Site-specific modification of the 5′-terminal fragment of *PGY1/MDR1* gene mRNA by reactive conjugates of antisense oligonucleotides

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The site-specific modification of the 5′-terminal fragment of *PGY1/MDR1* mRNA by oligodeoxyribonucleotide conjugates bearing residues of bleomycin A_5 (Blm), cobalt(II) tetracarboxyphthalocyanine (Phcn), 4-[*N*-(2-chloroethyl)-*N*-methylamino]benzylamine (RCl), or perfluoroarylazide (Az) was studied. Conjugates of oligonucleotides complementary to the RNA sequences 123—138 and 155—166 selectively modify RNA in the vicinity of these regions. The highest efficacy (up to 50%) was achieved in reactions with alkylating and perfluoroarylazide conjugates of oligonucleotides. Conjugates of perfluoroarylazide with 2′-*O*-modified oligonucleotides are much more efficient than analogous conjugates with oligodeoxyribonucleotides (extents of RNA modification are 40—50% and 20%, respectively).

Key words: RNA, affinity modification, oligonucleotide derivatives, bleomycin, Co^{II} tetracarboxyphthalocyanine, perfluoroarylazide.

The design of chemical agents, which are capable of selectively affecting particular nucleic acids under physiological conditions are of considerable interest for solving a number of important problems of molecular biology and chemotherapy. These agents can be used for the design of therapeutic drugs, which are able to inactivate nucleic acids of infectious agents and control gene expression. A direct approach to the synthesis of compounds, which interact selectively with particular nucleotide sequences, is based on the synthesis of oligonucleotide conjugates containing groups active in reactions with RNA and DNA.[1,2] In such constructs, an oligonucleotide serves as an address, which provides specific interactions with the complementary sequence of the target nucleic acid and performs the selective delivery of the reactive group to the chosen sequence. Alkylating[1,3,4] and photoactivated (arylazides, perfluoroarylazides, psoralens, porphyrins, *etc.*)[5—11] groups, whose reactions give rise to covalent bonds between the oligonucleotide and nucleic acid, and catalytically active groups, which cleave nucleic acids,[12—18] can be used as the reactive fragment of conjugates.

Conjugates containing 4-[*N*-(2-chloroethyl)-*N*-methylamino]benzylamine as the alkylating group were well studied in the reactions of site-specific modification of both short synthetic[1,4,19] and high-molecular-weight natural RNAs and DNAs.[1,20,21] Conjugates of oligonucleotides with aromatic azides are of interest because the rate of photomodification of biopolymers by these reagents are much higher than the rate of modification by other reagents, and these photomodification reactions can be initiated under an external action.[5,22] A series of arylazides with different structures, including perfluoroarylazides, were synthesized.[6,10,11,23] These compounds exhibit high efficiency in reactions with RNA and DNA. Conjugates of oligonucleotides with the antibiotic bleomycin[15,17] as well as with bleomycin itself [16] can catalytically cleave DNA[24] and, with a lower efficiency, RNA.[25] A series of conjugates of oligonucleotides with groups generating active forms of oxygen (Fe-, Co-, and Mn-porphyrins or phthalocyanines), which also cleave nucleic acids, were synthesized and it was demonstrated that short synthetic DNA targets can be site-specifically cleaved in the vicinity of the conjugate-binding region.[18,26,27]

Most of the above-mentioned reactive oligonucleotide conjugates were used for the site-specific cleavage or modification of short synthetic DNA targets.[4,6,10,23,26,27] Some conjugates were tested in reactions with short synthetic RNA.[4,10,11,25,26] The studies in which oligonucleotide conjugates were used for the site-specific modification of natural RNA are few in number.[20,21] No comparative studies of the efficacy of conjugates of different nature in reactions with RNA were performed.

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In the present study, we carried out a comparative investigation of the efficacy of site-specific modification of the 5′-terminal *PGY1/MDR1* mRNA fragment by conjugates of antisense oligonucleotides bearing bleomycin A_5 (Blm), cobalt(II) tetracarboxyphthalocyanine (Phcn), 4-[*N*-(2-chloroethyl)-*N*-methylamino]benzylamine (RCl), or perfluoroarylazide (Az) at the 5′-phosphate.

Experimental

The study was carried out with the use of calf intestine alkaline phosphatase (CIP), ribonuclease T1, DNase I (Boehringer Mannheim, Germany), T4 polynucleotide kinase, ribonuclease H (Fermentas, Lithuania), restrictase Dra I (Sibenzim, Russia), reverse transcriptase AMV (Life Science, USA), T7 phage RNA polymerase (Novosibirsk Institute of Bioorganic Chemistry, Siberian Branch of the Russian Academy of Sciences, Russia), acrylamide, *N*,*N*′-methylenebisacrylamide, agarose, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, tris(hydroxymethyl)aminomethane (Tris), dithiotreitol, piperidine, diethyl pyrocarbonate (DEPc), ethylenediaminetetraacetic acid (edta) and its disodium salt (Na_2edta), ribonucleoside 5′-phosphates, deoxyribonucleoside 5′-phosphates, dideoxyribonucleoside 5′-triphosphates (Sigma, USA), and γ-[^{32}P]-ATP with a specific activity of higher than 0.1 PBq mol^{-1} (Biosan, Russia).

All solutions were prepared with the use of water purified on a MilliQ system (MilliPore, USA). The buffers were sterilized by treating with diethyl pyrocarbonate (250 μL of DEPC per liter of the solution, 60 °C, 10 h) or by autoclaving.

The compositions of the buffers used were as follows: **buffer A**, 40 *mM* Tris-HCl, pH 7.5, 1 *mM* spermidine, 8 *mM* $MgCl_2$; **buffer B**, 50 mM Tris-HCl, pH 8.5, 0.1 *mM* Na_2edta; **buffer C**, 50 *mM* Tris-HCl, pH 7.6, 10 *mM* $MgCl_2$, 5 *mM* DTT, 0.1 *mM* spermidine, 0.1 *mM* Na_2edta; **buffer D**, 2 *M* imidazole, pH 7.0, 1 *mM* Na_2edta, 250 μg mL^{-1} of the total yeast tRNA as the carrier; **buffer E**, 6 *M* urea, 25 *mM* sodium citrate, pH 4.5—4.8, 1 *mM* Na_2edta, 100 μg mL^{-1} of the RNA carrier; **buffer F**, 50 *mM* Tris-HCl, pH 8.3, 6 *mM* $MgCl_2$, 40 *mM* KCl; **buffer G**, 50 *mM* sodium cacodylate, pH 7.2, 0.2 *M* KCl, 100 μg mL^{-1} of the RNA carrier; **buffer H**, 50 *mM* Tris-HCl, pH 7.2, 0.2 *M* KCl, 100 μg mL^{-1} of the RNA carrier; **buffer I**, 0.1 *M* NaCl, 0.01 *M* Tris-HCl, pH 7.4, 1 *mM* Na_2edta.

Experiments on the photomodification were carried out with the use of a DRK-120 high-pressure mercury lamp of an OI-18A illuminator (LOMO, Russia) equipped with an UFS-2 light filter to separate emission lines in the range of 303—365 nm.

The temperature was maintained with the use of a Multitemp II Thermostatic Circulator (LKB, Sweden).

Oligonucleotides. The pCGACCTCGCGCTCCTT (**dB**), 24-mer TTCCAAGGAGCGCGAGGTCGGGAT (**dON24**), and 17-mer AAGGGGACCGCAATGGA (**dON17**) oligodeoxyribonucleotides were synthesized by the standard phosphoramidite method on an ASM-102U synthesizer (BIOSSET, Novosibirsk, Russia) in the Novosibirsk Institute of Bioorganic Chemistry of the Siberian Branch of the Russian Academy of Sciences. The oligoribonucleotide $C^mC^mA^mU^mU^mG^mC^mG^mG^mG^mU^mC^mC^mp$ (**mK**) containing the methoxy group at position 2′, the oligoribonucleotide $C^tC^tA^tU^tU^tG^tC^tG^tG^tG^tU^tC^tC^tp$ (**tK**) containing the 2′-*O*-tetrahydropyranyl group, and their deoxyribo analog CCATTGCGGGTCCp (**dK**) were synthesized by the solid-phase H-phosphonate method.[28,29] A CPG-500 controlled pore glass (Sigma, USA) bearing residues of 2-[2-(4,4′-dimethoxytrityloxy)ethylsulfonyl]ethanol was used as the polymeric carrier. The oligonucleotides were isolated by HPLC. The oligonucleotide purities were tested by electrophoresis in 12% polyacrylamide gel (PAAG) under denaturating conditions followed by visualization using the Stains-All dye. The oligoribonucleotide AAGGGGACCGCAAUGGA (rON17) was kindly supplied by M. N. Repkova (Novosibirsk Institute of Bioorganic Chemistry, Siberian Branch of the Russian Academy of Sciences).

