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New approach to enhancing the efficiency and specificity of interaction in duplexes by the use of tandem structure

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Abstract: Examined in this work is ssDNA recognition by tetranucleotides and their alkylating derivatives in the presence of effectors (native oligonucleotides and their derivatives bearing a duplex stabilizing group such as phenazinium). Though tetranucleotides themselves are not capable of interacting with DNA, the effective site specific DNA recognition by tetranucleotides and their alkylating reagents is caused by the presence of flanking effector pair. The influence of diphenazinium octanucleotide derivatives on the interaction of tetranucleotide or its reactive derivatives with DNA by far exceeds the influence of native octanucleotides. The level of DNA modification by the alkylating moiety of tetranucleotide depends on hybridization properties of the effector. Tetranucleotide forms complexes with the same DNA-target site with T_m changing from 0°C to 38°C. Besides, the tetranucleotide reagent modifies that site. The extent of modification varies from 0% to 60%, depending on a type of the effectors used. Having itself several binding sites, the tetranucleotide reagent is made to interact with DNA target exclusively in one binding site determined by the sequence of the flanking effector pair. A new method based on the usage of flanking effector pairs is thus proposed for enhancing the efficiency and specificity of DNA recognition by short oligonucleotides and for discriminating their binding sites.

Introduction

The efficiency of site-specific recognition of nucleic acids by oligonucleotides and their derivatives are known to be defined by their hybridization properties depending mainly on the length of the oligonucleotide strand. However, the possibility of forming imperfect strong complexes increases with the size of oligonucleotide too. *In vitro* these imperfect duplexes can be destroyed by special methods, for example, temperature increase. The other problem arises in *in vivo* systems where significant changes of conditions are not acceptable. In this case the formation of incomplete duplexes of long oligonucleotides and DNA exerts a negative effect on the specificity of interaction of oligonucleotides and DNA. Certain difficulties also arise when short oligonucleotides are used. The decrease of oligonucleotide length results in increased number of possible binding sites and loss of hybridization efficiency. As a result although the imperfect complexes formed by DNA and short oligonucleotides are not observed at 37°C the short oligonucleotides also do not interact effectively with DNA at that temperature. This work deals with the development of an approach to increasing the efficiency and specificity of oligonucleotide-DNA recognition under physiological conditions.

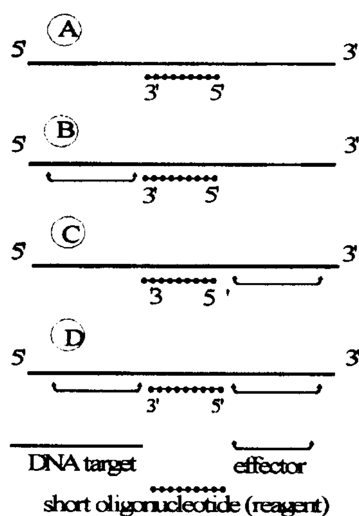
We have earlier demonstrated the possibility of improving the efficiency of the site specific modification of DNA by alkylating reagents on the basis of hexanucleotides by introducing an effector, which is a mono- or diphenazinium oligonucleotide derivative complementary to the sequence contiguous to the target sequence of the reagent (1-9). Other examples have appeared recently where native oligonucleotides are used as auxiliary moieties ("helpers" or "facilitators") to enhance specificity of some processes based on nucleic acid - oligonucleotide recognition (in designing the long oligonucleotide probes by enzymatic and chemical ligation (10-12), ribozyme cleaving (13), photomodification of DNA and RNA-target by psoralen oligonucleotide derivatives (14), sequencing by using joined primers (15)).

In this work we demonstrate the possibility of effective and site-specific DNA-target recognition by such short oligonucleotides as tetramers, which themselves are not capable of forming complexes with DNA-target. The result is achieved by introducing a flanking pair of effectors; the latter are represented by auxiliary native oligonucleotides or their derivatives bearing stabilizing groups.

