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N-(2-Hydroxyethyl)phenazinium derivatives of oligonucleotides as effectors of the sequence-specific modification of nucleic acids with reactive oligonucleotide derivatives

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It has been found that mono- and especially diphenazinium derivatives of oligonucleotides complementary to the DNA sequence adjacent to the target sequence of the addressed alkylation of DNA, significantly enhance the extent and specificity of alkylation with *p*-(*N*-2-chloroethyl-*N*-methylamino)benzylamide derivatives of the addressing oligonucleotides, thus playing the role of effector of the sequence-specific (complementary addressed) modification.

Sequence-specific alkylation; Phenazinium oligonucleotide derivative; Effector

1. INTRODUCTION

Modification of nucleic acids in the vicinity of definite sequences with reactive derivatives of oligonucleotides complementary to these sequences was proposed over 20 years ago by N.I. Grineva as a general approach to sequence-specific ('complementary addressed') modification of the nucleic acid strands [1–3]. Most of the investigation was carried out using derivatives bearing the *N*-2-chloroethyl-*N*-methylaminophenyl (ClR) group. These derivatives were shown to alkylate single-stranded DNA fragments in the regions adjacent to the target sequences [4,5]. However, definite indications were obtained that reaction may proceed to some extent in the regions of incomplete complementarity of the target sequences and oligonucleotide moiety (address) of the reagent [6]. Obviously the variety of such regions should increase with the growth of the length of the addressing moiety. At the same time short oligonucleotides do not form duplexes of sufficient stability and the extent of modification by their derivatives is low [5]. The reaction yield may be enhanced by binding some duplex stabilizing radical, e.g. phenazinium to the terminal residue of the addressing oligonucleotide opposite the location of the reactive group [5,6].

In the present paper we describe another approach to enhancement of the extent and specificity of the sequence-specific alkylation of single-stranded DNA with reagents having short oligonucleotide address. It is based upon the lengthening of the double-stranded region formed by DNA and reagent with 'effector' oligonucleotide complementary to the DNA sequence adjacent to the target one. To make the nicked duplex formed more stable, phenazinium derivatives of the effector oligonucleotide were used.

2. MATERIALS AND METHODS

A single-stranded DNA fragment was obtained and ^{32}P-labeled at the 3′ end as described [5]. Oligonucleotide derivatives **I–VI** used are presented in fig.1. Starting

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oligonucleotides containing either 5′-phosphate (for the preparation of reagents **I, II** and derivatives **III** and **V**) or both 3′- and 5′-phosphates (for the preparation of reagents **V** and **VI**) were prepared by the solid phase phosphoramidite procedure using automated synthesator Victoria 4M, USSR [7]. Terminal monophosphate groups were introduced by conversion of uridine-5′-phosphate residues by periodate oxidation and subsequent β-elimination as described [8]. To prepare oligonucleotide containing these residues $(iPr)_2N\text{-}P(OCH_3)\text{-}OU(Bz)_7$ was used at the last step and (DMTr)U(Ac) at the first step of the oligonucleotide synthesis. The structure of oligonucleotides was confirmed by the Maxam-Gilbert procedure [9]. *N*-hydroxyethylphenazinium (Phn) and *p*-(*N*-2-chloroethyl-*N*-methylamino)benzylamide ($ClRCH_2NH$) residues were attached to phosphates using the phosphate activation procedure described in [6,10]. Concentration of the derivatives used was measured spectrophotometrically, taking molar extinction as the sum of molar extinctions of oligonucleotide and respective radicals ($\epsilon_{260} = 14.7 \times 10^3\ M^{-1}\ cm^{-1}$ for Phn and $\epsilon_{260} = 10 \times 10^3\ M^{-1}\ cm^{-1}$ for $ClRCH_2NH$). Alkylation conditions and treatment of the specimens are described in the legends to figs 2 and 3.

