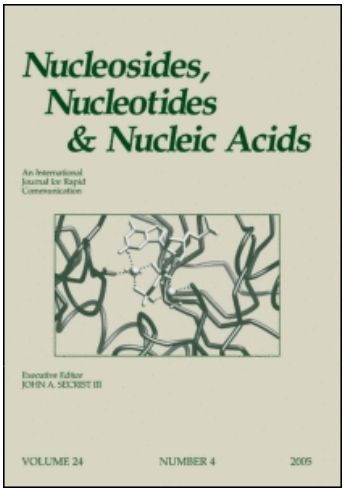

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D. V. Pyshnyi[a]; S. G. Lokhov[a]; E. M. Ivanova[a]; V. F. Zarytova[a]
[a] Novosibirsk Institute of Bioorganic Chemistry, Novosibirsk, Russia

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SPECIAL PROPERTIES OF MODIFICATION OF ssDNA TARGET BY ALKYLATING DERIVATIVES OF OLIGONUCLEOTIDES IN TANDEM COMPLEXES

D.V.Pyshnyi, S.G.Lokhov, E.M.Ivanova, V.F.Zarytova*
Novosibirsk Institute of Bioorganic Chemistry, Novosibirsk, Russia

ABSTRACT: The influence of an effector (di-N-(2-hydroxyethyl)-phenazinium derivative of oligonucleotide) on modification of the DNA target by alkylating derivatives of oligonucleotides having various hybridization properties was studied. Being adjacent to the alkylating group of the reagent, the effector enhances the target modification if the oligonucleotide reagent has low hybridization properties and suppresses the modification if the reagent can form the stable complex with the DNA target at the used conditions.

INTRODUCTION

Recently auxiliary oligonucleotides or their derivatives (effectors) are applied to enhance of the thermal stability of the target complexes formed by oligonucleotides and NA. The effector increases the extent of association of the adjacent oligonucleotide in the tandem complex due to cooperative interactions of tandem's components [1]. These auxiliary oligonucleotides are used to study processes which include the complex formation of NA and oligonucleotides [2], to modify the target sequence of DNA [3-5].

In this work we study how hybridization properties of the oligonucleotide reagents affect on modification of DNA in the tandem complexes in the presence of effector.

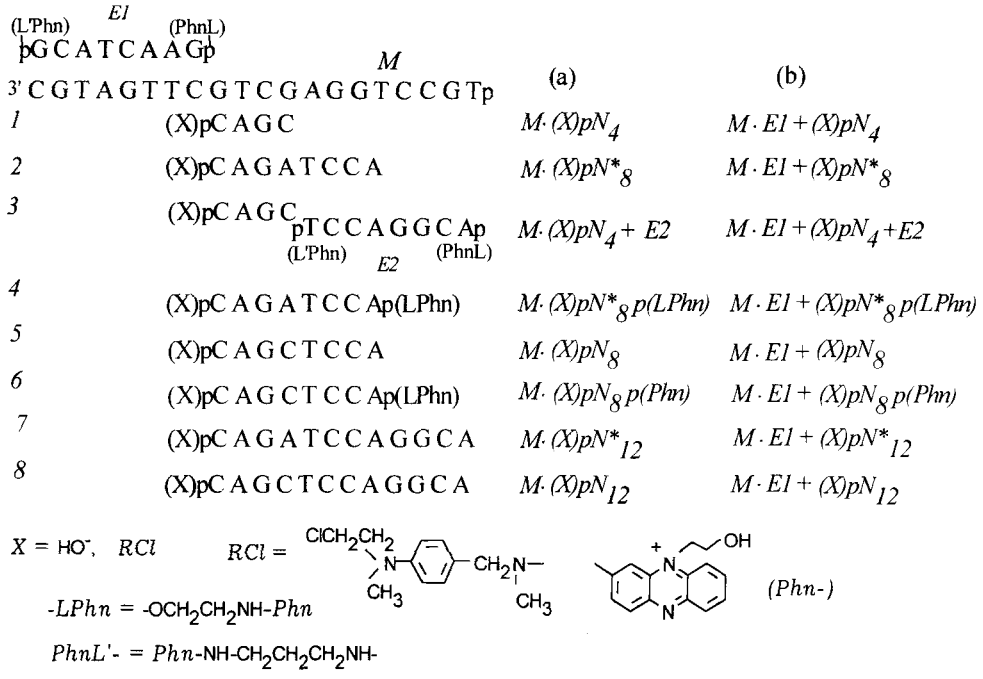
* FAX: 007 3832 35 16 65; e-mail: zarytova@niboch.nsc.ru