Reactive oligonucleotide derivatives. The conjugate of the oligonucleotide with bleomycin (**dB-Blm**) was synthesized according to a procedure described earlier.[24] The conjugate of the oligonucleotide with 4-[*N*-(2-chloroethyl)-*N*-methylamino]benzylamine (**dB-RCl**) was synthesized according to a known procedure.[30] The conjugate of the oligonucleotide with cobalt tetracarboxyphthalocyanine (**dB-Phcn**) was prepared according to a procedure described earlier.[26] The conjugates of the oligonucleotides (**tK-Az**, **mK-Az**, **dK-Az**) containing the 4-azidotetrafluorobenzamide group were synthesized as described earlier.[10,31]

Preparation of the 5′-[^{32}P]-labeled 190-mer in vitro transcript of PGY1/MDR1 RNA. The preparative cleavage of the pMDR670 plasmid by restriction endonuclease *Dra I*, purification of linearized DNA, and the preparation of the *in vitro* transcript of *PGY1/MDR1* mRNA were performed as described earlier.[32] The RNA transcript was isolated by electrophoresis in 6% PAAG under denaturating conditions, eluted from the gel with 0.3 *M* AcONa (pH 5.0), and precipitated with ethanol. The RNA that precipitated was dried, dissolved in MilliQ water, and stored at −20 °C. The concentration of RNA was determined spectrophotometrically.

Dephosphorylation of the RNA transcript was carried out as described earlier.[33] The reaction mixture (50 μL) containing RNA (400 pmol), buffer B, 2% formamide, 2.5 *mM* DTE, and calf intestine alkaline phosphatase in concentration of 12 units mL^{-1} and RNasin in concentration 200 units mL^{-1} (Frementas, Lithuania) was incubated at 37 °C for 30 min. Then the same amount of alkaline phosphatase was added and the mixture was again incubated at 37 °C for 30 min after which the mixture was extracted with a phenol—$CHCl_3$ mixture (1 : 1), and RNA was precipitated with ethanol.

The 5′-[^{32}P]-labeled RNA was synthesized according to a standard procedure.[34] The labeled RNA was separated by electrophoresis in 8% PAAG under denaturating conditions. The RNA band was cut from the gel by autoradiography, eluted, and precipitated as described above.

The 5′-[^{32}P]-label was introduced into the oligonucleotides according to a standard method.[35]

The RNA hydrolysis in an imidazole buffer and with RNase T1. *Statistical RNA hydrolysis in an imidazole buffer.*[36] 5′-[^{32}P]-Labeled RNA was incubated in a 2 *M* imidazole buffer (buffer D) at 90 °C for 10 min and precipitated from the reaction mixture by the addition of ten volumes of a 2% solution of $LiClO_4$ in acetone. The RNA precipitate was dissolved in 8 *M* urea and stored at −20 °C until it was subjected to polyacrylamide gel electrophoresis.

Hydrolysis with RNase T1.[37] [^{32}P]-Labeled RNA was incubated in buffer E at 55 °C for 10 min in the absence of RNase T1. Then RNase T1 was added to the reaction mixture to the con-

centration of 100—150 units mL^{-1} and the mixture was incubated at 55 °C for 10 min. The reaction was quenched by the addition of a Tris-borate buffer (1 μL; 1 *M* Tris-borate buffer, pH 8.3, 20 *mM* EDTA). The resulting mixture was subjected to polyacrylamide gel electrophoresis without pretreatment.

***Probing of RNA·oligonucleotide heteroduplexes with RNase H.*[38]** The reaction mixture (10 μL) containing 5′-[^{32}P]-*PGY1/MDR1* mRNA at a concentration of $1.5 \cdot 10^{-8}$ mol L^{-1}, buffer G, and the oligonucleotide or the corresponding conjugate at a concentration of $1 \cdot 10^{-6}$—$5 \cdot 10^{-5}$ mol L^{-1} was incubated at 37 °C for 30 min. Then RNase H was added to the concentration of 1 unit mL^{-1} and the reaction mixture was again incubated at 37 °C for 30 min. After incubation, RNA was precipitated with ethanol. The RNA pellet was centrifuged, washed with 80% EtOH, and dissolved in 8 *M* urea. The hydrolysis products were analyzed by electrophoresis in 8% PAAG under denaturating conditions. After electrophoresis, the gel was dried and autoradiographed on an X-ray film for 1—2 days.

Primer-directed reverse transcription. Primer-directed reverse transcription was carried out according to a known procedure[39] with the use of 0.05 μg of RNA and 60 pmol of the 5′-[^{32}P]-labeled primer oligonucleotide in each reaction. The elongation reaction was carried out at 37 °C for 1 h. To determine the RNA sequence, the elongation reaction of the primer in the presence of 50 μM dideoxyribonucleoside phosphates (ddNTP) was performed in parallel. The reaction was quenched by precipitation with ethanol. The DNA precipitate was separated by centrifugation (15 min, 14000 rpm) and dissolved in formamide containing leading dyes. The samples were incubated at 100 °C to remove RNA and then subjected to polyacrylamide gel electrophoresis in 8% PAAG under denaturating conditions. After electrophoresis, the gel was dried and autoradiographed on an X-ray film for 1—2 days.

Site-specific cleavage/modification of RNA190 and DNA by oligonucleotide conjugates.

The conjugate of the oligonucleotide dB with bleomycin (dB-Blm). The reaction mixture (10 μL) contained the [^{32}P]-labeled fragment of *PGY1/MDR1* mRNA or DNA (**dON24**) at a concentration of $1.5 \cdot 10^{-8}$ *M*, buffer G, $5 \cdot 10^{-5}$ *M* conjugate **dB-Blm**, $5 \cdot 10^{-4}$ *M* Moore salt ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$), and 0.05 *M* β-mercaptoethanol. A solution of the Moore salt was prepared directly before use in water saturated with argon. The reaction was carried out at 37 °C for 1, 3, or 5 h and then quenched by precipitation with ethanol. The RNA and DNA cleavage products were analyzed under denaturating conditions in 8% and 20% PAAG, respectively. After completion of electrophoresis, the gel was dried and autoradiographed on an X-ray film for 1—2 days. To obtain quantitative data, the autoradiograph of the gel was analyzed with the use of the Gel-Pro Analyzer program package (Media Cybernetics, Inc., USA). The relative experimental error was no higher than 10%.

Conjugate of oligonucleotide dB with cobalt(II) tetracarboxyphthalocyanine (dB-Phcn). The reaction mixture (10 μL) contained the [^{32}P]-labeled fragment of PGY1/MDR1 mRNA or DNA (**dON24**) at a concentration of $1.5 \cdot 10^{-8}$ mol L^{-1}, buffer H, $5 \cdot 10^{-5}$ *M* conjugate **dB-Phcn** or $1 \cdot 10^{-5}$ *M* phthalocyanine, and $5 \cdot 10^{-3}$ *M* β-mercaptoethanol. The samples were incubated at 37 °C for 30 min, 1, 3, 5, or 10 h. The reaction was quenched by the addition of 3 *M* AcONa (1 μL, pH 5.8) and EtOH (40 μL). The samples containing DNA were treated with piperidine to reveal a "hidden" modification. For this purpose, the DNA precipitate obtained after the modification was dissolved in 1 *M* piperidine (50 μL) and incubated at 95 °C for 40 min. The RNA and DNA cleavage products were analyzed as described above.

Conjugate of oligonucleotide dB with 4-[*N*-(2-chloroethyl)-*N*-methylamino]benzylamine (dB-RCl). The reaction mixture (10 μL) contained 5′-[^{32}P]-labeled DNA or RNA at a concentration of $1.5 \cdot 10^{-8}$ mol L^{-1}, buffer H, 0.1 *mM* EDTA, and conjugate **dB-RCl** at a concentration of $1 \cdot 10^{-5}$ mol L^{-1}. The samples were incubated at 37 °C for 1, 5, or 10 h. The reaction was quenched by the addition of 3 *M* AcONa (1 μL, pH 5.8) and EtOH (40 μL). The reaction products were analyzed as described above.

Conjugates of oligonucleotides (dK-Az, tK-Az, and mK-Az) containing the *p*-azidotetrafluorobenzamide group. ***A. Photomodification of the 17-mer synthetic fragment of PGY/MDR1 mRNA (region 151—167) (rON17) and its DNA analog (dON17).*** The reaction mixture (10 μL) containing the 5′-[^{32}P]-labeled RNA or DNA target at a concentration of $1 \cdot 10^{-7}$ mol L^{-1} and the corresponding perfluoroarylazide reagent at a concentration of $1 \cdot 10^{-5}$ mol L^{-1} in buffer I was placed in cylindrical wells (4 mm in diameter) of immunological plates. The plates were covered and irradiated for 10 min at 5, 20, or 40 °C with the use of a DRK-120 high-pressure mercury lamp (at a distance of 5 cm) equipped with an UFS-2 light filter to separate emission lines in the range of 303—365 nm; the total power density of the flux $W = 0.5 \cdot 10^{-4}$ W cm^{-2}. After irradiation, the reaction mixture was precipitated with 2% $LiClO_4$ and subjected to electrophoresis in denaturating 20% PAAG. The results of electrophoretic assays were visualized by autoradiography at 4 °C. To obtain quantitative data, the autoradiograph of the gel was digitized using the Gel-Pro Analyzer program package (Media Cybernetics, Inc., USA). The relative experimental error was no higher than 10%. The extent of formation of covalent adducts was estimated as the ratio of the peak areas of the covalent adducts to the sum of the peak areas of the covalent adducts and the peak of the starting oligonucleotide.