3. RESULTS

Single-stranded DNA fragment 303 nucleotides

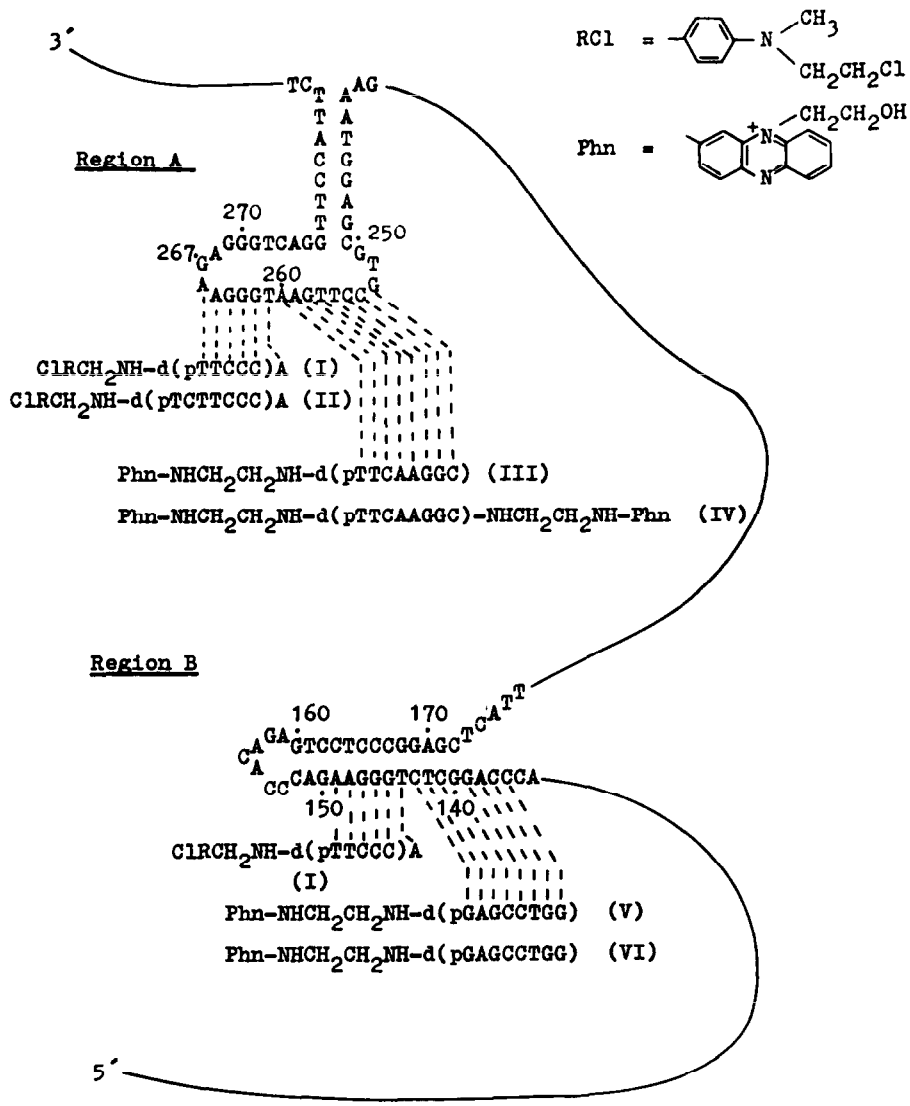

Fig.1. The structures of the target regions of the DNA fragment, reagents and effectors used. Broken lines represent tentative hydrogen bondings.

in length described in [5] was used in this investigation. The fragment contains two identical oligonucleotide sequences TGGGAA in positions 144–149 and 261–266. The regions adjoining these sequences and their tentative secondary structure estimated according to [11] are presented in fig.1, as well as the reagents used and phenazinium