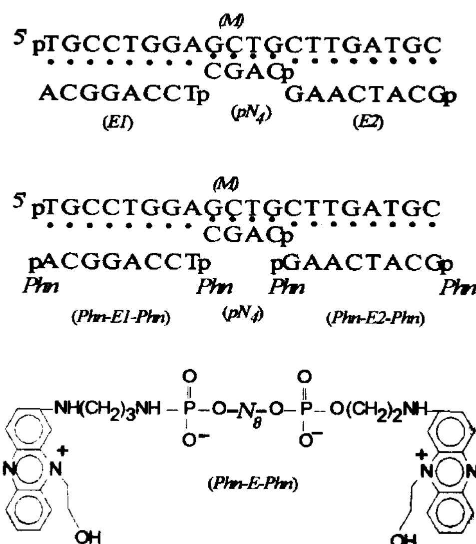
Results and discussion

The influence of an effector on DNA-target recognition by tetranucleotides is investigated by two approaches: 1) examination of thermostability of duplexes formed by DNA and tetranucleotide, and 2) examination of DNA modification by alkylating reagent attached to a tetranucleotide in the presence of an effector.

To handle these problems we have studied the following model duplexes (Scheme 1): system A - DNA-target + tetranucleotide (or its alkylating reagent); system B - DNA-target + tetranucleotide (or reagent) + 3'-flanking effector; system C - DNA-target + tetranucleotide (or reagent) + 5'-flanking effector; system D - DNA-target + tetranucleotide (or reagent) + pair of 3'-,5'-flanking effectors. Short native oligonucleotides and their derivatives containing stabilizing groups such as phenazinium were used as the effectors.



SCHEME 1



SCHEME 2

The interaction of oligonucleotide (*M*) (20-mer) with tetranucleotide pN_4 complementary to the former in its 9-12 site has been examined. Octanucleotides *E1* and *E2* (complementary to sequences 1-8 and 13-20, correspondingly of the target *M* and flanking pN_4 in the duplex) and their diphenazinium derivatives *Phn-E1-Phn* and *Phn-E2-Phn* were used as effectors (Scheme 2).

Stability of complexes formed by the target M with the effectors

At first thermal denaturation of complexes formed by DNA-target and effectors, octanucleotides *E1* and *E2* and their diphenazinium derivatives, was examined (Table 1). Duplexes *M* + *E1* (complex 1) and *M* + *E2* (complex 2) have close T_m°C (33°C and 35°C). Diphenazinium octanucleotide derivatives *Phn-E1-Phn* and *Phn-E2-Phn* produce with *M* target significantly more stable complexes 3 and 4 in accordance with the data of work (17). The complex formed by octanucleotide *E2 (Phn-E2-Phn)* is more stable than the complex formed by octanucleotide *E1 (Phn-E1-Phn)* (ΔT=2°C). The effector pair, *E1* + *E2* (or *Phn-E1-Phn* and *Phn-E2-Phn*), and DNA-target form two duplex regions separated by four bases gap (complex 5 or complex 6). On the melting curves of duplex *M* + *E1* + *E2* (complex 5) there is a single maximum at the temperature close to T_m of the complex formed by target *M* and one of the octanucleotides, *E1* or *E2*. The temperature corresponding to this maximum was denoted as T_m for duplexes of *M* target - effector pairs. The analogous result was obtained for complex of *M* target and diphenazinium derivatives *M* + *Phn-E1-Phn* + *Phn-E2-Phn* (complex 6) (for example, Fig. 1, curve 2) (Table 1).

The influence of the effector on the stability of the DNA-target - tetranucleotide complex

The investigation of thermal stability of *M* + pN_4 duplex shows that tetranucleotide has different hybridization properties in A-D systems (Table 2). Without any effector (in A system), the formation of complex *M* + pN_4 is not registered. A differential curve of thermal denaturation of the mixture *M*

Table 1. T_m°C of complexes formed by *M* target and effector

Complex No	Effector 1	Effector 2	T_m°C
1	*E1*	-	33
2	-	*E2*	35
3	*Phn-E1-Phn*	-	52
4	-	*Phn-E2-Phn*	54
5	*E1*	*E2*	35
6	*Phn-E1-Phn*	*Phn-E2-Phn*	54

Table 2. T_m°C (calculated*) of complex *M* + pN_4 in B-D systems

Complex No	System	Effector 1	Effector 2	T_m°C
7	B	*E1*	-	-
8	C	-	*E2*	-
9	D	*E1*	*E2*	23
10	B	*Phn-E1-Phn*	-	20
11	C	-	*Phn-E2-Phn*	24
12	D	*Phn-E1-Phn*	*Phn-E2-Phn*	38

*See "Materials and Methods"

