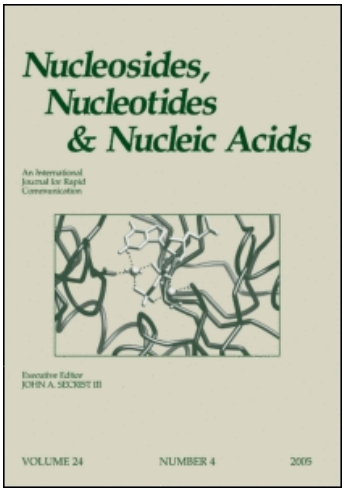

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Mini-antisense Oligonucleotides

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MINI-ANTISENSE OLIGONUCLEOTIDES

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ABSTRACT A new strategy of selective DNA target modification was proposed. The using of reactive derivatives of short oligonucleotides in the presence of flanking effector pair allows one to modify DNA target only when the perfect complementary complex of DNA target and oligonucleotide tandem is formed.

The important problem remaining to be resolved is the enhancement of specificity and selectivity of the DNA recognition by oligonucleotide probes. The long oligonucleotides have high hybridization properties and they are able to form sufficiently stable unperfect complexes with DNA. The short oligonucleotides have many possible binding sites within DNA sequence and their complexes are extremely unstable. Recently we have shown that site-specific interaction between short oligonucleotide and DNA can be intensified by a pair of flanking effectors, diphenazinium derivatives of short oligonucleotides[1].

In this work comparative study of selective DNA recognition by alkylating derivatives of oligonucleotides of various length in the presence of effector has been carried out.

The modification of *M* target (20-mer) by alkylating (*RCl*) reagent based on dodeca-, octa-, and tetranucleotide without effectors or in the presence of both native octanucleotide and its phenazinium (*Phn*) derivative in the complexes 1-3 was investigated.

In the perfect complex 1.1a reagent *RCl*-pN_{12} modified the *M* target efficiently (FIG.1a) but unfortunately the alkylation of several nucleotide bases was registered (FIG.2, line 1) (complex *M*+ pN_{12} (***X***=C), T_m=56°C). Whereas in the presence of effector *E1(Phn)*

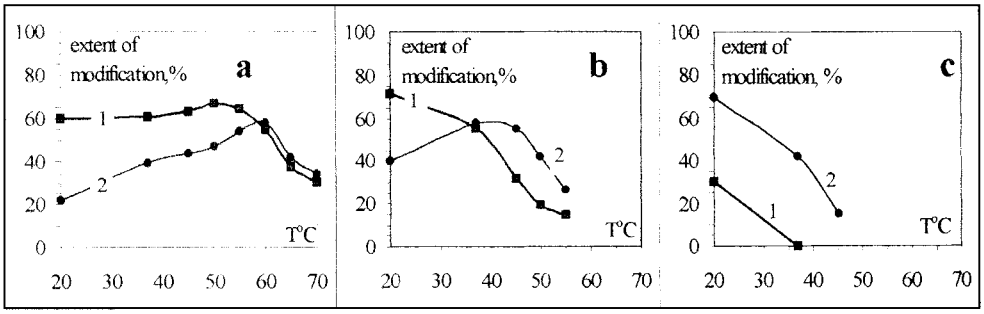


FIG.1. Temperature dependence of *M* target modification extent in perfect complexes (*X*=C): **a) 1.1a** - curve 1, **1.2a** - curve 2; **b) 2.1** - curve 1, **2.2** - curve 2; **c) 3.1a** - curve 1, **3.2a** - curve 2 in the buffer 0.1 M NaCl, 0.01 M tris-HCl (pH 7.2), 1 mM EDTA. The concentration of *M* target was 5 x 10^{-7} M, the concentrations of the oligonucleotide components were 1 x 10^{-5} M.

(complex 1.2a) only one base G9 of the target was alkylated and the extent of the whole target modification by the reagent was significantly decreased at temperature lower than

1.1) 3' C G T A G T T C G T C G A G G T C C G T p 5'
RCl-pC A G*X* T C C A G G C A
$RCl\text{-}pN_{12}(X)$

1.2) C G T A G T T C G T C G A G G T C C G T p
RCl-pC A G*X* T C C A G G C A
pG C A T C A A Gp
PhnI *E1(Phn)* *l'Phn*

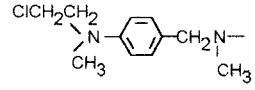
2.1) C G T A G T T C G T C G A G G T C C G T p
RCl-pC A G C T C C A
$RCl\text{-}pN_8(X)$

2.2) C G T A G T T C G T C G A G G T C C G T p
RCl-pC A G C T C C A
pG C A T C A A Gp
PhnI *l'Phn*

3.1) C G T A G T T C G T C G A G G T C C G T p
RCl-pC A G*X*
$RCl\text{-}pN_4(X)$
pG C A T C A A G pT C C A G G C A
E1 *E2*

