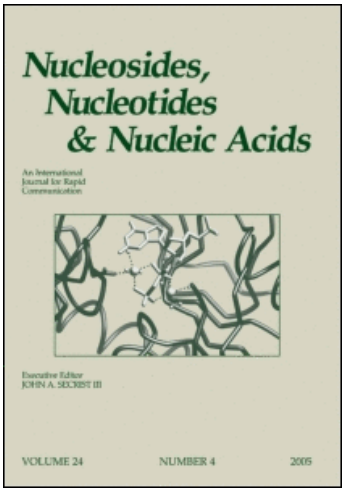

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New Photoreactive Oligoribonucleotide Conjugates: Hybridization and Modification Assays

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ABSTRACT

An evaluation of hybridization and modification abilities of new types of photoactivatable oligoribonucleotide conjugates bearing *p*-azidotetrafluorobenzamide group at the N4 of cytosine is presented.

Key Words: Oligoribonucleotides; Conjugates; *p*-Azidotetrafluorobenzamide group; N4 of cytosine; Hybridization; Photomodification of nucleic acids.

INTRODUCTION

Designing novel photoactivatable oligoribonucleotide conjugates is of great importance for studying of RNA-NA and RNA-protein interactions. An approach to the synthesis of oligoribonucleotide conjugates containing functionalized adenine, uracil and cytosine was developed.[1–3] Some properties of these oligoribonucleotide conjugates have been described.[3,4] The modification ability of the new oligoribonucleotide conjugates bearing the photoactivatable *p*-azidotetrafluorobenzamide group at

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the N4 of cytosine as well as the hybridization properties of these photoreagents in comparison with corresponding amino-containing precursors were investigated in this work.

RESULTS AND DISCUSSION

With the aim of performing a comparative study of the properties of the modified oligomers, we constructed a model system consisting of the ribopentadecamer, its deoxyribo analog, and complementary oligoribonucleotides containing modified cytosine at the 3′- or 5′-terminus or within the chain (Fig. 1).

A series of oligoribonucleotides carrying an aliphatic amino group at N4 of the cytosine residue was synthesized by the solid phase H-phosphonate approach[3] and then transformed into corresponding perfluoroarylazide oligoribonucleotide derivatives by analogy with.[2] First of all we investigated the hybridization properties of the modified oligoribonucleotides (Table 1). In the case of amino-containing oligomers (**ON1–ON5**) RNA*–RNA duplexes are more stable than the hybrid RNA*–DNA duplexes, probably because of their more rigid structure. The attachment of aliphatic aminolinkers with different length at the N4 of cytosine weakly affects the T_m of the duplexes independent of the modified nucleoside location (Table 1). This fact results from a combination of electronic, electrostatic and conformational effects of the protonated aliphatic amino group.[5] The presence of a *p*-azidotetrafluorobenzamide group at N4 of the cytosine residue decreased the duplex stability for all reagents (**1–5**)

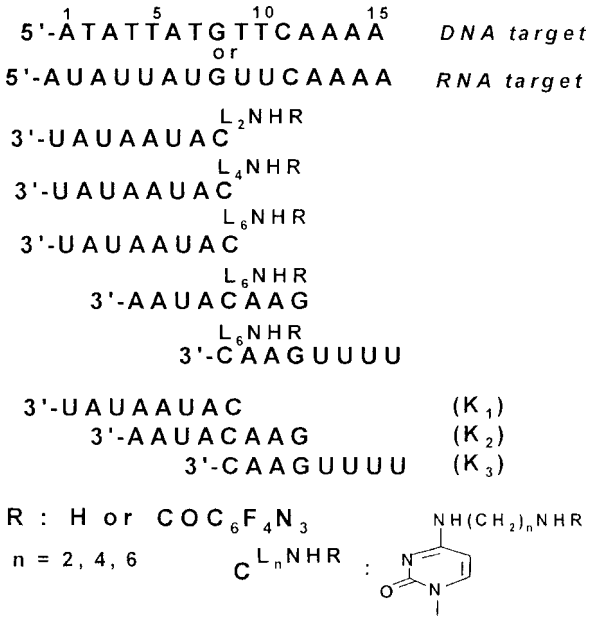

Figure 1. Model system for the study of properties of oligoribonucleotide conjugates containing modified cytosine at the different positions of the chain.

Table 1. The melting temperatures (°C) of duplexes formed by the modified oligoribonucleotides and complementary pentadecanucleotides 5′—AUAUUAUGUUCAAAA and 5′—ATATTATGTT-CAAAA.