B. Photomodification of the 190-mer fragment of PGY1/MDR1 RNA by conjugates tK-Az, mK-Az, and dK-Az. The reaction mixture (10 μL) contained 5′-[^{32}P]-labeled RNA at a concentration of $5 \cdot 10^{-8}$ mol L^{-1}, buffer H, and the oligonucleotide conjugate at a concentration of $1 \cdot 10^{-5}$ mol L^{-1}. The samples were incubated at 37 °C for 10 min and irradiated with filtered light using a mercury lamp at 37 °C for 1, 3, 6, or 10 min. Then the samples were precipitated with ethanol and analyzed as described above.

Results and Discussion

RNA target and conjugates of antisense oligonucleotides

The aim of the present study was to compare the efficacy of RNA modification/cleavage by reactive conjugates of oligonucleotides with chemical groups of different nature, *viz.*, with bleomycin A_5, Co^{II} phthalocyanine, perfluoroarylazide, and 4-[*N*-(2-chloroethyl)-*N*-methylamino]benzylamine. The 5′-terminal fragment of mRNA of the human multi-drug resistance gene *mdr1*

(PGY1/MDR1 mRNA) (bases 1—190) was used as the RNA target. This fragment contains the 5′-nontranslated region, the translation initiation site, and a small fragment of the coding region of this mRNA.[32] The synthetic 17-mer oligoribonucleotide rAAGGGGACCGCAATGGA (rON17), which is a fragment of *PGY1/MDR1* mRNA (bases 155—170), was used as the RNA target, which does not have a pronounced secondary structure. The oligonucleotides dTTCCAAGGAGCGCGAGGTCGGGAT (**dON24**) and dAAGGGGACCGCAATGGA (**dON17**) corresponding to the regions 119—142 and 155—170 of this mRNA were used as models for estimating the possibilities of the modification of the chosen NA sequences by the conjugates synthesized.

Earlier,[32] the secondary structure of the 5′-terminal fragment of *PGY1/MDR1* mRNA containing 670-nucleotides was proposed. Since the shortened RNA fragment (190 nucleotides) might have a different secondary structure, we compared the structures of the fragments of *PGY1/MDR1* mRNA containing 190 and 670 nucleotides by probing with ribonucleases T1 and ONE.[40] A comparison of the data from probing revealed the identity of the sites of hydrolysis by ribonucleases T1 and ONE in both fragments (results of probing are not reported), which indicates that the 190-mer sequences in the spatial structures of these RNA have similar foldings (Fig. 1).

The chemical groups involved in the oligonucleotide conjugates are presented in Table 1. The antisense oligonucleotides for the synthesis of conjugates were chosen taking into account the specificity of action of different groups. According to the data published in the literature,[1,19] alkylation of RNA and DNA by alkylating oligonucleotide conjugates most efficiently proceeds at the guanine residues adjacent to the conjugate-binding region. Perfluoroarylazides show the highest efficacy in modification of sequences containing several guanine residues in succession both in RNA and DNA.[6—8,23] Precise data on the sequence specificity of the reactions involving phthalocyanine-containing oligonucleotide conjugates are lacking. However, it was demonstrated[27,41] that such reagents modify DNA predominantly at sequences containing guanine residues. Besides, metalloporphyrins can oxidize the C(5′) atom of deoxyribose of a nucleoside located at the 3′-terminus of an AT sequence.[27] It was found[17,42,43] that the DNA cleavage both by free bleomycin and bleomycin bound to an oligonucleotide proceeds predominantly at sequences containing the 5′-GT-3′ and 5′-GC-3′ fragments. Analysis of the data on the RNA cleavage by free bleomycin showed that the reactivity of nucleotides toward this reagent depends substantially on the RNA structure. Thus, the cleavage proceeds most efficiently at the junction between single- and double-strandeded regions of RNA.[17] In double-stranded regions of RNA, the cleavage by bleomycin proceeds predominantly in the G-Pyr fragments.[17]

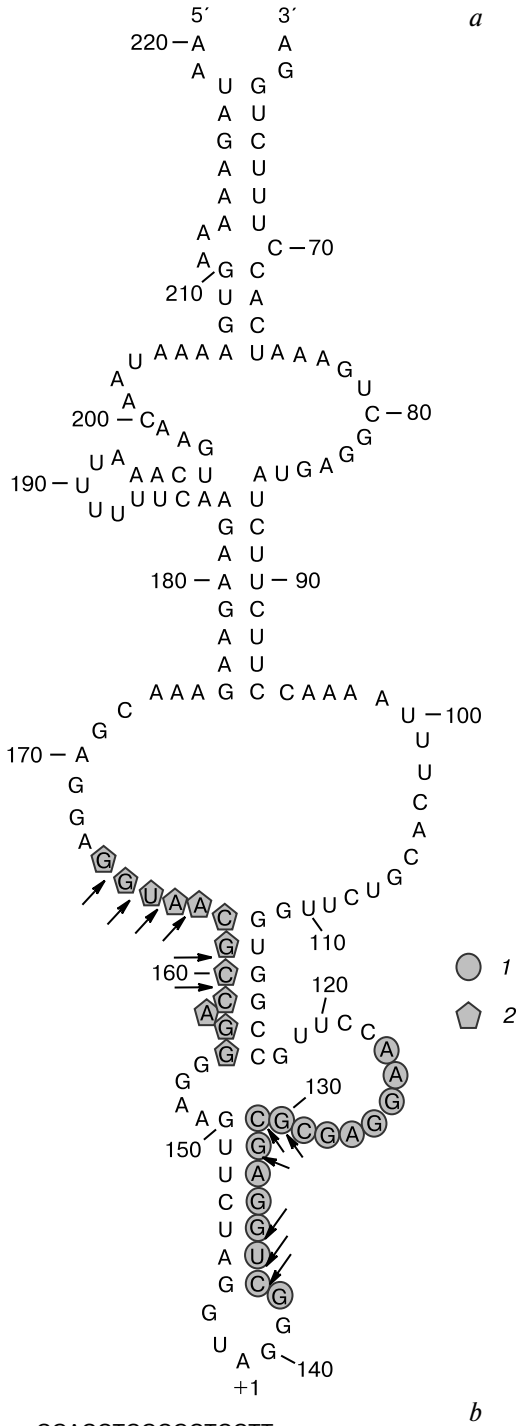
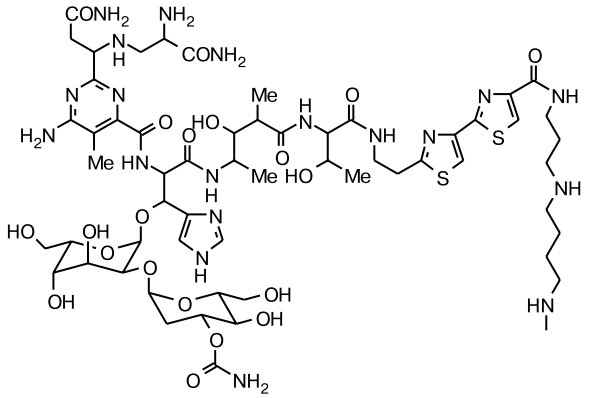


Fig. 1. *a*. Secondary structure of the 5′-terminal fragment of *PGY1/MDR1* mRNA and complementary regions of antisense oligonucleotides **dB** (*1*) and **dK** (*2*). The sites of RNA hydrolysis by RNase H in the RNA·oligonucleotide heteroduplexes are indicated by arrows; the translation initiation site of P glycoprotein is marked with +1. *b*. The sequences of oligonucleotides **dB** and **dK**.

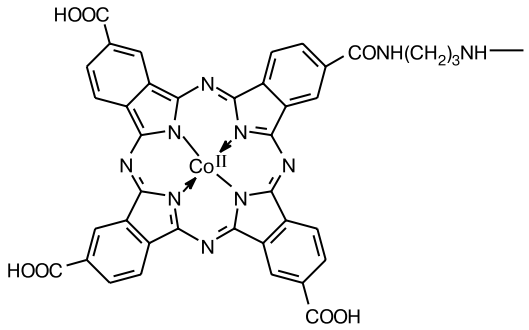
Table 1. Oligonucleotide conjugates and their efficacy in reactions with the fragments of *PGY1/MDR1* mRNA and oligodeoxyribonucleotides *in vitro*

Oligonucleotide[a]	RNA complementary region	Active group	Efficacy of cleavage/modification[b] (%)		Oligonucleotide[a]	RNA complementary region	Active group	Efficacy of cleavage/modification[b] (%)	
			RNA	DNA				RNA	DNA
dB	123—138	**Blm**	~5	60 [c]	**dK**	155—166	**Az**	20	58 [d]
	123—138	**Phcn**	~1	15 [c]	**tK**	155—166	**Az**	51	60 [d]
	123—138	**RCl**	56	62 [c]	**mK**	155—166	**Az**	40—50	42 [d]

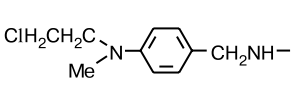
[a] **d** is oligodeoxyribonucleotide, **t** is oligo(2′-*O*-tetrahydropyranylribonucleotide), and **m** is oligo(2′-*O*-methylribonucleotide).
[b] The conditions for the modification/cleavage are given in the Experimental section. The error of the determination of the extent of modification or cleavage of nucleic acids was no higher than 10%.
[c] Deoxyribo analog **dON24** of the *PGY1/MDR1* mRNA region 120—143.
[d] Deoxyribo analog **dON17** of the region *PGY1/MDR1* mRNA 151—167.



