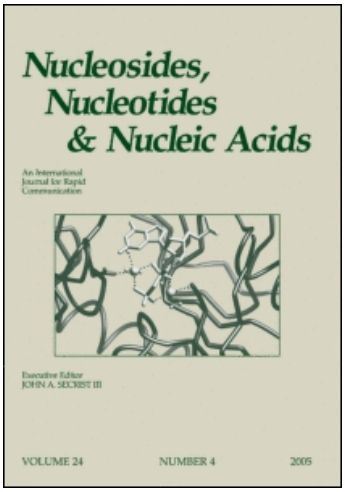

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Quantitative Parametrs of Cooperative Interactions of the Oligodeoxyribonucleotides on the Complementary Template

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QUANTITATIVE PARAMETRS OF COOPERATIVE INTERACTIONS OF THE OLIGODEOXYRIBONUCLEOTIDES ON THE COMPLEMENTARY TEMPLATE

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ABSTRACT. The cooperative interactions of oligonucleotides on the complementary template were studied using the quantitative analysis of the template alkylation with the oligonucleotides bearing covalently attached 4-[N-(2-chloroethyl)-N-methylamino]benzyl group at 5'-end. The influence of the mismatched nucleotides and the stabilizing N-(2-hydroxyethyl)phenazinium group at the 5'- and 3'-ends of the oligonucleotides on the parameters of cooperativity was evaluated.

It is known that two (or more) oligonucleotides are cooperatively bound at neighboring sites of polynucleotides. The specific binding of each oligonucleotide is increased in this case. Earlier the approach of quantitative investigation of cooperative interaction of oligonucleotides was developed using method of complementary-addressed modification titration (CAMT) [1,2]. In present paper this approach was used for the study of the influence of chemical groups and mismatched nucleotides at the 5'- and 3'-ends of oligonucleotides on the parameters of cooperative interaction.

Three targets (T10, T'22 and T22) had a complementary binding site for any of three antisense oligonucleotides, bearing covalently attached reactive 4-[N-(2-chloroethyl)-N-methylamino]benzyl group $ClRCH_2NH$- at 5'-end (6-meric reagent X6, 8-meric reagent X8, 8-meric reagent $X8^m$ forming TT-mismatch with the target):

Targets:

d(pTGAATGGGAAGAGGGTCAGGTT) (T22)

d(pTTTGCCTTGAATGGGAAGAGTT) (T'22)

d(pTGGGAAGAGT) (T10)

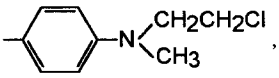
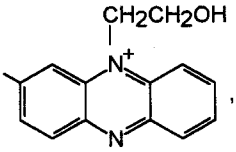
Reagents:

ClRCH$_2$NH-d(pTTCCCA) (X6)

ClRCH$_2$NH-d(pTCTTCCCA) (X8)

ClRCH$_2$NH-d(pTCTTCCCT) (X8^m)

where

RCl =  , Phn =  , L = -NH-CH$_2$CH$_2$-NH-

The target T'22 had additional one binding sites for neighboring oligonucleotide E$_1$ pd(TTCAAGGC) (effector E$_1$) or for its diphenazinium derivative Phn-L-pd(TTCAAGGC)p-L-Phn (effector E$_1^{Phn}$) bearing N-(2-hydroxyethyl)-phenazinium residue Phn- at both 5'- and 3' - ends covalently linked by ethylenediamine linker. Reagents X6, X8, X8^m and effectors E$_1$ and E$_1^{Phn}$ formed complementary tandem sequences E$_1$-X6, E$_1^{Phn}$-X6, E$_1$-X8, E$_1^{Phn}$- X8^m, E$_1$- X8^m on the target T'22. Target T22 had additional one binding sites for neighboring effector E$_2$ pd(TGACCCTC) or for its diphenazinium derivative Phn-L-pd(TGACCCTC)p-L-Phn (effector E$_2^{Phn}$) forming complementary tandem sequences X6 - E$_2$ and X6 - E$_2^{Phn}$ on the target T22.

Using the dependencies of the modification extents of alkylation of the targets at $t\rightarrow\infty$ on the initial concentration of the reagents the association constants of the reagents with the targets K_x were determined (Table 1). Experimental details were described previously [1,2]. Parameters of cooperativity α characterizing the efficiency of mutual interactions between the reagents X6, X8, X8^m and effectors E$_1$, E$_2$, E$_1^{Phn}$, E$_2^{Phn}$ have been found as the ratio of the association constants of the reagents in the presence and in the absence of corresponding effector (Table 1).

Quantitative results pointed to the following conclusions. The values of cooperativity parameters did not depend on oligonucleotide lengths. The efficiency of cooperative interaction increased by factor 3 in the presence of Phn-group covalently attached to

TABLE 1. The values of association constants K_x of the reagents with the complementary targets and parameters of cooperativity α at 25°C*.

Complex	Association constant K_x, M^{-1}	Type of contact	α
T10•X6	$(4.2\pm0.7)\cdot10^4$		
T10•X8	$(1.1\pm0.3)\cdot10^6$		
T10•X8^m	$(3.4\pm0.1)\cdot10^5$		
T'22•X6	$(1.1\pm0.3)\cdot10^4$		
T22•X6	$(4.2\pm0.7)\cdot10^4$		
T'22•E_1•X6	$(2.2\pm0.5)\cdot10^5$	(5')–A—T–(3') (3')–Tp A–(5')	5.2
T'22•E_1•X8	$(4.0\pm0.1)\cdot10^6$	-"-	3.6
T'22•E_1^{Phn}•X6	$(6.8\pm0.1)\cdot10^5$	(5')–A—T–(3') (3')–Tp A–(5') L—Phn	16.2
T'22•E_1^{Phn}•X8	$(1.5\pm0.4)\cdot10^7$	-"-	13.6
T'22•E_1•X8^m	$(3.3\pm0.1)\cdot10^5$	(5')–A—T–(3') (3')–Tp T–(5')	0.97
T'22•E_1^{Phn}•X8^m	$(3.7\pm0.2)\cdot10^6$	(5')–A—T–(3') (3')–Tp T–(5') L—Phn	10.8
T22•X6•E_2	$(4.0\pm0.6)\cdot10^4$	(5')–A—G–(3') (3')–Tp pC–(5') NH CH_2—RCl	0.95
T22•X6•E_2^{Phn}	$(4.2\pm0.7)\cdot10^5$	(5')–A—G–(3') (3')–Tp pC–(5') NH Phn CH_2—RCl	10.0

*Buffer: 0.16M NaCl, 0.02M Na_2HPO_4 (pH 7.5 at 25°C), 0.1 mM EDTA.

oligonucleotides and locating at the junctions, whereas presence of alkylating group $ClRCH_2NH$- at the junctions eliminated it. Sufficient effective cooperative interaction occurred in the case of simultaneous presence of both Phn- and $ClRCH_2NH$- groups at the junctions. The presence of the TT-mismatch at the junction prevented cooperative interaction, apparently, due to the elimination of base stacking in this case. Whereas, cooperative interaction occurs in the case of simultaneous presence of Phn- group covalently attached in effector E_1^{Phn} and the TT-mismatch base pair formed by the reagent $X8^m$ and target T'22 at complex formation. In this case any interaction has been suggested to occur between the Phn - group and mismatched thymidine base residue in the reagent $X8^m$.

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