotide (Figure 6C). In the case of LNA3, the parallel G-quadruplex was observed under all studied cationic conditions (Figure 6D). These experiments showed that our hypothesis concerning the effect of point LNA modifications on G4 structure was justified and that such modifications can induce formation of parallel G-quadruplex. To see if hemin itself can force DNA oligonucleotide to form a G-quadruplex structure we also measured the CD spectra of oligonucleotides with hemin addition in the presence and absence of potassium cation (Figure 7). The results showed that hemin is not able to stabilize G-quadruplex in the absence of a metal cation. The conclusion is that cation are necessary for the G-quadruplex structure formation of LNA-modified oligonucleotides.

In order to verify stability of LNA-modified G-quadruplexes, we determined melting temperatures using CD melting experiments (Table 4). In the presence of potassium cation, we first observed an increase in the T_m with increasing number of LNA modifications but surprisingly LNA3 possessed melting temperature even lower than unmodified HT22. Considering that T_m can be sensitive to both the length of loops and *syn-anti* conformation of guanines, we cannot deliberate on the stabilization effect because different structures

Figure 5: Scheme of LNA point-modifications of 5' G-quartet of telomeric G-quadruplex. Blue squares represents DNA guanine nucleotides and red squares represents LNA guanine nucleotides.

Figure 6: CD spectra of HT22 DNA oligonucleotide (A) and oligonucleotides with 1 (B), 2 (C) and 3 (D) LNA modifications in various cationic conditions: brown – potassium, orange – sodium, yellow – ammonium. Conditions: 10 mM Tris-HCl (pH=8.0), 100 mM salt, 2 μ M DNA.

Figure 7: CD spectra of DNA oligonucleotides: LNA1 (A), LNA2 (B), LNA3 (C) and HT22 (D) with addition of hemin with (orange) and without (brown) potassium cations. Conditions: 10 mM Tris-HCl (pH=8.0), 100 mM KCl, 2 μ M DNA, 2 μ M hemin.

Table 4: Melting temperatures (T_m) and hemin protonation constants (pK_a) for HT22 and LNA-modified oligonucleotides.

Name	pK_a	T_m [°C], K^+	T_m Na^+	T_m NH_4^+
HT22	7.5 ± 0.1	66.1 ± 0.9	52.7 ± 0.5	39.7 ± 0.7
LNA1	8.1 ± 0.1	71.5 ± 4.0	45.5 ± 3.5	32.8 ± 0.1
LNA2	>10	75.2 ± 1.8	48.0 ± 2.0	51.7 ± 0.1
LNA3	>10	64.0 ± 0.5	57.8 ± 0.5	48.2 ± 0.4

Figure 8: Catalytic activity of HT22 and LNA-modified DNAzymes in the reaction of ABTS oxidation by hydrogen peroxide. Color code: brown-potassium, orange-sodium, green-ammonium cations. Conditions: 10 mM Tris-HCl (pH=8.0), 100 mM KCl, NaCl or NH_4Cl , 0.0016% Triton X-100, 1 μ M DNA, 1 μ M hemin, 1 mM ABTS, 1 mM H_2O_2 .

may be present in the studied oligonucleotide systems. In the presence of sodium cation we observed reverse relationship. LNA1 and LNA2 had T_m lower than HT22 by ca. 7 and 5°C, respectively. However in this case we observed an increase in the melting temperature with the number of LNA point modifications and LNA3 was characterized by the T_m higher than that for HT22. In the case of the experiments performed in the presence of ammonium cation we did not observe any rational dependence of T_m , which again can be explained by the formation of mixture of different species.

3.2.2 pK_a of hemin/LNA-modified oligonucleotides

As it was mentioned earlier, the feature that is the best correlated with the activity of the DNAzyme is the pK_a of hemin in the complex with G4 oligonucleotide. The higher the constant, the higher activity of DNAzyme that this oligonucleotide forms. We determined pK_a values for HT22 and LNA-modified oligonucleotides in the presence of potassium cation and the pK_a values are presented

in Table 4. HT22 shows the lowest pK_a and all LNA-modified oligonucleotides possess higher values and pK_a increases with the number of LNA modifications. One can expect that the LNA-modified oligonucleotides should form DNAzymes with higher peroxidase activity than unmodified HT22.

3.2.3 Activity of LNA-modified DNAzymes

The last part of this research includes the measurement of DNAzymes activity. For this purpose we have chosen the reaction of ABTS oxidation. In Figure 8 we present the initial velocities for the DNAzyme-catalyzed oxidation of ABTS with hydrogen peroxide. As it was proved already, All DNAzymes showed low activity in the presence of sodium cation. HT22 also possessed low activity in the presence of potassium or ammonium cations. However, oligonucleotides with LNA modifications exhibited much higher activity for these two cations and the increase in catalytic power was in line with the number of modifications. The activity in the presence of ammonium cation was much higher than that in the presence of potassium cation. Interestingly, despite the fact that LNA3 formed parallel G4 in the presence of Na^+ , the activity in these conditions was very low. These results show that the topology or G4 stability is not the determining factor when it comes to the DNAzyme activity. The parallel topology of the G-quadruplex is connected with higher activity but is not a necessary condition. The value that is more informative about DNAzyme activity level is the pK_a . In Table 5 we collected the ratios of the activities (V_{ox}/V_{ohemin}) for conditions presented in Figure 8 in the presence of sodium, potassium and ammonium cations and additionally without presence of any cation and in the presence of mixture of potassium and ammonium cations. For all DNAzymes, the highest catalytic activities were observed when both potassium and ammonium cations were present (K^+/NH_4^+ mixture). These results are in good agreement with our previous report [23]. This effect can be explained by the fact that potassium cation is necessary for the formation of the G-quadruplex and ammonium cation promotes peroxidase activity of DNAzyme. It was also noticed by Sintim et al. that ammonium cation is favored by the ABTS oxidation by DNAzyme in the comparison with other substrates [24]. Presented results proved that LNA point modification can effect DNAzyme activity and change oligonucleotide with low initial activity into a very efficient DNAzyme. Additionally, such modifications are desirable for perspective applications in biological systems.

Table 5: Comparison of relative activities (V_{ox}/V_{ohemin}) of HT22 and LNA-modified DNAzymes.

	HT22	LNA1	LNA2	LNA3
Without M ⁺	1.21	1.26	1.20	1.04
K ⁺	1.51	4.67	9.07	9.77
Na ⁺	0.81	1.00	0.98	1.07
NH ₄ ⁺	3.12	9.56	13.39	21.83
K ⁺ /NH ₄ ⁺	4.57	15.20	16.77	23.73

4 Conclusion

In conclusion we thoroughly examined the influence of analogue modifications on the topology, stability and activity of G4/hemin DNAzyme (ribozyme). Our results showed that application of the 2'-OMe-RNA analogue on the length of oligonucleotide and LNA-point modifications can enhance peroxidase activity of the G4/hemin complex. These modifications can also turn poor DNAzyme like HT22 into a system with very high activity. All modifications favoured the formation of parallel topology which is connected to the high activity of DNAzyme. These results are important from the point of possible applications for probe design for living organisms as well as the application of DNAzymes that normally possess low activity. We are currently working on the development of probes using described modified DNAzymes.

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Conflict of interest: Authors declare no conflict of interest.

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