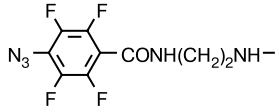
Blm



Phcn



RCl



Az

Note. Blm is the residue of bleomycin A_5, Phcn is the residue of cobalt(II) tetracarboxyphthalocyanine, RCl is the residue of 4-[*N*-(2-chloroethyl)-*N*-methylamino]benzylamine, and Az is the residue of perfluoroarylazide.

The oligonucleotide-binding region was chosen in such a way that the complementary site would contain a single-stranded region of RNA composed of 4—6 bases, which is necessary for efficient binding with the RNA target,[44,45] and would be located in the vicinity of the required sequence. Oligonucleotide **dB** complementary to the fragment 123—138 of *PGY1/MDR1* mRNA was chosen for the synthesis of conjugates with bleomycin A_5 (**dB-Blm**), phthalocyanine (**dB-Phcn**), and RCl (**dB-RCl**) and would be expected to deliver the reactive groups to the ^{135}GUCGGG^{140} sequence. Oligonucleotide **dK** complementary to the region 155—166 directs the arylazido group of the conjugate to the sequence of four guanine residues G_{153}—G_{156}, which exhibit the highest sensitivity to such reagents.[8] The complementary sites of the oligonucleotides at the *PGY1/MDR1* mRNA fragment (hereinafter, RNA190) are presented in Table 1 and Fig. 1.

Study of binding specificity of oligonucleotides and their conjugates with RNA190

The ability of the oligonucleotides and conjugates under consideration to form duplexes with complementary sequences of RNA190 was tested by probing with RNase H. 5′-[^{32}P]-Labeled RNA190 was hydrolyzed in the complex with the oligodeoxyribonucleotide or conjugate using RNase H. The reaction products were subjected to electrophoresis in 8% polyacrylamide gel under denaturating conditions (Fig. 2). As expected, oligonucleotides **dB** and **dK** taken at a concentration of 10^{-6} mol L^{-1} were bound to the fragment of *GY1/MDR1* mRNA only in the target regions (Fig. 2, lanes *8—12*). The insertion of a reactive group into an oligonucleotide may substantially change its affinity for RNA. It appeared that the addition of phthalocyanine or bleomycin to oligonucleotide **dB** and the addi-

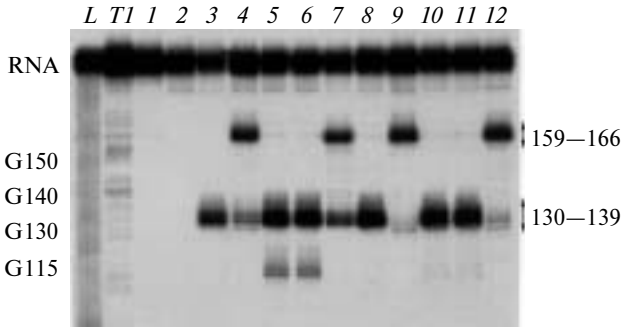


Fig. 2. Probing of the heteroduplexes of the 5′-[^{32}P]-labeled fragment of *PGY1/MDR1* mRNA (RNA190) with the oligonucleotides and conjugates using RNase H. The autoradiograph of denaturating 8% PAAG. Lanes *L* and *T1*, statistical RNA190 hydrolysis in a 2 *M* imidazole buffer and by RNases T1 under denaturating conditions, respectively; *1*, control, RNA190; *2*, RNA190+RNase H; *3—12*, RNA190 hydrolysis by RNase H in the presence of oligonucleotides **dB** (*3*, *8*) and **dK** (*4*, *9*) and conjugates **dB-Blm** (*5*, *10*), **dB-Phcn** (*6*, *11*), and **dK-Az** (*7*, *12*) at concentrations of $1 \cdot 10^{-5}$ (*3—7*) and $1 \cdot 10^{-6}$ mol L^{-1} (*8—12*). The nucleotides of *PGY1/MDR1* mRNA are numbered.

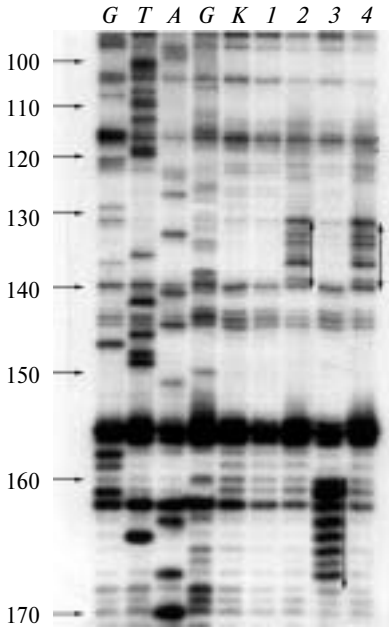


Fig. 3. Regions of hybridization of the *PGY1/MDR1* mRNA fragment (RNA190) with the oligonucleotides as assayed by RNase H and primer-directed reverse transcription. Lanes *C*, *T*, *A*, and *G*, RNA190 sequence reactions; lane *K*, control, RNA190; *1—4*, RNA190 incubated with RNase H in the absence of oligonucleotides (*1*) and in the presence of oligonucleotides **dB** (*2*) and **dK** (*3*) and conjugate **dB-Blm** (*4*) ($1 \cdot 10^{-6}$ mol L^{-1}). The numbers indicate nucleotides of RNA190. The sites of cleavage by RNase H are indicated by double arrows.

tion of perfluoroarylazide to oligonucleotide **dK** led to an enhancement of their affinity for RNA. When the concentrations of conjugates **dB-Blm**, **dB-Phcn**, and **dKAz** were increased to 10^{-5} mol L^{-1}, these conjugates exhibited the ability to be additionally bound to RNA190 in the regions 113—124 and 130—139, although with low efficacy (Fig. 2, lanes *5—7*). At low concentrations, no interaction of the conjugates with these RNA regions was observed.

The precise identification of the oligonucleotide-binding regions of RNA190 was carried out by primer-directed reverse transcription. For this purpose, we performed RNA reverse transcription after its treatment in the RNA•oligonucleotide heteroduplexes with RNase H using the 5′-[^{32}P]-labeled primer. As can be seen from Fig. 3, oligonucleotides **dB** and **dK** and conjugate **dB-Blm** were bound to RNA190 at the complementary sequences (regions 123—138 and 158—166, respectively; Fig. 3, lanes *2—4*). The data on RNA190 hydrolysis by RNase H involved in the heteroduplexes are summarized in Fig. 1.

Reactions of fragments of *PGY1/MDR1* mRNA and DNA with oligonucleotide conjugates

Conjugate of oligonucleotide dB with 4-[*N*-(2-chloroethyl)-*N*-methylamino]benzylamine (dB-RCl). Experiments on site-specific alkylation of RNA190 with conjugate **dB-RCl**, like the reactions with other conjugates, were carried out according to a standard scheme. 5′-[^{32}P]-Labeled RNA190 at a concentration of $1.5 \cdot 10^{-8}$ mol L^{-1} was incubated in the presence of the conjugate in the corresponding buffer for 1—10 h at different concentrations of the conjugate. As the control experiment, RNA was incubated in the same buffer in the presence of only the oligonucleotide, only bleomycin or phthalocyanine, or in the presence of the conjugate but without the addition of the required cofactors. The reaction products were subjected to electrophoresis in 8% PAAG under denaturating conditions. The quantitative data were obtained by processing the autoradiographs of the gels with the use of the Gel-Pro program package (see the Experimental section).

The characteristic features of site-specific alkylation of RNA and DNA targets by conjugates of oligonucleotides with 4-[*N*-(2-chloroethyl)-*N*-methylamine]benzylamine were well studied.[1,3,4,19,21] We used site-specific alkylation of RNA190 and **dON24** with the aim of revealing the accessibility of the chosen RNA region to modification. As mentioned above, the nucleic acid sequence adjacent to the oligonucleotide-binding region as well as the accessibility of the binding region in the RNA structure can be of decisive importance for the efficacy of RNA modification.[20,21] The experiments demonstrated that under the conditions used, RNA190 and **dON24** were modified by conjugate **dB-RCl** with similar efficacy (62 and 56%, respectively) (Table 1). These data indicate that the chosen RNA190 region is accessible to modification.