+ pN_4 is similar to one of *M* target having a weak duplex structure at T_m = 7°C. In the presence of one effector (in B or C systems) the tetranucleotide forms a complex with *M* target. Stability of that complex changes depending on the type of the effectors used (octanucleotides or their dye derivatives). The investigation of thermal stability of the complexes of *M* target and tetranucleotide in the presence of one of the octanucleotides, *E1* or *E2* (complexes 7 and 8), has revealed that only denaturation of complexes *M* + *E1* or *M* + *E2* is clearly reflected in the melting curves of mixtures *M* + pN_4 + *E1* or *M* + pN_4 + *E2*. These data demonstrate that native octanucleotide alone can not practically intensify the interaction between tetranucleotide and DNA target. The influence of diphenazinium derivatives *Phn-E1-Phn* or *Phn-E2-Phn* on the hybridization properties of tetranucleotide is significantly stronger. The differential melting curve of the complex *M* + pN_4 + *Phn-E1-Phn* (complex 10) has one particularly pronounced maximum (52°C) corresponding to T_m of the complex formed by *M* target with effector *Phn-E1-Phn* and a second less pronounced maximum (20°C) corresponding to T_m of *M* + pN_4 duplex. The *Phn-E2-Phn* effector forms a stronger complex with *M* target, increasing T_m of *M* + pN_4 duplex up to 24°C (complex 11).

Thus, influence of the effector on stability of the complex formed by target *M* and the tetranucleotide pN_4 depends directly on the hybridization properties of the effector.

In the presence of a flanking effector pair (system D), the stability of complex *M* + pN_4 drastically increases. If one of the octanucleotides, *E1* or *E2*, does not effect the hybridization properties of the tetranucleotide, their simultaneous usage (complex 9) facilitates the formation of a relatively strong somplex *M* + pN_4 (T_m = 23°C). The tetranucleotide pN_4 forms with target *M* the most stable complex in the presence of diphenazinium effector pair (complex 12) (Fig. 1).

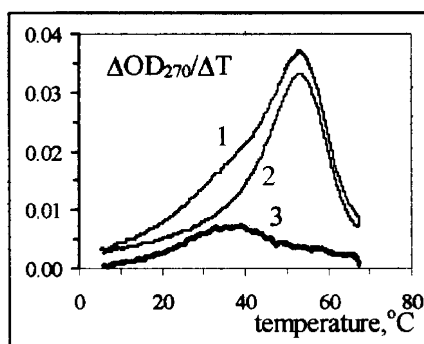


Fig. 1. Differential melting curves of complexes: 1 - *M* + (*Phn-E1-Phn* + pN_4 + *Phn-E2-Phn*) (complex 12); 2 - *M* + (*Phn-E1-Phn* + *Phn-E2-Phn*) (complex 6); 3 - *M* + pN_4 in the presence of effector pair *Phn-E1-Phn* + *Phn-E2-Phn* (calculated curve)

So the effector enhancement of tetranucleotide hybridization properties increases in the following order: *E1* ~ *E2* < *E1* + *E2* ~ *Phn-E1- Phn* ~ *Phn-E2-Phn* < *Phn-E1-Phn*+*Phn-E2-Phn*.

A pair of effector (either native octamers or their diphenazinium derivatives) affects the stability of the complex *M* + pN_4 more significantly than does a single effector. Besides the action of a single diphenazinium effector (either *Phn-E1-Phn* or *Phn-E2-Phn*) is close to the action of the native octamer pair *E1* + *E2*. The data obtained show that even such short oligonucleotide as tetramer with its extremely low hybridization properties forms a strong complementary complex with DNA-target when assisted by a pair of diphenazinium derivatives of oligonucleotides complementary to the flanking tetranucleotides on the DNA-strand.

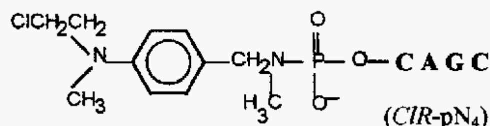
Influence of the effector on modification of target M by reagent ClR-pN4

The influence of the effector on the DNA-target recognition by short oligonucleotides may be investigated not only by determining the stability of a target-oligonucleotide complex in the presence of effectors but also by comparing the limits of target modification by oligonucleotide derivative bearing the reactive residue. As a reactive group we use alkylating 4-(N-2-chloroethyl-N-

methylamino)benzylmethylamine (*ClR*) that can not practically alkylate the DNA-target under conditions of addressed modification (25). The main features of *M* target modification were examined by the usage of tetranucleotide alkylating reagent *ClR*-pN_4 (Table 3). Without any effectors (complex 13) no modification of *M* target by reagent *ClR*-pN_4 has been found (Fig. 2a). In the presence of one effector (in B-C systems) *M* target modification is not registered when the effector used is an octanucleotide, *E1* or *E2* (complexes 14 and 15).

