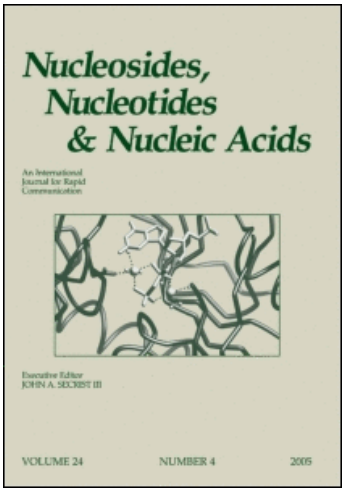

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Comparative Study of Modification of DNA and RNA by Oligo(2'-O-Methylribonucleotide) Derivatives

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COMPARATIVE STUDY OF MODIFICATION OF DNA AND RNA BY OLIGO(2'-O-METHYLRIBONUCLEOTIDE) DERIVATIVES

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ABSTRACT: The results of modification of the model DNA and RNA targets by the alkylating derivatives of 2'-O-methylribo-, ribo-, and deoxyhexanucleotides in the presence and absence of effectors (N-(2-hydroxyethyl)phenazinium derivatives of the same type of octanucleotides) are presented. It has been shown that the alkylating 4(N-methyl-N-2-chloroethyl-)benzylmethylamidophosphate derivatives of oligo(2'-O-methylribonucleotides) are the high effective reagents for the site specific modification of nucleic acids especially RNA.

INTRODUCTION

Oligonucleotides and their derivatives are widely used now as potential therapeutic and diagnostic agents and as unique tools for understanding cellular processes at the molecular level. The important features of oligo(2'-O-methylribonucleotides) are their resistance towards nucleases and high hybridization properties. The introduction of reactive groups into these oligonucleotide analogs allows one to design the effective reagents for sequence specific modification of nucleic acids, especially RNA which is the main target in the antisense approach.

It was shown earlier that short oligo(2'-O-methylribonucleotides) and their N-(2-hydroxyethyl)phenazinium derivatives possess high affinity towards complemen-

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DNA target

1 5 10 15 20

5' d (T T A C A C A C A C T G G G A A G T T T) 3'

A1 A C C C T T p(**RCl**)
T G T G T G T Gp(L**Phn**)

A2 A C C C U U p(**RCl**)
U G U G U G U Gp(L**Phn**)

A3 $A^mC^mC^mC^mU^mU^m$ p(**RCl**)
$U^mG^mU^mG^mU^mG^mU^mG^m$p(L**Phn**)

RNA target

1 5 10 15 20

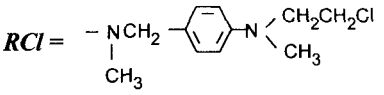
5' r (U U A C A C A C A C U G G G A A G U U U) 3'

B1 A C C C T T p(**RCl**)
T G T G T G T Gp(L**Phn**)

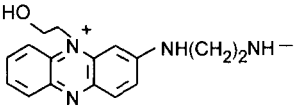
B2 A C C C U U p(**RCl**)
U G U G U G U Gp(L**Phn**)

B3 $A^mC^mC^mC^mU^mU^m$ p(**RCl**)
$U^mG^mU^mG^mU^mG^mU^mG^m$p(L**Phn**)

RCl =



LPhn =



Scheme

tary RNA and DNA targets [1,2].

RESULTS AND DISCUSSION

In this work the alkylation of model ribo- and deoxyribo-eicosanucleotides by 4(N-methyl-N-2-chloroethyl)benzylmethyl-amidophosphate derivatives of 2'-O-methylribo-, ribo- and deoxyhexanucleotides both in the presence and absence of effectors has been studied at different (20-60 °C) temperatures. The N-(2-hydroxyethyl) phenazinium derivatives of the same type of octanucleotides were used as effectors (Scheme).

The synthesis of these types of oligonucleotide derivatives has been carried out using the method of Zarytova V.F. et al [3].

The extent of the DNA target modification by all three types of reagents in the absence of effectors is very small and does not exceed 20% at 20 °C. According to efficacy of modification the reagents can be arrange in the order d > r > m. In the presence of effectors the order remains valid but the substantial increase of the extent of modification for all three types of reagents is observed (80-85% at 20°C) (FIG. 1a).