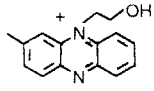
3.2) C G T A G T T C G T C G A G G T C C G T p
RCl-pC A G*X*
pG C A T C A A Gp pT C C A G G C Ap
PhnI *l'Phn* *PhnI* *l'Phn*
E2(Phn)

X = а) **C**, б) **A**

RCl = 

-l'Phn = -OCH_2CH_2NH-*Phn*

PhnI- = *Phn*-NH-$CH_2CH_2CH_2NH$-



(Phn-)

55°C (FIG.1a, 2, line 2). Because the complex *M* + *E1(Phn)* had T_m = 55°C, it was suggested that in the presence of *E1(Phn)* the reagent *RCl*-pN_{12} alkylated the target at

20-55°C preferably in tandem complex and at temperature higher 55°C did it from complex *M* + *RCl*-pN_{12}.

The main features of *M* target modification by octanucleotide reagent *RCl*-pN_8 were the same as in the case of the dodecanucleotide reagent (FIG.1b). The alkylating reagent *RCl*-pN_8 modified effectively at temperatures lower 35°C the wide number of the nucleotide bases of *M* target. (Complex *M* + pN_8, T_m = 35°C). In the presence of effector *E1(Phn)* only one base G9 of the target was modified and the extent of *M* target alkylation decreased at temperature lower then 35°C.

The other influence of effectors on the target modification was observed for tetranucleotide reagent *RCl*-pN_4. The tetranucleotide pN_4 has extremely weak hybridization properties and cannot form any complex with *M* target; that is why the reagent *RCl*-pN_4 cannot modify the target[2]. The data obtained showed that in the presence of effector pairs *E1+E2* or *E1(Phn) +E2(Phn)* (complexes 3.1a and 3.2a) the reagent *RCl*-pN_4 modified *M* target (FIG.1c) and only one base G9 of the target was alkylated (for example, FIG.2, line 6). The extent of target modification is in good correlation with the hybridization properties of effectors. The highest alkylation was observed when phenazinium effector pair was used (complex 3.2a). Thus, the diphenazinium effectors positively affect on the modification of the target by the tetranucleotide reagent in contrast to their effect on longer oligonucleotide reagents.

The comparison of the *M* target modification in the unperfect complexes was fulfilled using the tetra- and dodecanucleotide alkylating reagents in the mismatched duplexes 1b and 3b (***X***=A). The equal efficiency of *M* target modification at 37°C by the dodecanucleotide reagent in the both perfect 1.1a and mismatched 1.1b complexes (60% and 61%, respectively) was shown (FIG.2, lines 1,3). The same results of modification were obtained in the presence of effector *E1(Phnl)*; only in complexes 1.2a and 1.2b the extent of modification of *M* target was decreased till 35% (FIG.2, lines 2,4).

On the other hand the tetranucleotide reagent was very sensitive to the presence of mispair in the full length 20-mer tandem duplex. No modification of the target was observed in mismatched complex 3.1b (FIG.2, line 7), while in perfect complex (3.1a) the extent of modification was 40% (FIG.2, line 4).

Thus, the usage of cooperative tandem of short oligonucleotide derivatives effector-reagent-effector provides the superselective modification of DNA target under physi-

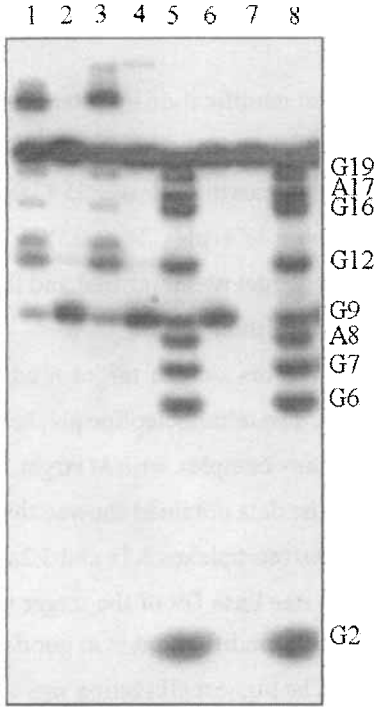


FIG.2. Autoradiogram of 20% denaturating PAGE of *M* target modification products (after piperidine treatment)
in the perfect omplexes (*X*=C): 1.1a- line 1; 1.2a - line 2; 3.2a - line 6;
in the unperfect complexes (*X*=A) 1.1b - line 3; 1.2b - line 4; 3.2b - line 7
at 37°C in the buffer: 0.1 M NaCl, 0.01 M Tris-HCl (pH7.2), 1 mM EDTA. The concentration of *M* target was 5 x 10^{-7} M, the concentrations of oligonucleotide components were 1 x 10^{-5} M
lines 5 and 8 - A+G.

ological conditions. Effectors should be applied to the nucleic acid modification by very short oligonucleotide reagents.

The use of "miniantisense" approach allows to modify DNA selectively avoiding the formation of unperfect reactive duplexes. That is practically impossible in the case of reagents based on long oligonucleotides.

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