Conjugate of oligonucleotide dB with bleomycin A_5 (dB-Blm). The cleavage of 5'-[^{32}P]-labeled RNA190 by the conjugate of oligonucleotide **dB** with bleomycin A_5

(**dB-Blm**) was carried out in the presence of Fe^{2+} ions and β-mercaptoethanol. The reaction products were analyzed by electrophoresis. The autoradiograph of the gel (Fig. 4, *a*) showed that the presence of conjugate **dB-Blm** gave rise to two RNA190 cleavage sites in the region of nucleotide bases 137—144, in the vicinity of the region of binding of oligonucleotide **dB**, and in the region of bases 106—109. The RNA cleavage in the region 106—109 occurs through binding of conjugate **dB-Blm** with RNA190 in the vicinity of this sequence, which was detected by probing with RNase H (Fig. 2). The efficacy of RNA190 cleavage by conjugate **dB-Blm** under the conditions used is low (no higher than 5%). In the absence of the conjugate, iron ions, or mercaptoethanol, the site-specific RNA cleavage was not observed. Free bleomycin also did not cleave RNA190 under these conditions (Fig. 4, *a*).

The cleavage of the 24-mer oligodeoxyribonucleotide (**dON24**) containing the region complementary to oligonucleotide **dB** under the action of conjugate **dB-Blm** in the presence of iron ions and β-mercaptoethanol led to accumulation of shortened DNA fragments (Fig. 4, *b*). The total efficacy of **dON24** cleavage by conjugate **dB-Blm** (56%) reached a plateau already after 60 min. Further incubation of **dON24** in the presence of conjugate **dB-Blm** did not lead to an increase in the extent of cleavage of the DNA target. Under the same conditions, **dON24** was also efficiently cleaved by free bleomycin (Fig. 4, *b*, lanes *3* and *4*).

The observed difference in the efficacy of DNA and RNA cleavage are most likely associated with substantial differences in the structure of the resulting complementary complexes. It is known that bleomycin is prone to cleave the B form of DNA,[16,17] whereas RNA and DNA/RNA heteroduplexes form double-stranded helixes of the A form.[46] Conceivably, it is due to these geometric characteristics of this complex, *viz.*, due to the differences in the sizes of the minor and major grooves and in the conformation of the carbohydrate residue, that the activated bleomycin cannot perform destruction of the ribose residue.[49] This fact may be responsible for the low efficacy of the reaction with RNA.[17] This inaccessibility of the substrate generally leads to self-oxidation followed by self-destruction of the antibiotic molecule.[16]

It should be noted that the mechanism of DNA cleavage by bleomycin has been studied in detail.[16,17,47] It is known with certainty that the mechanism of RNA cleavage differs from the mechanism of DNA cleavage, and these reactions afford different products.[16,48] It should also be taken into account that ribose and deoxyribose differ in sensitivity to oxidation.[12,49]

The results of our experiments demonstrated that the RNA cleavage by conjugate **dB-Blm** proceeded with low efficacy. The efficacy of RNA cleavage is more than an order of magnitude lower than the efficacy of DNA cleavage. The efficacy of RNA cleavage can be improved by optimizing the conjugate structure or by constructing

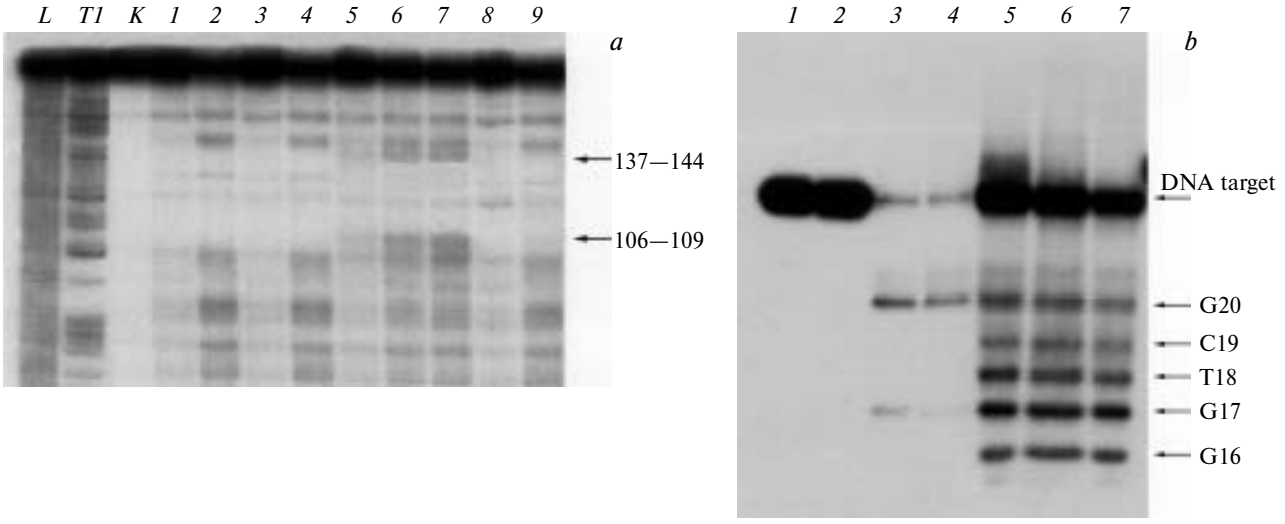


Fig. 4. Cleavage of RNA190 and **dON24** by the conjugate of oligonucleotide **dB** with bleomycin **(dB-Blm)**. *a*. Cleavage of the 5′-[^{32}P]-labeled fragment of *PGY1/MDR1* mRNA (RNA190) by conjugate **dB-Blm**: lanes *L* and *T1*, statistical RNA190 hydrolysis in a 2 *M* imidazole buffer (*L*) and with the use of RNase T1 (*T1*) under denaturating conditions; lanes *K* (control), *1—9*: RNA190 incubated in the buffer for 1 (*K*), 3 (*1*), and 5 h (*2*); RNA190 incubated in the presence of $5 \cdot 10^{-5}$ *M* bleomycin (*3*, *4*); $5 \cdot 10^{-5}$ *M* conjugate **dB-Blm**, $5 \cdot 10^{-4}$ *M* Fe^{2+} salt, and $5 \cdot 10^{-2}$ *M* β-mercaptoethanol for 1 (*5*), 3 (*6*), and 5 h (*7*); in the presence of only the Fe^{2+} salt and β-mercaptoethanol for 1 (*8*) and 5 h (*9*). The sites of RNA190 cleavage by conjugate **dB-Blm** are indicated by arrows. *b*. The cleavage of the 24-mer oligodeoxyribonucleotide (**dON24**) by conjugate **dB-Blm**: control (*1*), **dON24** control (*2*); **dON24** incubated with $1 \cdot 10^{-5}$ *M* bleomycin in the presence of $5 \cdot 10^{-2}$ *M* β-mercaptoethanol and $1 \cdot 10^{-4}$ *M* Fe^{2+} salt for 10 (*3*) and 90 min (*4*); in the presence of conjugate **dB-Blm**, Fe^{2+} salt, and β-mercaptoethanol for 1 (*5*), 3 (*6*), and 5 h (*7*). The concentrations of **dON24**, **dB-Blm**, Fe^{2+}, and β-mercaptoethanol were $1 \cdot 10^{-8}$, $1 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, and $5 \cdot 10^{-2}$ mol L^{-1}, respectively.

structures in RNA, which are favorable for the cleavage by bleomycin.[16,17,25]

Conjugate of oligonucleotide dB with cobalt(II) tetracarboxyphthalocyanine (dB-Phcn). Earlier, it has been demonstrated[26] that conjugates of oligonucleotides with cobalt tetracarboxyphthalocyanine can perform site-specific DNA cleavage with efficacy of 10—20%. Actually, the experimental data (Fig. 5, *a*) show that the direct DNA cleavage by the conjugate proceeded with efficacy of ~8% after incubation for 24 h and only in the presence of β-mercaptoethanol (see Fig. 5, *a*, lanes *3—6*). Treatment of **dON24** following its modification with 1 *M* piperidine revealed a hidden modification of the target, whose extent was also ~8% (50% of the total extent of DNA modification) (see Fig. 5, *a*, lanes *7—10*). Neither cleavage nor modification of **dON24** occurred upon its incubation with free phthalocyanine (Fig. 5, *a*, lanes *1* and *2*). The low yields achieved in this reaction are typical of DNA modifications by derivatives of metal complexes and are accounted for by a complex multistep process of radical formation.[13,50]

The 5′-[^{32}P]-labeled fragment of *PGY1/MDR1* mRNA was incubated with conjugate **dB-Phcn** under similar conditions at 37 °C in the presence of $5 \cdot 10^{-3}$ *M* β-mercaptoethanol. As can be seen from the analysis of the reaction products (see Fig. 5, *b*), the efficacy of RNA190 cleavage by conjugate **dB-Phcn** nearby the nucleotide 140 in the vicinity of the oligonucleotide **dB** binding site was about 1%. The lower extend of RNA190 cleavage as compared to DNA cleavage is, apparently, associated with higher resistance of ribose to oxidation.[12,51] It is possible that the modification gives rise to relatively stable products, which do not cause the direct cleavage of the sugar-phosphate backbone of RNA. It was demonstrated[51,52] that the efficacy of the cleavage of the DNA target by conjugates of oligonucleotides with porphyrins depends substantially on the structure and length of the linker between the metalloporphyrin and the oligonucleotide. Hence, one would expect that optimization of the conjugate structure might lead to an improvement of the efficiency of the reaction.