Table 3. The yield and site of M target modification by reagent *ClR*-pN_4 in A-D systems

Complex No	System	Effector 1	Effector 2	Yield,% 20°C	(Site) 37°C
13	A	-	-	0	0
14	B	*E1*	-	0	0
15	C	-	*E2*	0	0
16	D	*E1*	*E2*	30(G9)	0
17	B	*Phn-E1-Phn*	-	24(G9)	trace
18	C	-	*Phn-E2-Phn*	24(G9)	8(G9)
19	D	*Phn-E1-Phn*	*Phn-E2-Phn*	60(G9)	44(G9)



Different results are registered in the presence of diphenazinium derivative, *Phn-E1-Phn* or *Phn-E2-Phn* which increase the hybridization properties of pN_4 (complexes 17 and 18). At 20°C, i.e. close to T_m of complexes *M* + pN_4 in the presence of one of the effectors, *Phn-E1-Phn* or *Phn-E2-Phn*, the target alkylation reaches 24% and only one base G9 is practically modified. With reaction temperature raising to 37°C, the level of the target modification in the presence of one diphenazinium effector decreases: in complex 17 the modification yields traces of the product, the yield reaching 8% in complex 18. This distinction between the modification levels may be due to the fact that the sequence of octanucleotide *E1* includes trinucleotide sequence - *CAG*- complementary to the binding site of reagent *ClR-p<u>CAG</u>C* and the real concentration of duplex *M* + *ClR*-pN_4 under 37°C in the presence of effector *Phn-E2-Phn* is therefore somewhat higher than that in the presence of the effector *Phn-E1-Phn* which enhances the hybridization properties of tetranucleotide pN_4 to a smaller extent.

In the presence of effector pair (D system) the level of target modification runs up to 30% under 20°C in the case of pair *E1* + *E2* which increases T_m of complex *M* + pN_4 to 23°C; in the case of diphenazinium effector pair *Phn-E1-Phn* + *Phn-E2-Phn* the yield of *M* target modification reaches 60% at 20°C and 44% at 37°C (Figs. 2,3, Table 3, complexes 16 and 19).

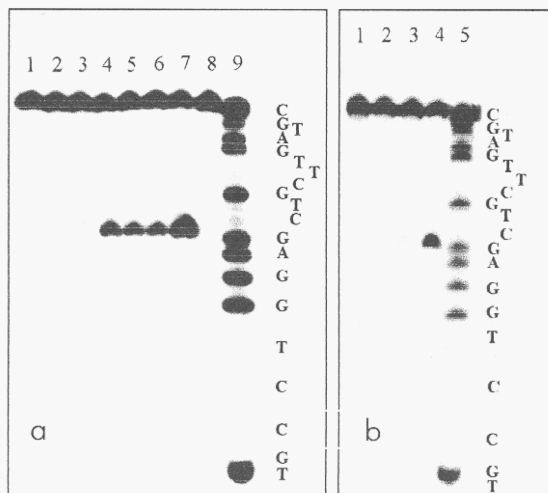
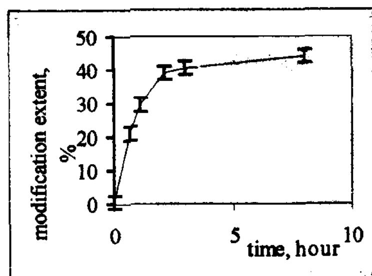
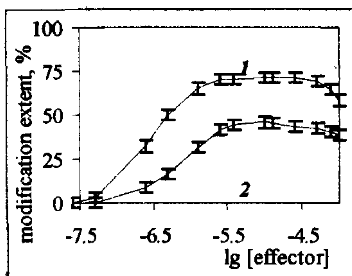


