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Synthesis and properties of cross-linked DNA duplexes

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Abstract A method has been devised to synthesize DNA duplexes with covalently connected strands. The structure of cross-linked duplexes was confirmed by a reaction with the restriction endonuclease *AluI*. The thermal stability of the resulting compounds was investigated.

Key words: 2'-Deoxy-2'-aminouridine; Modified oligonucleotides; Cross-linked duplexes

1. Introduction

Oligonucleotides can be covalently bound to a wide variety of molecules. A principal advantage of chemical modification of nucleic acids by reactive oligonucleotide derivatives is that they irreversibly cross-link the latter to the target, thus forming a stable duplex [1]. Covalently closed or cyclic oligonucleotides are able to form stable duplexes (dumbells) having natural nucleotides or non-nucleotide bridges in loop [2].

In our research covalent linkage between two strands of DNA duplexes is organized between 2'-amino function of 2'-amino-2'-deoxyuridine which was introduced in the one chain of the duplex and the carboxyl group located on the non-nucleoside insert of the other chain through the agency of a water soluble carbodiimide condensing agent [3].

2. Materials and methods

5'-*O*-(4,4'-Dimethoxytrityl)-*N*-acyl-2'-deoxynucleoside-3'-*O*-(β-cyanoethyl-*N*,*N*-diisopropylamido)phosphites were obtained from Applied Biosystems. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride from Sigma was used. *AluI* endonuclease (50 U/μl) was purchased from MBI Fermentas (Lithuania).

2.1. Synthesis and purification of oligonucleotides

Oligonucleotides were synthesized, deprotection was performed as described in [3]. Analyses of reaction mixtures and isolation of oligonucleotides, containing dimethoxytrityl group, were done by reversed phase HPLC as described earlier [5].

The carboxylation of the amino-containing oligonucleotides was performed as described in [3].

2.2. UV melting experiments

Absorbance vs. temperature profiles were recorded at 260 nm on Hitachi 150-20 spectrophotometer (Japan) equipped by thermostated cells. The duplex concentration was 10^{-4} M per monomer. The temperature was scanned at a heating rate of 0.5°C/min.

2.3. Cross-linking reaction

The cross-linking reaction was carried out in 0.05 M morpholinethylsulphonate buffer, pH 5.0, 20 mM $MgCl_2$. Oligonucleotides were mixed in stoichiometric ratio. 0.1 A_{260} units both oligonucleotides were dissolved in 40 μl buffer, the mixture was heated to 95°C, then cooled slowly to room temperature and 40 μl of 0.5 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in the same buffer was added. The reaction proceeded for 48 h at 20°C and the oligonucleotides were precipitated and analysed as described in [3].

Labeling and gel electrophoresis were performed as described in [6].

2.4. Interaction of DNA duplexes with the restriction enzyme

Hydrolysis of DNA duplexes by restriction endonuclease *AluI* was performed in 10 μl of the buffer containing 0.01 M Tris-HCl, 0.015 M $MgCl_2$, 0.15 M NaCl, 0.001 M dithiothreitol, 0.1 mg/ml bovine serum albumin. The duplex concentration was the same as in melting experiments. 25 U *AluI* were incubated with the corresponding duplexes for 2 h at 37°C. The reaction was stopped by heating at 95°C for 2 min. The reaction products were analysed by denaturing 20% PAGE.

3. Results and discussion

Two systems composed of 25-mers were under investigation. Duplex (A) contains the recognition site of *NFkB* transcription factor (Scheme 1). Duplex (B) contains mutated site of *NFkB* factor and recognition site of *AluI* restriction endonuclease (Scheme 1).