Conjugates of oligonucleotides of different types with perfluoroarylazide. Earlier,[10] the efficacy of photomodification by perfluoroarylazide derivatives of oligonucleotides depending on the type of the addressed oligonucleotide and the arrangement of the photoactivated group in the oligomeric chain has been studied for model 20-mer RNA and DNA targets. In the present study, we examined the site-specific modifications of *PGY1/MDR1* mRNA, the 17-mer synthetic fragment of the same mRNA (**rON17**), and its deoxyribo analog (**dON17**) by deoxyribo-, 2′-*O*-tetrahydropyranylribo-, and 2′-*O*-methyloligoribonucleotides bearing the *p*-azidotetafluorobenzamide group (**dK-Az**, **tK-Az**, and **mK-Az**, respectively) (Fig. 6). Analogs of oligonucleotides, which form highly stable complementary complexes,[53,54] such as 2′-*O*-modified oligoribonucleotides, have advantaged over usual oligodeoxyribonucleotides when they are used as antisense oligonucleotides or probes for their hybridization.

The photomodification was carried out upon irradiation with light in the wavelength region $280 < \lambda < 370$ nm.

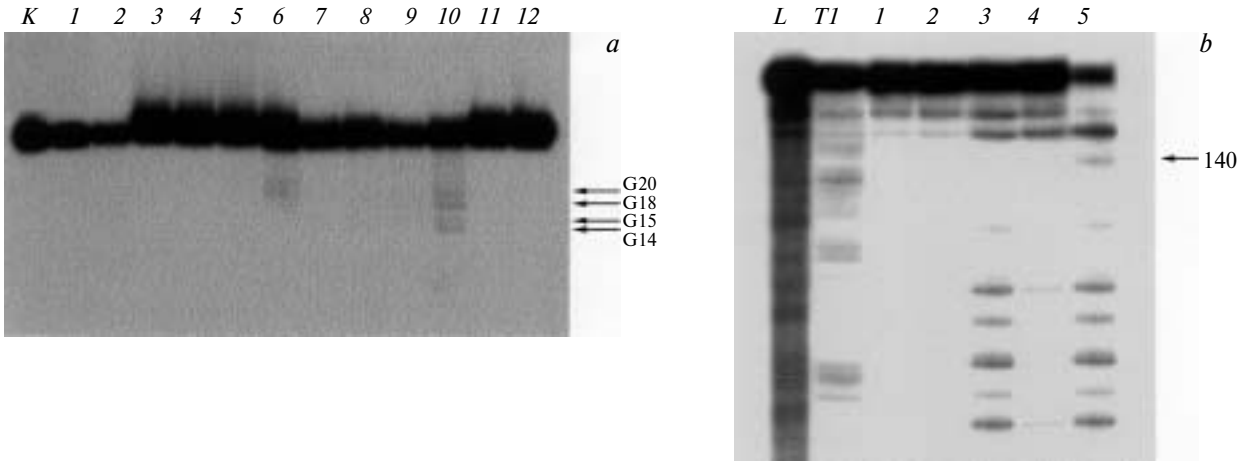


Fig. 5. Cleavage of RNA190 and **dON24** by the conjugate of oligonucleotide **dB** with cobalt(II) tetracarboxyphthalocyanine (**dB-Phcn**). *a*. Cleavage of the 24-mer oligodeoxyribonucleotide 5′-[^{32}P]-**dON24** by conjugate **dB-Phcn**. The autoradiograph of denaturating 18% PAAG: control (*K*), **dON24**; **dON24** incubated with phthalocyanine (*1*, *2*); with $1 \cdot 10^{-5}$ *M* conjugate **dB-Phcn** in the presence of $5 \cdot 10^{-5}$ *M* β-mercaptoethanol for 1 (*3*, *7*), 3 (*4*, *8*), 8 (*5*, *9*), and 24 h (*6*, *10*). After incubation and before electrophoresis, DNA was treated with 1 *M* piperidine (lanes *2*, *7—10*); DNA incubated only with $1 \cdot 10^{-5}$ *M* conjugate **dB-Phcn** for 24 h followed by treatment with piperidine (*11*) and without this treatment (*12*). The cleavage sites are indicated at the right of the figure. *b*. Cleavage of the 5′-[^{32}P]-labeled fragment of *PGY1/MDR1* mRNA (RNA190) by conjugate **dB-Phcn**: lanes *L* and *T1*, statistical RNA190 hydrolysis in a 2 *M* imidazole buffer and by RNase T1 under denaturating conditions, respectively; control, RNA190 (*1*); RNA190 incubated with $1 \cdot 10^{-5}$ *M* phthalocyanine in the presence of $5 \cdot 10^{-5}$ *M* β-mercaptoethanol for 1 (*2*) and 5 h (*3*); with conjugate **dB-Phcn** in the presence of $5 \cdot 10^{-5}$ *M* β-mercaptoethanol for 1 (*4*) and 5 h (*5*). The site of RNA190 cleavage by conjugate **dB-Phcn** is indicated by an arrow.

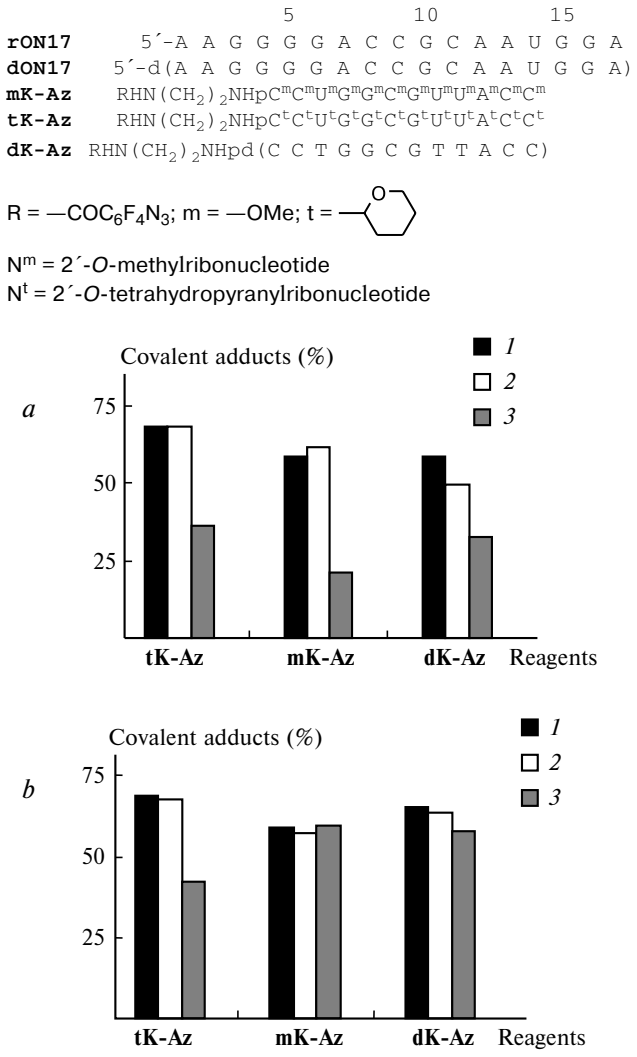


Fig. 6. Model duplexes and the extent of formation of covalent adducts upon photomodification of the synthetic 17-mer fragment of *PGY1/MDR1* mRNA (region 151—167) (*a*) and its deoxyribo analog (*b*) by reagents **tK-Az**, **mK-Az**, and **dK-Az** at different temperatures: 5 (*1*), 20 (*2*), 40 °C (*3*). The concentrations of the NA target and the reagents were $1 \cdot 10^{-7}$ and $1 \cdot 10^{-5}$ mol L^{-1}, respectively; the irradiation time was 10 min.

As can be seen from the histograms (see Fig. 6), both the RNA and DNA targets were equally efficiently modified by the reagents based on 2′-*O*-tetrahydropyranyl- and 2′-*O*-methyloligomers at 5 and 20 °C. An increase in the temperature to 40 °C led to a substantial decrease in the extent of formation of covalent adducts, which reflects a decrease in the percentage of the target involved in the complex with the conjugate (Fig. 7). Oligodeoxyribonucleotide-based reagent **dK-Az** modified the RNA target with a somewhat lower efficacy than the 2′-*O*-modified photoreagents. Apparently, the high efficacy of both the RNA and DNA modifications is attributed to the presence of a cluster composed of several guanosine residues in the immediate vicinity of the photogroup.

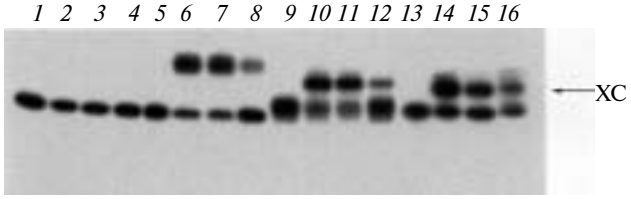


Fig. 7. Photomodification of the synthetic 17-mer fragment of *PGY/MDR1* mRNA (**rON17**) by reagents **dK-Az** (*5—8*), **tK-Az** (*9—12*), and **mK-Az** (*13—16*): **rON17** control (*1*), **rON17** after irradiation at 5 (*2*), 20 (*3*), and 40 °C (*4*), reaction mixtures before irradiation (*5*, *9*, *13*) and after irradiation at 5 °C (*6*, *10*, *14*), 20 °C (*7*, *11*, *15*), and 40 °C (*8*, *12*, *16*). The concentrations of **rON17** and the reagents were $1 \cdot 10^{-7}$ and $1 \cdot 10^{-5}$ mol L^{-1}, respectively; the irradiation time was 10 min.