Fig. 2. Autoradiogram of the 20% denaturating polyacrylamide gel-electrophoresis of *M* target modification after piperidine treatment in complexes
a) at 20°C, 1 - *M* + *ClR*-pN_4 (complex 13), 2 - *M* + (*E1* + *ClR*-pN_4) (complex 14), 3 - *M* + (*ClR*-pN_4 + *E2*) (complex 15), 4 - *M* + (*E1* + *ClR*-pN_4 + *E2*) (complex 16), 5 - *M* + (*Phn-E1-Phn* + *ClR*-pN_4) (complex 17), 6 - *M* + (*ClR*-pN_4 + *Phn-E2-Phn*) (complex 18), 7 - *M* + (*Phn-E1-Phn* + *ClR*-pN_4 + *Phn-E2-Phn*) (complex 19), 8 - *M* + (*Phn-E1-Phn* + *ClR*-pGTCG + *Phn-E2-Phn*), 9 - products of the (A+G) sequencing reaction (26);
b) at 37°C, 1 - *M* + (*E1* + *ClR*-pN_4 + *E2*) (complex 16), 2 - *M* + (*Phn-E1-Phn* + *ClR*-pN_4) (complex 17), 3 - *M* + (*ClR*-pN_4 + *Phn-E2-Phn*) (complex 18), 4 - *M* + (*Phn-E1-Phn* + *ClR*-pN_4 + *Phn-E2-Phn*) (complex 19), 5 - products of the (A+G) sequencing reaction (26).





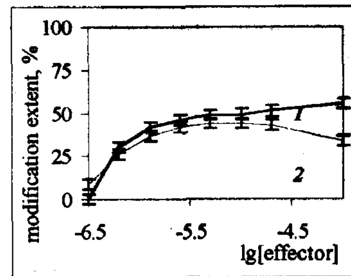


Fig. 3 Fig. 4a Fig. 4b

Fig. 3. Kinetic curve of accumulation of *M* target modification product in complex *M* +(*Phn-E1-Phn* + *ClR*-pN_4 + *Phn-E2-Phn*) (complex 19) at 37°C.

Fig. 4. The dependence of extent of *M* target modification by reagent *ClR*-pN_4 on concentration of effectors
a) 1 - at 20°C, 2 - at 37°C; [*Phn-E1-Phn*]=[*Phn-E2-Phn*] = 10^{-7} - 10^{-4} M;
b) at 37°C, 1 - [*Phn-E1-Phn*]=10^{-5} M - constant, [*Phn-E2-Phn*]= 10^{-7} - 10^{-4} M, 2 - [*Phn-E2-Phn*]=10^{-5} M - constant, [*Phn-E1-Phn*]=10^{-7} - 10^{-4} M.

The investigation of the dependence of the target alkylation extent on the concentration of diphenazinium effector pair has pointed out that the effector influence is revealed at the concentration of effector pair (2,5x10^{-7} M) twice lower than the concentration of the target and 40 times lower than the reagent concentration. The action of the effector pair is practically constant in a wide range of concentrations: 1x10^{-6} - 5x10^{-5} M (Fig. 4a). The relation of the modification level and the concentration of one effector at constant concentration of the other was studied since the effectors, *Phn-E1-Phn* and *Phn-E2-Phn*, have somewhat different influence on the target modification by reagent *ClR*-pN_4 at 37°C. The data obtained (Fig. 4b) demonstrate that the rise of concentration of *Phn-E2-Phn* ([*Phn-E1-Phn*]=10^{-5} M) leads to increase in the level of *M* target modification. Concentration rise of *Phn-E1-Phn* in 10^{-5} -10^{-4} M range ([*Phn-E2-Phn*]=10^{-5} M) suppresses the modification from 44% to 34%.

In all cases the *M* target modification by reagent *ClR*-pN_4 in the presence of effectors is strictly site specific since only the *G9* base is subjected to alkylation. The alkylation of bases *G12* and *C13* which are closer to the reactive group has not been detected.

The described above features of *M* target modification by reagent *ClR*-pN_4 in the presence of effectors, such as the dependence on stability of the complex formed by the target and the reagent; the effect of reaction temperature; the observed competition of effector *Phn-E1-Phn* with the reagent for the binding site - all these features indicate that the reactions are being carried out in complementary complexes. That conclusion has been confirmed by the fact that alkylating reagent *ClR*-pGCTG on the basis of the tetranucleotide non-complementary to *M* target is not capable of modifying the *M* target in systems A-D (Fig. 2a, line 8).