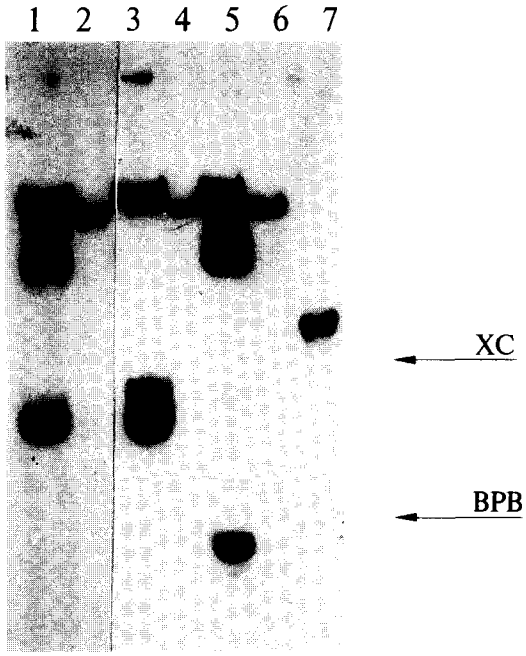

Fig. 1. Gel electrophoresis analysis (20% PAAG, 7 M urea) of cross-linked DNA duplexes cleavage by restriction endonuclease *AluI*. Lanes: 1, 3, 5 = cleavage of duplexes $\mathbf{J^{cl}}$, $\mathbf{H^{cl}}$ and $\mathbf{G^{cl}}$; lanes 2, 4, 6, 7 = duplexes $\mathbf{J^{cl}}$, $\mathbf{H^{cl}}$ and $\mathbf{G^{cl}}$, 25-mer oligonucleotide (controls).

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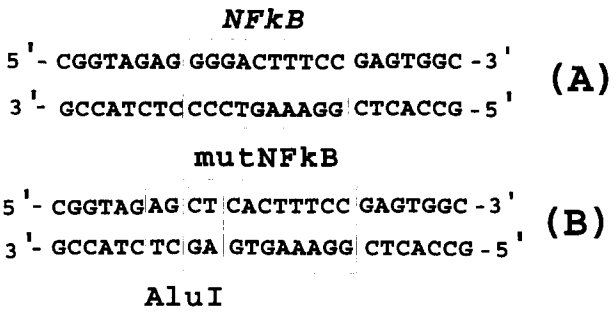
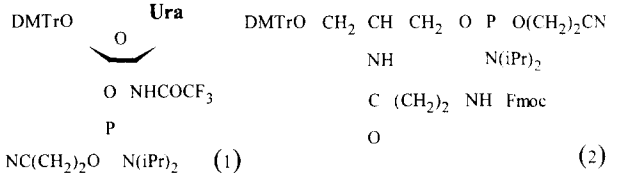


Scheme 1.

Modified oligonucleotides were synthesized using phosphoramidites **1** and **2** following standard phosphoramidite procedure (see Scheme 2 and Table 1).

The carboxyl group was introduced into appropriate amino-containing oligonucleotides by the reaction with succinic anhydride (Table 1).

Optimal reciprocal arrangement of the interacting groups was determined by molecular modelling. It was shown that modifications should be opposite each other or should be displaced one according the other for two nucleoside residues. In all cases the reaction between strands of duplexes was carried out using water soluble carbodiimide condensing agent (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride). The reaction mixtures were analysed and the aimed products were purified by ion-pair HPLC and electrophoresis in 20% denaturing PAAG. The results of the cross-linking reaction are presented in the Table 1.



Scheme 2.

The data of thermal stability of initial and cross-linked duplexes studied by UV-spectroscopy are displayed in the Table 1. Modified duplexes were marked by a decrease in thermal stability as compared to native duplexes in about 7°C. This fact is due to the destabilizing action of the modification induced, as well as to the elimination of the heterocyclic base in the site of introduction of the nonnucleoside insert.

The melting temperature for the cross-linked duplexes increased and proved to exceed not only that for the modified duplexes, but also that for original ones. Such increase of thermal stability for modified duplexes confirms the presence of the covalent linkage between two strands.

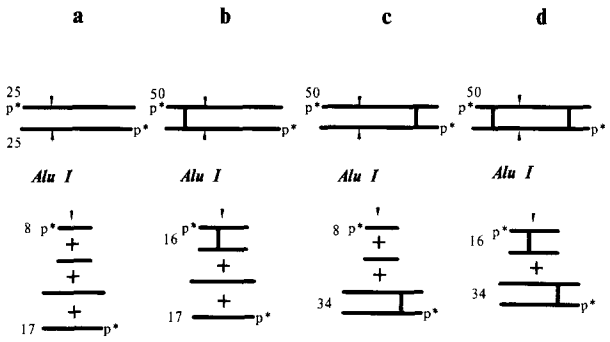
When comparing these results with the previously obtained data (the yield of cross-linked duplex was 26% when the melting temperature of initial duplex was 37°C [3]) it became obvious that the yield of aimed product depends on the stability of duplex-precursor. If the melting temperature of initial duplexes is about 80°C, the yield of cross-linked compounds reaches 80% (see Table 1).