The photomodification of the *PGY1/MDR1* mRNA fragment was carried out at 37 °C under analogous conditions. The autoradiograph of the gel obtained by separation of the products of RNA photomodification is shown in Fig. 8, *a*. On irradiation of RNA in the presence of conjugate **tK-Az**, the reaction of RNA proceeded with the highest efficiency and reached 51% (see Fig. 8, *a*, lanes *10—14*). In the course of electrophoretic separation, the products of the reaction of RNA with conjugate **mK-Az** have the electrophoretic mobilities similar to that of the starting RNA. Because of this, the extent of modification could not be determined quantitatively (Fig. 8, *a*, lanes *15—20*). The efficacy of the RNA modification by conjugate **dK-Az** is much lower (20%), which is, apparently, associated with the spatial organization of the RNA/DNA duplex. As can be seen from Fig. 8, *b*, the reaction proceeded rapidly and reached a plateau in a matter of minutes.

It was demonstrated[6,17,25,26,55,56] that conjugates of oligonucleotides with bleomycin, phthalocyanine, and perfluoroarylazide can perform the site-specific modification or cleavage of short model DNA with efficacy of up to 40—60%. Based on these data, one would expect that such oligonucleotide conjugates could be used for RNA modification. We found that the efficacy of RNA modification by the conjugates of oligodeoxyribonucleotides with bleomycin, phthalocyanine, and perfluoroarylazide is much lower than that observed upon modification of short DNA (Table 1). In addition, it appeared that the efficacy of modifications of both the 17-mer (**rON17**) and long (RNA190) RNA targets by the perfluoroarylazide conjugates of ribo- and oligodeoxyribonucleotides differ by more than a factor of two. Apparently, this is associated with the fact that the spatial organization of duplexes and, consequently, the arrangement of the reactive groups with respect to the major and minor grooves of the helix differ substantially for the RNA/RNA, RNA/DNA, and DNA/DNA duplexes.[48]

The efficacy of site-specific modification of RNA involved in a complementary complex under the action of

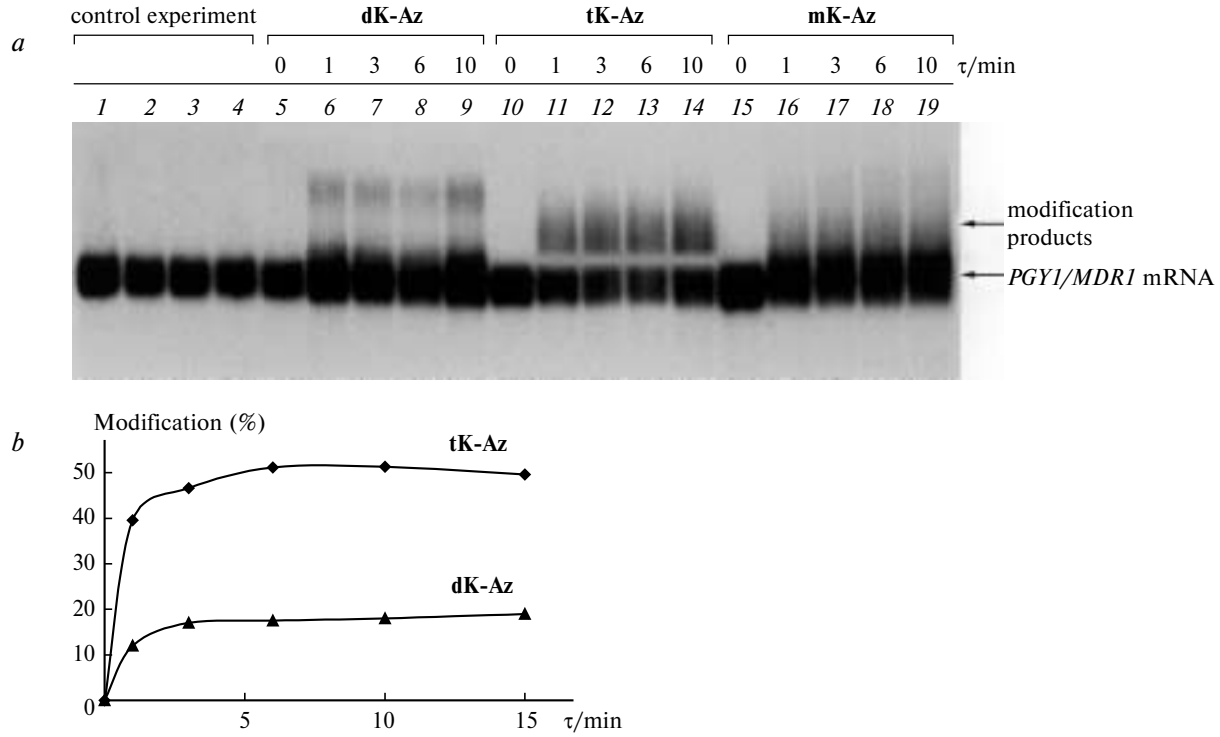


Fig. 8. Photomodification of the 5′-[^{32}P]-labeled fragment of *PGY1/MDR1* mRNA by the conjugates of 2′-*O*-tetrahydropyranyl-, 2′-*O*-methyloligoribonucleotides, and oligodeoxyribonucleotide with *p*-azidotetrafluorobenzamide (**tK-Az**, **mK-Az**, and **dK-Az**, respectively). *a*. Analysis of the modification products by electrophoresis in 8% PAAG under denaturating conditions: lane *1*, control, irradiation of RNA in the absence of conjugates; lanes *2—4*, RNA incubated in the presence of $1 \cdot 10^{-5}$ *M* conjugates without irradiation; lanes *5—19*, irradiation of RNA in the presence of $1 \cdot 10^{-5}$ conjugates *M* **dK-rAz** (*5—9*), **tK-Az** (*10—14*), and **mK-Az** (*15—19*). The irradiation conditions are given in the Experimental section; the irradiation time (τ/min): 0 (*5*, *10*, *15*); 1 (*6*, *11*, *16*); 3 (*7*, *12*, *17*); 6 (*8*, *13*, *18*), and 10 (*9*, *14*, *19*). *b*. Accumulation of covalent adducts formed through the photoaddition of conjugates **K-Az** to RNA.

the reactive group bound to the oligonucleotide is determined by different factors, *viz.*, the mechanism and kinetics of modification, the structure and stability of the complexes formed between RNA and the oligonucleotide conjugate, the RNA structure in the vicinity of the conjugate-binding site, the sensitivity of RNA bases located at the site of the reactive group to modification or cleavage, *etc*. The comparison of different conjugates demonstrated that some of these conjugates can be, in principle, used for the site-specific action on RNA. Alkylating derivatives of oligonucleotides and perfluoroarylazide conjugates of modified oligoribonucleotides enable one to perform highly efficient site-specific RNA modification. Perfluoroarylazide conjugates of oligonucleotides have a number of advantages, due to which they can be used for solving different structure-function problems, for example, for investigating the structures of complexly organized RNA-protein complexes *in vitro*.[22] The conjugates of oligonucleotides with bleomycin and phthalocyanine exhibited low efficacy in the reactions with RNA190. Conceivably, optimization of their structures might lead to the construction of conjugates, which will enable one to perform RNA modification with biologically significant efficacy.

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References

1. D. G. Knorre, V. V. Vlassov, V. F. Zarytova, A. V. Lebedev, and O. S. Fedorova, *Design and Targeted Reactions of Oligonucleotide Derivatives*, Boca Raton, CRC Press, 1994.
2. S. T. Crooke, *Biochim. Biophys. Acta*, 1999, **1489**, 31.
3. V. V. Vlassov, in *Antisense Research and Applications*, Eds. S. T. Crooke, and B. Lebleu, Boca Paton, CKC Press, 1993, 235.
4. Z. A. Sergeeva, A. G. Venyaminova, and V. F. Zarytova, *Nucleosides, Nucleotides*, 1998, **17**, 2153.
5. R. P. Wayne, *Principle and Applications of Photochemistry*, Oxford University Press, Oxford, NewYork—Tokio, 1988.