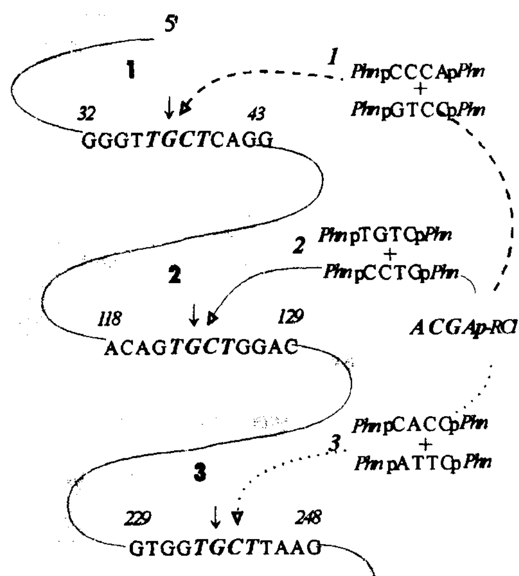
The efficiency of *M* target modification by alkylating reagent on the basis of tetranucleotide in A-D systems correlates with the stability of the duplex *M* +pN_4 in same systems and reaches its maximum in the presence of a flanking pair of diphenazinium effectors.

At the next step we have examined the possibility of discriminating the tetranucleotide binding sites by tetranucleotide reagent and the flanking diphenazinium effector pairs. Such phenomenon was observed during modification of DNA-302 fragment by reagent *ClR*-pAGCA on the basis of tetranucleotide which has three sites of the complementary binding with DNA-target: 36-39 in region 1, 122-125 in region 2, and 233-236 in region 3 (Scheme 3).

The reaction has been carried out in the presence of one of the three different pairs of diphenazinium derivatives of tetranucleotides. Each pair is complementary to two tetranucleotide sequences of target DNA flanking the binding site of the reagent.

When the effector pair *Phn*-pCCCAp-*Phn* and *Phn*-pGTCCp-*Phn* complementary to region 1 is used, the alkylation takes place with 21% yield only with base G37. In the presence of the effector pairs complementary to regions 2 or 3 the modification yields the alkylated bases G123 (26%) or G234 (39%), correspondingly (Fig. 5).

Thus the pair of diphenazinium effectors is capable of directing the tetranucleotide reagent to one out of several binding sites where the formation of perfect complex of the DNA target and the tandem "effector-reagent-effector" occurs. The site of "reagent fixation" on DNA is defined by



SCHEME 3

Scheme 3. The directive action of flanking effector pairs leading to selection of one binding site among the three sites of DNA by tetranucleotide reagent *ClR*-pAGCA.

the effectors complementary to sequence target sites flanking the selected binding site of the reagent. A similar "directive" action of the effector (mono- or diphenazinium derivatives of octanucleotide) has been observed earlier in the case of modification of DNA-target by the alkylating reagent on the basis of hexanucleotide having stronger hybridization properties than the tetranucleotide (1,2,40).

Though tetranucleotides can not interact with DNA themselves, as shown in this work, the effective and site specific DNA recognition by tetranucleotides and their alkylating reagents is carried out with the help of a flanking effector pair. Comparison of the thermostability of the complex formed by DNA-target and tetranucleotide in the presence of octanucleotides and their diphenazinium derivatives shows that T_m of the complex formed by DNA and the tetranucleotide changes from 0° to 38°C according to the type of effectors and the method of their usage. The level of DNA modification by alkylating reagent of tetranucleotide depends on hybridization properties of the effectors. The flanking pair of diphenazinium effectors causes the most significant enhancement of the interaction between DNA and tetranucleotide or its alkylating derivative. Having itself several binding sites, the tetranucleotide reagent is made to interact with DNA target exclusively in one binding site determined by the sequence of the flanking effector pair.

The data obtained serve for developing a new method of enhancing the efficiency and specificity of DNA target recognition by short oligonucleotides and discriminating the binding sites of latter. Alkylating reagents of short oligonucleotides were used throughout the work. Recently we have obtained preliminary data showing that the main principles of effector intensification studied in this work can be applied to short oligonucleotide derivatives containing other reactive residues, such as photoactive and cleaving groups.