Amino-containing oligonucleotide	RNA	DNA	Reagent	RNA	DNA
3′-UAUAAUAC^{L2NH2} (**ON1**)	17	13	3′-UAUAAUAC$^{L_2NHC(O)C_6F_4N_3}$ (**1**)	8	8
3′-UAUAAUAC^{L4NH2} (**ON2**)	22	15	3′-UAUAAUAC$^{L_4NHC(O)C_6F_4N_3}$ (**2**)	9	9
3′-UAUAAUAC^{L6NH2} (**ON3**)	16	13	3′-UAUAAUAC$^{L_6NHC(O)C_6F_4N_3}$ (**3**)	11	8
3′-AAUAC^{L6NH2} AAG (**ON4**)	35	24	3′-AAUAC$^{L_6NHC(O)C_6F_4N_3}$AAG (**4**)	23	16
3′-C^{L6NH2}AAGUUUU (**ON5**)	20	13	3′-C$^{L_6NHC(O)C_6F_4N_3}$AAGUUUU (**5**)	17	6
3′-UAUAAUAC (**K$_1$**)	20	15			
3′-AAUACAAG (**K$_2$**)	36	26			
3′-CAAGUUUU (**K$_3$**)	23	12			

L_2:$(CH_2)_2$; L_4:$(CH_2)_4$; L_6:$(CH_2)_6$.
The conditions of thermal denaturation are given in Experimental section.

in comparison with unmodified oligomers (Table 1). A similar effect was obtained when this photo group was introduced at the C8 of adenine or C5 of uracil of oligoribonucleotides.[4]

It is necessary to note that in the case under consideration RNA*–RNA duplexes are more sensitive to the changes both in a linker length (reagents **1–3**) and in a position of modified cytosine (reagents **3–5**) than hybrid duplexes. The most pronounced affect on stability of the duplexes is observed in the case of reagent (**4**), when the modified cytosine is located within the chain.

A comparative study of photomodification of RNA and DNA targets by reagents (**1–5**) bearing *p*-azidotetrafluorobenzamide group at the N4 of cytosine has been studied under conditions favoring formation of the duplexes (Table 2). The analysis of the reaction mixtures by denaturing PAGE after irradiation of corresponding duplexes revealed the presence of two bands corresponded to the initial target and the cross-linked products. The extent of covalent adducts formation was much higher for RNA than for DNA target, viz. $\sim$55% for RNA and $\sim$30% for DNA for reagents (**1–3** and **5**) bearing a photo group at the 3′- or 5′-end. The reagent (**4**) with the reactive group within the chain proved to be two times less efficient (25% for RNA and 11% for DNA) than the other reagents. We can conclude that the location of modified cytidine in the oligomeric chain has a more pronounced influence on the formation of covalent adducts with RNA and DNA than the lengthening of a linker. Evidently, the presence of the reactive group within the duplex results in certain spatial and conformational hindrances for the reaction of modification.

It is possible to carry out more detailed study of photomodification products by piperidine treatment in the case of a DNA target. Total modification of DNA by the

Table 2. Photomodification of 5′-AUAUUAUGUUCAAAA and 5′-ATATTATGTTCAAAA by reagents (**1–5**).

	RNA target	DNA target							
					Points of modification, %[a]				
					Covalent piperidine labile adducts	Hidden modification			
Reagent	Covalent adducts, %	Covalent adducts (stable and labile to piperidine), %	Covalent piperidine stable adducts, %	Hidden modification, %	G_8	T_{10}	T_9	G_8	T_7
(**1**)	56	27	11	28	7			9	6
(**2**)	54	27	11	25	5		5	9	
(**3**)	58	34	13	19	5			7	
(**4**)	25	11	5	38		8	12	11	
(**5**)	55	35	15	25	9		7	8	

The conditions of photomodification are given in Experimental section.
[a]Given only if more than or equal to 5%.

new photoactivatable conjugates of oligoribonucleotides is composed from several types of damage: the covalent linking of reagent to target that gives piperidine labile or piperidine stable adducts and the hidden modification, which shows only after piperidine treatment of a band coincided with the initial target (Table 2). In the case of covalent adducts the G8 residue of the DNA target, closest to the reactive group, was preferentially modified, giving the piperidine labile products. A hidden modification affects a wider spectrum of nucleobases. The highest level of the hidden modification has been obtained in the case of reagent (**4**).

There are no exact data about chemical structure of adducts formed by action of perfluoroarylazide derivatives of oligonucleotide on nucleic acids.[6]

Thus, it was shown that the new photoreagents bearing a *p*-azidotetrafluorobenzamide group at the N4 of cytosine modify nucleic acids efficiently and site-specifically and the photomodification of NA depends on the type of target, location of modified cytidine within the chain, and the structure of amino linker. The results obtained allow these photoactivatable oligoribonucleotide conjugates to be considered as promising effective reagents for site-specific modification of nucleic acids.