After piperidine treatment of the reaction mixture the products of modification are cleaved at the modified target sites. The G^{13}-G^{17} bases of the DNA target are alkylated in all tree cases (duplexes A1-A3) with different efficacy depending on the

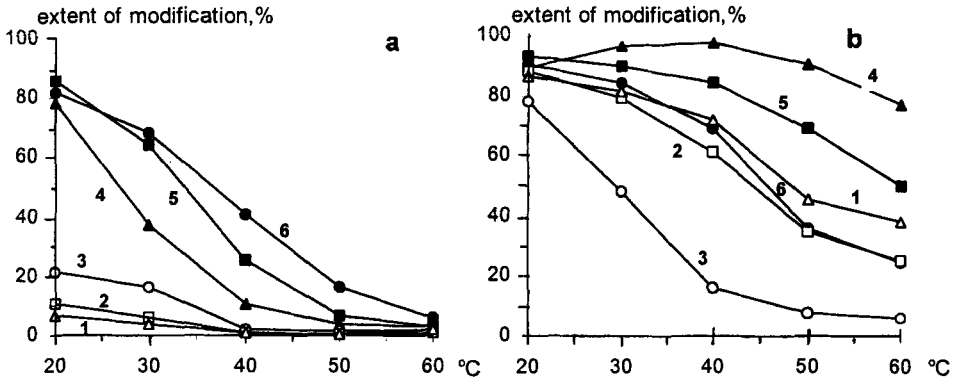


FIG.1. Dependence of the extent of DNA (**a**) and RNA (**b**) targets alkylation on temperature by reagents (ClR)pU^{m}U^{m}C^{m}C^{m}C^{m}A^{m}, (ClR)pUUCCCA and (ClR)pTTCCCA in the absence (curves 1-3, respectively) and in the presence of efectors (curves 4-6, respectively) in 0.1 M NaCl, 0.01 M Tris-HCl (pH 7.2), 0.1 mM EDTA. [Reagent or effector] = 1 x 10^{-5} M; [target] = 5 x 10^{-7} M. Time of alkylation: 60 h (20°C), 14 h (30°C), 5 h (40°C), 1.5 h (50°C), 0.3 h (60°C).

oligonucleotide type. The G^{17} base is modified predominantly (72%) in duplex A1 at 30 °C. The G^{17} and G^{13} bases are alkylated with close efficacy (26 and 27% respectively) in duplex A2. The G^{13} base is modified preferentially (16%) in the case of duplex A3. The relative distribution of the alkylating bases of the DNA target in A1-A3 complexes is presented in FIG. 2.

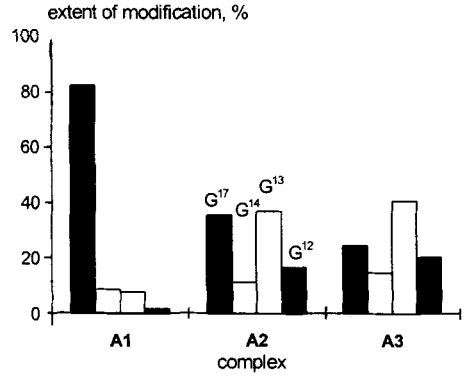


FIG. 2. The relative distribution of the alkylated bases of DNA target in the complexes of different types (30°C).

The efficacy of the RNA modification at the same conditions was much higher. The maximum extent of modification amounts to 80-90% at 20°C even in the absence of effectors. According to efficacy of RNA modification the reagents can be arrange in order m > r > d. The increase of the extent of RNA

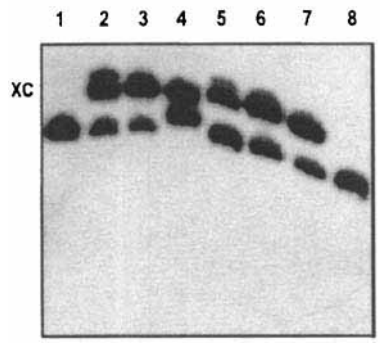


FIG. 3. Autoradiogram of the 20% denaturating PAGE of RNA target alkylation products at 30°C by reagents: (C1R)pTTCCCA in the presence of (PhnL)pG$(TG)_3$T (lane 2) and in its absence (lane 5); (C1R)pUUCCCA in the presence of (PhnL)pG$(UG)_3$U (lane 3) and in its absence (lane 6); (C1R)p$U^mU^mC^mC^mC^mA^m$ in the presence of (PhnL)pG^m $(U^mG^m)_3U^m$ (lane 4) and in its absence (lane 7); lane 1 and 8: control RNA target.

modification occurs in the presence of effectors (FIG.1b). The maximal extent of modification is achieved by using the derivatives of oligo(2'-O-methylribonucleotides) as reagent and effector. The additional covalent adduct is observed in this case (FIG. 3, lane 4).

The results obtained show that the 5'-alkylating derivatives of oligo(2'-O-methylribonucleotides) are the high effective reagents for the site specific modification of RNA.

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