The suggested approach may be used for stabilization of complementary complexes of short oligonucleotides and DNA; intensifying the recognizing specificity of nucleic acids and oligonucleotide derivatives; designing gene-directed preparations. In general the given approach may be used for all technologies where site-specific interaction of oligonucleotides with nucleic acids is necessary, for example, in molecular diagnostics, etc.

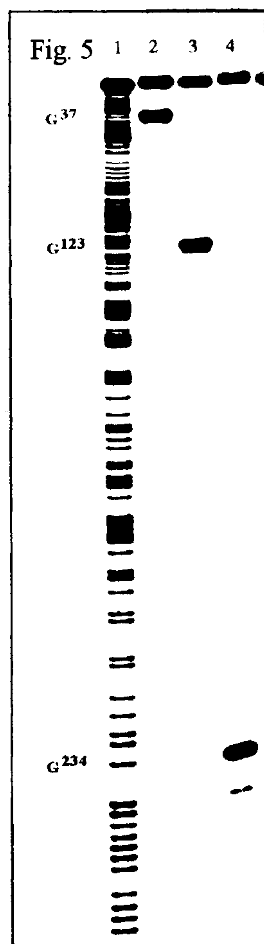


Fig. 5. Autoradiogram of the 8% denaturating polyacrylamide gel-electrophoresis of ssDNA (302 mer) modification (after piperidine treatment) with reagent *ClR*-pAGCA in the presence of: 2 - *Phn*-pACCCp-*Phn* + *Phn*-pCCTG-*Phn* (region 1), 3 - *Phn*-pCTGCp-*Phn* + *Phn*-pGTCC-*pPhn* (region 2), 4 - *Phn*-pCCACp-*Phn* + *Phn*-pCTTA-*pPhn* (region 3); 1 - products of the (A+G) sequencing reaction (26), [*ClR*-pAGCA] = [effector] = 5 x 10^{-5} M.

Materials and methods

Synthesis of oligonucleotides and their derivatives

Oligonucleotides were synthesized by using a phosphotriester method starting with 5'-*p*-chlorophenyl *N*-acyl-3'-*O*-levulinyl nucleoside 5'-phosphates and *p*-chlorophenyl β-cyanoethyl *N*-acyl nucleoside 5'-phosphates (16).
3',5'-Di-*N*(2-hydroxyethyl)phenazinium derivatives of oligonucleotides were obtained using approach (17).
Alkylating derivatives of oligonucleotides containing 4(*N*-2-chloroethyl,*N*-methylamine)benzylmethyl-amide were synthesized according to (18) and analyzed by HPLC at the LiChrosorb RP-18 using gradient of acetonitryle in 0.05 M $LiClO_4$ (19).

Physical measurements

The concentrations of oligonucleotides and their derivatives were measured spectrophotometrically using extinctions ε_{260} of mono- and dinucleotides (20), alkylating group (1.47×10^4 M^{-1} cm^{-1} (21)), *N*-(2-hydroxyethyl)phenazinium residue (1×10^4 M^{-1} cm^{-1} (17)).

The thermal denaturation of oligonucleotide complexes was performed in 10 mM sodium cacodylate (pH 7.4), containing 0.1 M NaCl, 1 mM EDTA, the concentration of each component of complex was 1.3×10^{-5} M. The absorption at 260 nm was detected for each oligonucleotide mixture as a function of increasing temperature, heating rate was 0.5-1°C/min on the UV detector of Milichrom liquid chromatograph in a thermoregulated cell specially designed for this purpose. The equilibrium optical melting curves were found on the basis of more than 600 experimental points with the step 10 point/°C and were completely reversible in heating-cooling processes. The first derivatives of optical melting curves versus temperature were calculated using a gradient of linear approximation by 10 experimental points. The melting curve of complex M + pN_4 (or its steroid derivatives) in the presence of effector pair was calculated by difference between the melting curves of complex M + pN_4 (or its steroid derivatives) + effector pair and of complex M + effector pair. T_m of complex M + pN_4 in the presence of this effector pair denotes T_{max} of calculated curve (for example, Fig.1).