The displace of modifications in the chains of duplexes one according the other for two nucleoside residues (Table 1, du-

Table 1
The description of the oligodeoxyribonucleotide duplexes used

The initial duplexes			The cross-linked duplexes		
Abbreviation	Sequences	Tm, °C	Abbreviation	Yield of the product, %	Tm, °C
A	5′-CGGTAGAGGGGACTTTCCGAGTGGC-3′ 3′-GCCATCTCCCCTGAAAGGCTCACCG-5′	77	–	–	–
B	5′-CGGTAG ***AGCT*** CACTTTCCGAGTGGC-3′ 3′-GCCATC ***TCGA*** GTGAAAGGCTCACCG-5′	78	–	–	–
C	5′-CGGTAGAGGGGACTTTCCGA N^c TGGC-3′ 3′-GCCATCTCCCCTGAAAGGCT U^n ACCG-5′	74	C^{cl}	52	84
D	5′-CGGT N^c GAGGGGACTTTCCGAGTGGC-3′ 3′-GCCA U^n CTCCCCTGAAAGGCTCACCG-5′	74	D^{cl}	82	85
E	5′-CGGT N^c GAGGGGACTTTCCGA N^c TGGC-3′ 3′-GCCA U^n CTCCCCTGAAAGGCT U^n ACCG-5′	70	E^{cl}	67	87
F	5′-CGGTAG N^c GGGGACTTTCCGAGTGGC-3′ 3′-GCCA U^n CTCCCCTGAAAGGCTCACCG-5′	75	F^{cl}	84	88
G	5′-CGGTAG ***AGCT*** CACTTTCCGA N^c TGGC-3′ 3′-GCCATC ***TCGA*** GTGAAAGGCT U^n ACCG-5′	72	G^{cl}	40	85
H	5′-CGGT N^c G ***AGCT*** CACTTTCCGAGTGGC-3′ 3′-GCCA U^n C ***TCGA*** GTGAAAGGCTCACCG-5′	73	H^{cl}	75	86
J	5′-CGGT N^c G ***AGCT*** CACTTTCCGA N^c TGGC-3′ 3′-GCCA U^n C ***TCGA*** GTGAAAGGCT U^n ACCG-5′	69	J^{cl}	62	88

U^n = 2′-amino-2′-deoxyuridine; N^c = nonnucleoside insert with carboxyl group.



Scheme 3.

plex F) does not influence considerably on efficiency of the reaction.

During cross-linking reaction in the case of duplex with two points of modification three products are identified.

The fact, we have obtained cross-linked duplexes is possible to illustrate by reaction of restriction endonuclease *Alu*I with original and modified duplexes as controls. In the process of endonuclease degradation of native duplex, obtained from two 25-mers, two 8-mers and two 17-mers appear (Scheme 3a). If both oligonucleotides of duplex contain ^{32}P-label, two cross-linkages can be observed on autoradiograph corresponding to 8-mer and 17-mer.

The composition of the reaction mixture after *Alu*I cleavage is determined by reciprocal arrangement of restriction site and cross-linking. A PAGE autoradiograph of enzymatically cleaved products for duplex $\mathbf{G}^{cl}$, $\mathbf{H}^{cl}$, $\mathbf{J}^{cl}$ is presented on Fig. 1. These data correspond to the Scheme 3, and confirm the presence of cross-links in presented duplexes. In the case of duplex $\mathbf{H}^{cl}$ (the both chains are labeled) there are 16-mer and 17-mer fragments on autoradiograph (Scheme 3b; lane 3, Fig. 1). The results of duplex $\mathbf{G}^{cl}$ cleavage are 8-mer and 34-mer oligonucleotides (Scheme 3c; lane 5, Fig. 1), and of duplex $\mathbf{J}^{cl}$ – 16-mer and 34-mer (Scheme 3d; lane 1, Fig. 1).

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