6. M. I. Dobrikov, V. F. Zarytova, N. I. Komarova, A. S. Levina, S. A. Lokhov, T. A. Prikhod´ko, G. V. Shishkin, D. R. Tabatadze, and M. M. Zaalishvili, *Bioorg. Khim.* 1992, **18**, 540 [*Russ. J. Bioorg. Chem.*, 1992, **18** (Engl. Transl.)].
7. M. I. Dobrikov, S. A. Gaidamakov, A. A. Koshkin, N. P. Luk´yanchuk, G. V. Shishkin, and V. V. Vlassov, *Dokl. Akad. Nauk,* 1995, **344**, 122 [*Dokl. Chem.*, 1995 (Engl. Transl.)].
8. M. I. Dobrikov, *Usp. Khim.*, 1999, **68**, 1062 [*Russ. Chem. Rev.*, 1999, **68** (Engl. Transl.)].
9. G. D. Cimino, H. B. Gamper, S. T. Isaaks, and J. E. Hearst, *Annu. Rev. Biochem.*, 1985, **54**, 1151.
10. D. Novopashina, M. Kuznetsova, and A. Venyaminova, *Nucleosides, Nucleotides and Nucleic Acids*, 2001, **20**, 903.
11. A. S. Levina, M. V. Berezovskii, A. G. Venjaminova, M. I. Dobrikov, M. N. Repkova, and V. F. Zarytova, *Biochimie*, 1993, **75**, 25.
12. D. S. Sigman, A. Mazumder, and D. M. Perrin, *Chem. Rev.*, 1998, **98**, 1089.
13. W. K. Pogozelski and T. D. Tullius, *Chem. Rev.*, 1998, **98**, 1089.
14. V. N. Sil´nikov and V. V. Vlassov, *Usp. Khim.*, 2001, **70**, 491 [*Russ. Chem. Rev.*, 2001, **70** (Engl. Transl.)].
15. S. M. Hecht, *Bioconjugate Chem.*, 1994, **5**, 513.
16. R. M. Burger, *Chem. Rev.*, 1998, **98**, 1153.
17. D. S. Sergeev and V. F. Zarytova, *Usp. Khim.*, 1996, **65**, 377 [*Russ. Chem. Rev.*, 1996, **65** (Engl. Transl.)].
18. D. Dolphin, *Can. J. Chem.*, 1994, **72**, 1005.
19. G. G. Karpova, *Izv. SO Akad. Nauk SSSR, Ser. Khim.*, 1987, **12**, 82 [*Bull. Sib. Branch Russ. Acad. Sci., Div. Chem. Sci.*, 1987, **12** (Engl. Transl.)].
20. M. A. Zenkova, G. G. Karpova, A. S. Levina, S. V. Mamaev, and V. V. Solov´ev, *Bioorg. Khim.* 1990, **16**, 788 [*J. Bioorg. Chem. USSR*, 1990 **16** (Engl. Transl.)].
21. M. Zenkova, C. Ehresmann, J. Caillet, M. Springer, G. Karpova, B. Ehresmann, and P. Romby, *Eur. J. Biochem.* 1995, **231**, 726.
22. D. M. Graifer and G. G. Karpova, *Mol. Biologiya*, 2001, **35**, 584 [*Russ. Mol. Biol.*, 2001, **35** (Engl. Transl.)].
23. M. N. Repkova, A. G. Venyaminova, and V. F. Zarytova, *Nucleosides Nucleotides*, 1997, **16**, 1797.
24. V. F. Zarytova, D. S. Sergeyev, and T. S. Godovikova, *Bioconjugate Chem.*, 1993, **4**, 189.
25. J. B. Smith, A. S. Levina, P. E. Vorobjev, V. F. Zarytova, and E. Wickstrom, *Proc. Amer. Assoc. for Cancer Research Annual Meeting*, Philadelphia, Pennsylvania, April 10—14, 1999, 133.
26. V. V. Koval´, A. A. Chernonosov, T. V. Abramova, T. M. Ivanova, O. S. Fedorova, and D. G. Knorre, *Bioorg. Khim.* 2000, **26**, 118 [*Russ. J. Bioorg. Chem.*, 2000, **26** (Engl. Transl.)].
27. B. Mestre, A. Jakobs, G. Pratviel, and B. Meunier, *Biochemistry*, 1996, **35**, 9140.
28. Z. A. Sergeeva, S. G. Lokhov, and A. G. Ven´yaminova, *Bioorg. Khim.* 1996, **22**, 916 [*Russ. J. Bioorg. Chem.*, 1996, **22** (Engl. Transl.)].
29. M. A. Kuznetsova, D. V. Pyshnyi, M. N. Repkova, and A. G. Ven´yaminova, *Bioorg. Khim.* 2000, **26**, 78 [*Russ. J. Bioorg. Chem.*, 2000, **26** (Engl. Transl.)].
30. V. F. Zarytova, T. S. Godovikova, I. V. Kutyavin, and L. M. Khalimskaya, in *Biophosphates and Their Analogues — Synthesis, Structure, Metabolism and Activity*, Eds. K. S. Bruzik and W. J. Stec, Elsevier Science Publishers, Amsterdam, 1987, 149.
31. M. N. Repkova, T. M. Ivanova, R. V. Filippov, and A. G. Ven´yaminova, *Bioorg. Khim.* 1996, **22**, 432 [*Russ. J. Biorg. Chem.*, 1996, **22** (Engl. Transl.)].
32. E. V. Kostenko, R. Sh. Bibilashvili, V. V. Vlassov, and M. A. Zenkova, *Mol. Biologiya*, 2000, **34**, 67 [*Russ. Mol. Biol.*, 2000, **34** (Engl. Transl.)].
33. M. Silberklang, A. Prochiantz, A. L. Haenni, and U. L. Rajbhandary, *Eur. J. Biochem*, 1977, **72**, 465.
34. D. A. Peattie, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1760.
35. T. Maniatis, E. F. Fritsch, and J. Sambrook, *Molecular Cloning. A Laboratory Manual,* 2d Ed., Gold Spring Harbor Laboratory Press, New York, 1989, 568.
36. A. V. Vlassov, V. V. Vlassov, and R. Giege, *Dokl. Akad. Nauk,* 1996, **349**, 411 [*Dokl. Chem.*, 1996 (Engl. Transl.)].
37. H. Donis-Keller, A. M. Maxam, and W. Gilbert, *Nucleic Acids Res.*, 1977, **4**, 2527.
38. A. S. Mankin, E. A. Skripkin, N. V. Chichkova, A. M. Kopylov, and A. A. Bogdanov, *FEBS Lett.*, 1981, **131**, 253.
39. L. Lempereur, M. Nicoloso, N. Riehl, C. Ehresmann, B. Ehresmann, and J.-P. Bachellerie, *Nucleic Acids Res.*, 1985, **13**, 8339.
40. C. Ehresmann, F. Baudin, M. Mougel, P. Romby, and J.-P. Ebel, *Nucleic Acids Res.*, 1987, **15**, 9109.
41. B. Mestre, M. Pitie, C. Loup, C. Claparols, G. Pratviel, and B. Meunier, *Nucleic Acids Res.*, 1997, **25**, 1022.
42. A. D. D´Andrea and W. A. Haseltine, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 3608.
43. M. Takeshita, A. P. Grollman, and H. Ohtsubo, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 5983.
44. V. Petuyk, M. Zenkova, R. Giege, and V. V. Vlassov, *FEBS Lett.*, 1999, **444**, 217.
45. V. A. Petyuk, M. A. Zenkova, R. Giege, and V. V. Vlassov, *Mol. Biologiya*, 2000, **34**, 879 [*Russ. Mol. Biol.*, 2000, **34** (Engl. Transl.)].
46. H. H. Klump, in *Biochemical Thermodynamics*, Ed. M. N. Jones, Elsevier, Amstredam—Tokyo—Oxford—New York, 1988, 100.
47. A. Hüttenhofer, S. Hudson, H. F. Noller, and P. K. Mascharac, *J. Biol. Chem.*, 1992, **267**, 24471.
48. M. V. Keck and S. M. Hecht, *Biochemistry*, 1995, **34**, 12029.
49. D. G. Knorre, O. S. Fedorova, and E. I. Frolova, *Usp. Khim.*, 1993, **62**, 70 [*Russ. Chem. Rev.*, 1993, **62** (Engl. Transl.)].
50. G. Pratviel, M. Pitie, J. Bernadou, and B. Meunier, *Nucleic Acids Res.*, 1991, **19**, 6283.
51. P. Bigey, G. Pratviel, and B. Meunier, *Nucleic Acids Res.* 1995, **23**, 3894.
52. B. Mestre, A. Jakobs, G. Pratviel, and B. Meunier, *Biochemistry*, 1996, **35**, 9140.
53. K.-H. Altmann, N. M. Dean, D. Fabbro, S. M. Freier, T. Geiger, R. Häner, D. Hüsken, P. Martin, B. P. Monia, M. Müller, F. Natt, P. Nicklin, J. Phillips, U. Pieles, H. Sasmor, and H. E. Moser, *Chimia*, 1996, **50**, 168.
54. E. A. Lesnik and S. M. Freier, *Biochemistry*, 1995, **34**, 10807.
55. D. S. Sergeyev, T. S. Godovikova, and V. F. Zarytova, *Nucleic Acids Res.*, 1995, **23**, 4400.
56. M. I. Dobrikov, S. A. Gaidamakov, A. A. Koshkin, T. I. Guinutdinov, N. P. Luk´yanchuk, G. V. Shishkin, and V. V. Vlassov, *Bioorg. Khim.* 1997, **23**, 191 [*Russ. Bioorg. Chem.*, 1997, **23** (Engl. Transl.)].

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