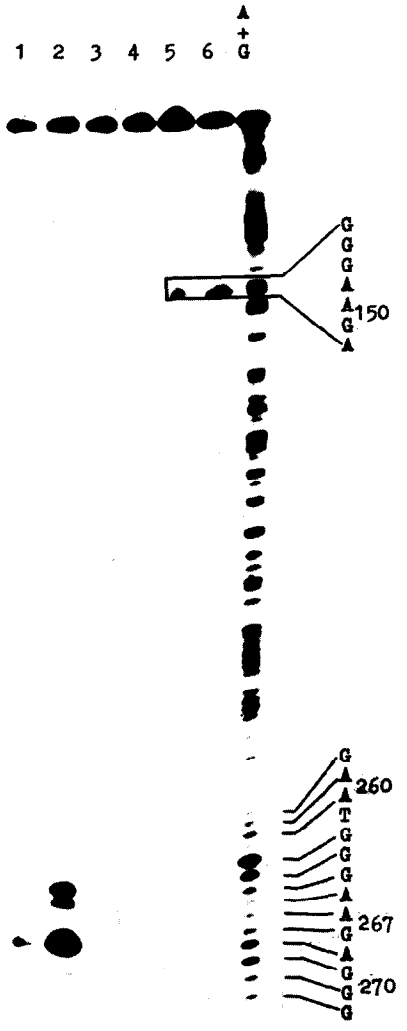

Fig.2. Autoradiogram of the 8% denaturating polyacrylamide gel-electrophoresis of 3′ ^{32}P-labeled DNA fragment modified by reagent **I** after 10% piperidine treatment at 100°C for 30 min. Modifications were carried out in 0.16 M NaCl, 0.1 mM EDTA, 0.02 M Na_2PO_4, pH 7.4, t = 37°C, time = 18 h, [DNA] = 1×10^{-8} M, [**I**], [**III**], [**IV**], [**V**], [**VI**] = 1.7×10^{-5} M). Reaction probes additionally contained the following: lane 1: **I** and **III**; lane 2: **I** and **IV**; lane 3: none; lane 4: **I**; lane 5: **I** and **V**; lane 6: **I** and **VI**.

oligonucleotide derivatives tried as potential effectors. The latter contain oligonucleotide moieties complementary, respectively, to sequences 136–143 and 253–260 adjacent upstream to TGGGAA fragments.

No alkylation was observed at 37°C after treatment of 10^{-8} M fragment with 1.7×10^{-5} M of reagent **I** (fig.2, lane 4). Addition to the same reaction mixture of an equimolar amount of **III** resulted in the appearance of a slight alkylation of G^{267} (fig.2, lane 1). Addition of the same amount of **IV** resulted in significant alkylation of G^{267} and some alkylation of G^{263} and G^{264} (fig.2, lane 2). No reaction was observed in this region upon addition of **VI**. In the presence of the latter diphenazinium derivative definite alkylation in the oligopurinic region $G^{145}GGAAGA^{151}$ was also found (fig.2, lane 6). Modification level calculated as the ratio of radioactivity of the spots in the modification area to the sum of the counts of these spots and the spot at the start for diphenazinium derivatives **III** and **VI** were found to be 45 and 25%, respectively. Being related to the total radioactivity of the gel these values are significantly lower (14 and 5%, respectively).

The quantitative dependence of the modification extent on the effector concentration was studied using reagent **II** and monophenazinium derivative **III**. In this case alkylation proceeded mainly at G^{269}. The results are presented in fig.3. It is seen that at a concentration of reagent **II** insufficient for any measurable alkylation in the absence of effector nearly complete alkylation may be achieved by increase of the effector concentration up to 10^{-4} M.

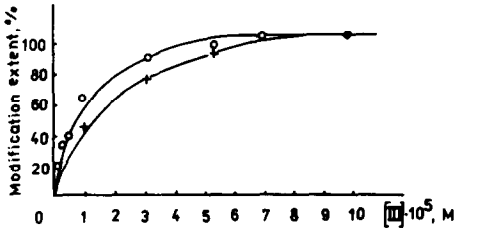

Fig.3. The dependence of the DNA fragment modification extent by reagent **II** on concentration of effector **III** [DNA] = 1×10^{-8} M, [**II**] = 1×10^{-5} M, t = 25°C (○), 37°C (×). The modification extents were calculated as the ratio of radioactivity of the spot of product to the sum of radioactivities of the spots of product and the unchanged DNA fragment.

4. DISCUSSION

It is shown that the modification yield and specificity of the complementary addressed alkylation of DNA with oligonucleotide derivatives containing aromatic 2-chloroethylamino group may be increased significantly by phenazinium oligonucleotide derivatives lengthening the duplex formed by addressing part of the reagent and target sequence of DNA. Thus these derivatives may be considered as effectors (activators) of such modification. Specificity is enhanced since both complete and incomplete complexes formed by effector outside the target region, do not lead to modification due to the absence of reactive group, whereas complexes formed by reagent outside this region are insufficiently stable to result in measurable extent of alkylation due to the absence of adjacent effector. It is noteworthy that addition of effectors **V** and **VI** permitted alkylation of guanines to be carried out within the stem of tentative hairpin which never were found to be modified by previously studied reagents [5]. Therefore, it may be expected that the use of effectors would permit sequence-specific modification to be performed within double-stranded regions of DNA molecules. Although demonstrated for alkylation, the approach does not seem to depend on the nature of the reactive group and we believe that it will be useful for the other types of reactions used for the sequence specific modification of nucleic acids.

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