RESULTS AND DISCUSSION

For the investigation we used the 20-mer DNA fragment (*M*) as a target (Scheme). The octanucleotide derivative containing N(2-hydroxyethyl)phenazinium groups at 3'- and 5'-phosphates was used as effector (*E1*). The melting point of the $M \cdot E1$ complex is 55 °C [5]. The tetranucleotide pCAGC (pN_4) was used as oligonucleotide with extremely low hybridization properties (T_m of the $M \cdot pN_4$ complex is less than 5°C). The ability of the tetranucleotide to form the complex was increased by some ways: by lengthening up to the octamer pCAGATCCA (pN^*_8) the sequence of which had one base substitution (T_m of the complex $M \cdot pN^*_8$ is 10 °C); by using the effector *E2* which was adjacent to 3'-end of the tetramer (T_m of the duplex $M \cdot pN_4$ in the presence of *E2* effector is 24 °C); by coupling phenazinium residue to 3'-phosphate of the octamer (pN^*_8p(LPhn)) (T_m of complex $M \cdot pN^*_8p(LPhn)$ is 31 °C); by lengthening up to the correct octamer pCAGCTCCA (pN_8) (T_m of complex $M \cdot pN_8$ is 35 °C) and coupling the phenazinium residue to its 3'-phosphate (pN_8p(LPhn)) (T_m of the complex $M \cdot pN_8p(LPhn)$ is 44 °C); by lengthening up to the incorrect and correct 12-mer (pN^*_{12} and pN_{12}, recpectively) (T_m of the complexes $M \cdot pN^*_{12}$ and $M \cdot pN_{12}$ are 46 and 55 °C). The alkylating derivatives of these oligonucleotides containing 4(N-methyl-N-2-chloroethyl)benzylmethylamide group at 5'-terminus phosphate, (RCl)pN_n, form in the complexes $M \cdot (RCl)pN_n$ both without and with the *E1* effector the same reactive center as depicted in Scheme.

The modification of *M* target was carried out at 20 °C since at this temperature all reagents in the presence of *E1* effector form with the target the tandem complexes (1b-8b).

At first we studied the modification of *M* target by the reagents in the complexes 1a-8a without effector. It has been found that the extent of modification of the target is correlated with the melting points of the $M \cdot pN_n$ complexes only in the case of using the reagents hybridization properties of which provide the quick exchange of the reagent on the *M* target (the extent of association the target with the reagent less than 1) (the complexes 1a-5a). The maximal extent of the target modification (72%) is observed if the reagent (RCl)pN_8 was used. The further increase of hybridization properties of the oligonucleotides leads to the falling of the extent of modification and in the case of the (RCl)pN_{12} reagent which forms the most stable complex with the target the yield of alkylation is 60%. Thus, if the extent of association of the target with the reagent is close to 1, the efficacy of modification may be not as much as possible. It testifies that for the obtaining of the high yield of target modification the reaction conditions must provide the existence of the *target · reagent* complex in the mobile equilibrium (near T_m). It may be a consequence of the different conforma-



Scheme

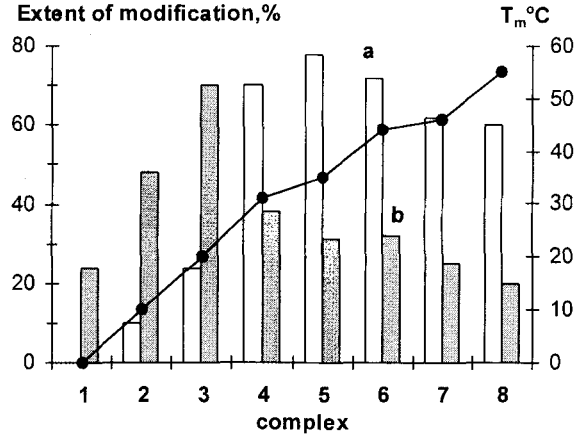


FIG. The extent of modification of the *M* target by the alkylating reagent of oligonucleotides in the complexes 1a-8a without the *E1* effector (**a**) and in the complexes 1b-8b in the presence of the *E1* effector (**b**) at 20°C and the melting points of the $M \cdot pN_n$ complexes (line). Modification: 0.01 M Tris-HCl (pH 7.2), 0.1 M NaCl, and 1 mM EDTA at 20°C for 48 hours, the concentration of the target in reaction mixtures was 0.5 μM, the concentrations of the oligonucleotide derivatives (the reagent or effector) were 10 μM each. Termal denaturation: 0.1 M NaCl, 0.01 M sodium cacodylate (pH 7.4), 1 mM EDTA, the concentration of each oligonucleotide component was $1.3 \cdot 10^{-5}$ **M**.

tions of reactive complexes *target · reagent* with the different stability since the stronger complex of oligonucleotides has the more rigid structure which promotes less realization of the reaction in the complex.

The similar feature of the target modification was observed in complexes 1b-8b in the presence of *E1* effector. The *E1* effector, being adjacent to the alkylating group of the oligonucleotide reagent, increases the efficacy of alkylation in the complexes 1b-3b, if the oligonucleotide reagent forms the labile *target·reagent* complex as it is depicted in FIG. On the contrary, if the oligonucleotide reagent has the high hybridization properties, the *E1* effector suppresses significantly the target modification (complexes 4b-8b). The extent of target modification by the reagent $(RCl)pN_{12}$ in the presence of the effector in the complex 8b falls to 24 % in comparison with 60% in the complex 8a. Furthermore the *E1* effector changes the sites of the target modification. The main alkylated site of *M* target in complexes 2a, 4a-8a is the C^{13} base and in the complexes 2b, 4b-8b is the G^9 base. The tetranucleotide reagent $(RCl)pN_4$ in all its complexes (1b, 3a-b) modifies only one G^9 base of the target. These data allow us to suggest that the different influence of the effector on the modification is connected to the different conformation of the *target · reagent* duplex. The increase of a rigidity of its structure may result in the decrease of the efficiency of the target modification by the oliginucleotide reagent in the tandem complexes.

Thus, the effector influence on DNA modification is the most positive in the case of using the reactive derivative of short oligonucleotide, which forms itself extremely labile complex with DNA target.

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