Modification of DNA fragments with alkylating oligonucleotide derivatives

5'-[^{32}P]-Labeled oligonucleotide *M* was obtained in accordance with protocol (22). 3'-[^{32}P]-ssDNA-fragment (302-mer) obtained using method (23) was prepared by S.Mamaev (Novosibirsk Institute of Bioorganic Chemistry, RAS, Russia).
The modification of M target DNA (20-mer) was carried out in buffer: 0.1 M NaCl, 0.01 M *tris*-HCl (pH 7.2), 1 mM EDTA at 20°C for 48 hours and at 37°C for 8 hours (>5 half-times of the reaction of ionization of C-Cl-bond in the reagents (21)). The concentration of *M* target in reaction mixtures was 5×10^{-7} M, the concentrations of the oligonucleotide derivatives were: the reagent - 1×10^{-5} M, the effectors - 1×10^{-4} M at 20°C and 1×10^{-5} M at 37°C.
The modification of ssDNA-fragment (302-mer) was carried out in buffer: 0.16 M NaCl, 0.02 M Na_2HPO_4, 0.1 mM EDTA (pH 7.4) at 25°C for 24 hours; the concentration of DNA-fragment was equal to 1×10^{-8} M.
To cleave the modified DNA fragment at positions of alkylated bases it was treated with 1 M piperidine at 100°C for 30-50 min (24) The cleavage products were analyzed by electrophoresis in the denaturing 8% or 20% polyacrylamide 7 M urea gel. After autoradiography the radioactive spots were cut off the gel and counted by liquid scintillation on a counter Mark III ("Nuclear Chicago", USA). The modification extents were calculated as the ratio of radioactivity of the spot of product to the sum of radioactivities of the spots of product and unchanged DNA target.

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References

1. V.F.Zarytova et al. *Dokl. Akad. Nauk SSSR.* 302, 102-104 (1988).
2. I.V.Kutyavin et al. *FEBS Lett.* 238, 35-38 (1988).
3. N.V.Amirakhanov et al. *Bioorgan. Khim.* 16, 1523-1530 (1990).
4. V.F.Zarytova et al. Bioorgan. Khim. 16, 1653-1660 (1990).

5. V.F.Zarytova et al. *Collection special issue of Czechoslovak. Chem. Comm.* 55, 245-248 (1990).
6. E.M.Ivanova et al. *Collection special issue of Czechoslovak. Chem. Comm.* 55, 217-220 (1990).
7. V.F.Zarytova. *Nucleic Acids Res. Symposium ser.* 24, 103-106 (1991).
8. V.F.Zarytova et al. *Bioorgan. Khim.* 18, 895-900 (1992).
9. O.S.Fedorova et al. *Bioorgan. Khim.* 20, 932-943 (1994).
10. S.A.Strobel and P.B.Dervan. *J.Am.Chem.Soc.* 111, 7286 (1989).
11. N.Colocci et al. *J.Am.Chem.Soc.* 115, 4468-4473 (1993).
12. S.A.Gryaznov et al. *Nucleic Acids Res.* 22, 2366-2369 (1994).
13. J.Goodchild. *Nucleic Acids Res.* 20, 4607-4612 (1992).
14. E.Pascolo et al. *Biochim.Biophys.Acta.* 1219, 98-106 (1994).
15. T.L.Azhikina et al. *Dokl. Akad. Nauk SSSR.* 331, 751-753 (1993).
16. V.F.Zarytova et al. *Bioorgan. Khim.* 9, 516-521 (1983).
17. S.G.Lokhov et al. *Bioconjugate Chem.* 3, 414-419 (1992).
18. V.F.Zarytova et al. *Biophosphates and their analogues - synthesis, structure, metabolism and activity.* p. 149-164, Elsevier Science Publishers, Amsterdam, (1987).
19. N.I.Grineva et al. *Bioorgan. Khim.* 3, 210-214 (1977).
20. C.R.Cantor and I.J.Tinoco. *J.Mol.Biol.* 13, 65-72 (1965).
21. G.I.Baram et al. *Bioorgan. Khim.* 12, 613-620 (1986).
22. K.L.Berkner and W.R.Folk. *J.Biol.Chem. 252*, 3176-3184 (1977).
23. V.V.Vlassov et al. *Nucleic Acids Res.* 14, 4065-4076 (1986).
24. A.M.Maxam and W.Gilbert. *Methods in Enzymology*, 65. pp. 499-560. Acad. Press. New York-London, (1980).
25. D.G.Knorre and V.V.Vlassov. Affinity modification of biopolymers. Roca Raton, CRC Press Inc. Florida, pp. 129-165 (1989).
26. D.G.Korobko et al. Bioorgan. Khim. 4, 1281-1283 (1978).