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Complementary addressed modification of nucleic acids with the alkylating derivatives of oligothymidylate ethyl phosphotriesters

Effect of the phosphotriester fragments' configuration

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The alkylating derivatives of four individual diastereomers of the oligonucleotide $[dTp(Et)]_3dTpU$ and two individual diastereomers of oligonucleotide $[dTp(Et)dTp]_4$ have been synthesized. The reagents with the phosphorus atoms in the enantiomeric p″ configuration are shown to be more efficient in reacting with poly(dA) and with nucleic acids in Krebs-2 ascites carcinoma cells compared to those with the phosphorus atoms in the p′ configuration.

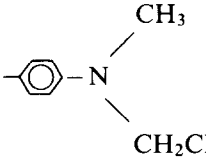
Nucleic acid; Oligonucleotide derivative; Affinity modification

1. INTRODUCTION

The most important potential applications of the affinity modification of nucleic acids with reactive derivatives of oligonucleotides (complementary addressed modification) [1] concern specific damage to cellular nucleic acids. The modification of cellular nucleic acids can most easily be achieved by using nonionic oligonucleotide derivatives such as phosphotriesters of oligonucleotides, since they readily penetrate into animal cells [2–4]. These derivatives are synthesized as different diastereomers which can vary in ability to interact with complementary nucleotide sequences. Here, we report results of experiments on the affinity modification of poly(dA) and cellular biopolymers with alkylating oligothymidylate derivatives (I–VI) synthesized from individual diastereomers of oligothymidylate ethyl phosphotriesters.

dTp′ (Et)dTp′ (Et)dTp′ (Et)dTpU(=CH-RCl) (I)
dTp′ (Et)dTp″(Et)dTp′ (Et)dTpU(=CH-RCl) (II)
dTp″(Et)dTp′ (Et)dTp″(Et)dTpU(=CH-RCl) (III)
dTp″(Et)dTp″(Et)dTp″(Et)dTpU(=CH-RCl) (IV)
dTp′ (Et)dTpdTp′ (Et)dTpdTp′ (Et)dTpdTp′ (Et)-dTp(NH-CH_2-RCl) (V)
dTp″(Et)dTpdTp″(Et)dTpdTp″(Et)dTpdTp″(Et)-dTp(NH-CH_2-RCl) (VI)

where RCl is a reactive alkylating group



Designations p′ and p″ correspond to tetrahedral phosphorus atoms with enantiomeric configurations.

2. MATERIALS AND METHODS

Oligothymidylate ethyl phosphotriesters possessing phosphorus atoms in the differing enantiomeric configurations p′ and p″ were synthesized from individual diastereomers of the dinucleotides which were isolated by silica gel chromatography [5,6]. The absolute configurations of the phosphorus atoms p′ and p″ have not yet been established; 2D NMR and chromatographic experiments designed to identify their con-

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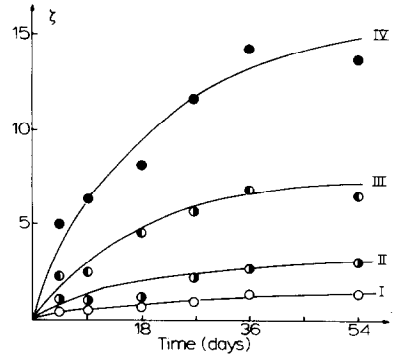

Fig.1. Kinetic curves of poly(dA) alkylation with reagents I–IV at 5°C in 0.2 M NaCl, 0.01 M $MgCl_2$, 0.01 M Tris-HCl (pH 7.4). Concentrations are listed in table 1. ζ, extent of alkylation (in per cent of ζ_∞).

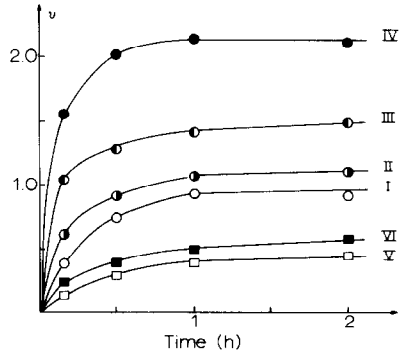

Fig.2. Binding of alkylating oligonucleotide derivatives with Krebs-2 ascites carcinoma cells. Cells were incubated in standard medium (see section 2) with reagents I–IV at 25°C and reagents V and VI at 37°C. U, binding of ^{14}C label to the cells (per cent).

figurations according to Stec, Zon and co-workers [7–9] are in progress.