EXPERIMENTAL

The reagents and sorbents were purchased from Sigma (USA), Fluka (Switzerland), and Merck (Germany). Pentadecaribonucleotide was synthesized using phosphoramidite chemistry and automatic synthesizer ''ASM-102U'' (Biosset, Russia).

Oligoribonucleotides bearing aliphatic amino group at the N4 of cytosine (ON1–ON5) were synthesized by the H-phosphonate method as described previously.[3]

***p*-Azidotetrafluorobenzamide derivatives of oligoribonucleotides (1–5)** were synthesized by the reaction of amino-containing oligomers with N-hydroxysuccinimide ester of 4-azidotetrafluorobenzoic acid by analogy with.[2] Characteristics of the conjugates (**3–5**): sequence (No); the yield; the homogeneity according to the data from RP HPLC; the retention time/in comparison with the retention time of corresponding **ON**; the spectral ratio at four wavelengths (250/260; 270/260; 280/260; 290/260): 3′-UAUAAUAC $^{L6NHC(O)C_6F_4N_3}$ (**3**); 85%; 99%; 16.4 min/11.4 min; 0.82; 0.79; 0.38; 0.10; 3′-AAUAC$^{L6NHC(O)C_6F_4N_3}$AAG (**4**); 83%; 97%, 15.2 min/12.7 min; 0.87; 0.78; 0.41; 0.13; 3′-$^{L6NHC(O)C_6F_4N_3}$ AAGUUUU (**5**); 80%; 98%; 15.1 min/11.4 min; 0.86; 0.81; 0.44; 0.14.

Thermal denaturation of oligonucleotide duplexes was studied at the wavelength of 270 nm by means of a specially designed device with a temperature-controlled optical microcuvette, based on a UV detector of the chromatograph ''MiliChrom'' (Russia). The rate of heating was $0.7–1.0°C \cdot min^{-1}$. The concentration of each oligonucleotide component was $1.3 \cdot 10^{-5}$ M in a buffer containing 100 mM NaCl, 1 mM Na_2EDTA, 10 mM Na cacodylate, pH 7.4. Molar absorbances of amino-modified oligonucleotides were calculated by analogy with.[3] Molar absorbances of *p*-azidotetrafluorobenzamide oligonucleotides were calculated as a sum of ε_{260} of

corresponding aminomodified oligonucleotide and molar absorbance of the $N_3C_6F_4CO$-group (23200 $M^{-1} \cdot cm^{-1}$).[3]

Photomodification of RNA and DNA. The sample (10 μl) containing 5′-[^{32}P]phosphorylated DNA or RNA ($1 \cdot 10^{-7}$ M) and corresponding photoreagent ($1 \cdot 10^{-5}$ M) in buffer (100 mM NaCl, 1 mM Na_2EDTA, 10 mM Tris-HCl, pH 7.4) was put into a well of immunological plate and irradiated with a high-pressure Hg-lamp using glass filters (303–365 nm, $W = 5 \cdot 10^{-4}$ $W \cdot cm^{-2}$) for 10 min at the distance of 10 cm at 5°C. After irradiation, the reaction mixture was precipitated by 2% $LiClO_4$ in acetone and analyzed by 20% denaturing PAGE. To obtain quantitative data, the autoradiograph of a gel was digitized with the use of the Gel-Pro Analyzer program package (Media Cybernetics, Inc., USA). The relative experimental error was no higher than 20%. The extent of covalent adducts was calculated as a ratio of radioactivity in the corresponding band to the overall radioactivity in the lane. In the case of DNA, the fraction coincided with the cross-linked products was eluted from the gel, precipitated by 2% $LiClO_4$ in acetone, treated with 10% aqueous piperidine (30 min at 100°C) and analyzed by 20% denaturing PAGE. The extent of modification at a particular position of the DNA in the case of covalent adducts, and the extent of piperidine stable adducts formation after piperidine treatment was calculated using the equation $(I_i \cdot P_Y / \Sigma I_i) \cdot 100\%$, where ΣI_i-radioactivity in the lane, I_i-radioactivity in the corresponding band, P_Y-share of the covalent adducts in the reaction mixture. The fraction coincided with the initial target was isolated from gel as described above. The hidden modification extent was calculated after piperidine treatment of this fraction using the equation $([\Sigma I_i - I_0] \cdot P_X / \Sigma I_i) \cdot 100\%$, where ΣI_i—radioactivity in the lane, I_0—radioactivity in the band corresponding to initial target, P_X—share of the initial target in the reaction mixture. The hidden modification extent at a particular position of the DNA target was calculated using the equation $(I_i \cdot P_X / \Sigma I_i) \cdot 100\%$, where I_i—radioactivity in the corresponding band.

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