Alkylating reagents I–IV were prepared by coupling ^{14}C-labeled 4-(*N*-2-chloroethyl-*N*-methylamino)benzaldehyde to the *cis*-diol group of the 3′-terminal ribose of the oligonucleotides [5]. To prepare reagents V and VI, the phosphate-activated oligonucleotides were reacted with ^{14}C-labeled 4-(*N*-2-chloroethyl-*N*-methylamino)benzylamine to yield 4-(*N*-2-chloroethyl-*N*-methylamino)benzyl phosphamides of oligonucleotides [10].

Ascites tumor Krebs-2 cells were maintained in CC57BR mice. Cells were washed three times by centrifugation in medium 199 supplemented with 10 mM Hepes (pH 7.2), 50 μg/ml streptomycin and 30 U/ml (standard medium), and incubated with the oligonucleotide derivatives. Incubation conditions for reagents I–IV were: 2 weeks at 5°C; concentration of cells, 3.6×10^6 cells/ml; concentration of reagents, 0.5 μM; for reagents V and VI: 2.5 h at 37°C; 5×10^6 cells/ml; concentration of reagents, 0.67 μM. In experiments investigating the uptake of oligonucleotide derivatives, the cells were incubated with the ^{14}C-labeled derivatives in the standard medium, washed three times by centrifugation in the same medium, and counted in a scintillation counter. Isolation and quantitation of the alkylated nucleic acids and proteins from the cells were performed as described in [4,5,11].

3. RESULTS AND DISCUSSION

Fig.1 shows the kinetics of poly(dA) alkylation with reagents I–IV. It is observed that the rates of alkylation vary among the reagents. Treatment of the kinetic data according to the scheme of affinity modification [5] gives values of the limit extent of alkylation (ζ_∞, extent of modification; achieved at incubation time $\rightarrow \infty$), the association constants of the reagents and polymer (K_x), and ratios of the rates of intracomplex and nonspecific bimolecular reactions (f) (table 1). In all cases, the highest values for these parameters, reflecting a more efficient reaction, are obtained for reagent IV with the phosphorus atoms in the p′′ configuration.

Table 1

Kinetic parameters of poly(dA) alkylation with reagents I–VI

Reagent	Concentration (μM)	ζ_∞ ($\times 10^2$)			K_x (M^{-1})			f		
		5°C	20°C	37°C	5°C	20°C	37°C	5°C	20°C	37°C
I	57	1.1			12			3.3		
II	50	2.3			25			6.8		
III	47	6.8			76			20		
IV	39	18			230			53		
V	6.3		30	4.6		430	48		86	13
VI	6.3		56	40		1300	670		160	120

[a] Poly(dA) concentration: 1000 μM (per nucleoside residue)

Table 2

Modification of biopolymers in Krebs-2 ascites carcinoma cells treated with alkylating derivatives of oligothymidylates

Reagent	Uptake of reagent by cells (pmol/10^6 cells)	Covalent binding of reagent (pmol/10^6 cells)				R^a
		Total binding to biopolymers	Binding to RNA	Binding to DNA	Binding to proteins	
I	1.4	0.26	0.06	0.06	0.14	0.9
II	1.1	0.30	0.09	0.09	0.12	1.5
III	2.1	0.83	0.31	0.31	0.21	3.0
IV	3.3	1.60	0.77	0.47	0.33	3.8
V	0.54	0.08	0.03	0.02	0.03	1.7
VI	0.8	0.17	0.06	0.05	0.06	1.9

[a] R, selectivity factor (reagent bound to nucleic acids/reagent bound to proteins)

Similar results were obtained for reagents V and VI (table 1). These findings are in agreement with the earlier observation that the melting temperature for the complex poly(dA)·[dTp''(Et)dTp]$_4$ is 20°C higher than that for poly(dA)·[dTp'(Et)dTp]$_4$ [6]. Fig.2 shows the kinetics of uptake of reagents I–VI by Krebs-2 ascites carcinoma cells. Among isomers I–IV, reagent IV is the most efficient in penetrating into the cells and in alkylation of cellular nucleic acids (table 2). In terms of reactivity toward nucleic acids vs proteins (selectivity factor R, table 2), reagent IV shows a 4-times greater specificity to nucleic acids compared to reagent I which has all ethylated phosphorus atoms in the opposite configuration. A similar, although less pronounced, difference was observed for reagents V and VI (table 2). In contrast to the reaction with nucleic acids, the efficiency of protein modification is not influenced by the configuration of the ethylated phosphate fragments of the reagents. The results obtained demonstrate that reactive derivatives of ethyl esters of oligonucleotides with phosphorus atoms in the p'' configuration are considerably more efficient in reacting with nucleic acids compared to the corresponding isomers with the phosphorus atoms in the p' configuration. This fact should be taken into account in the designing of phosphotriester oligonucleotide derivatives for the specific inhibition of certain nucleic acid functions.

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