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SEQUENCE-SPECIFIC MODIFICATION OF DNA FRAGMENTS BY OLIGONUCLEOTIDE DERIVATIVES CONTAINING ALKYLATING GROUPS AT THE C5 POSITION OF DEOXYURIDINE.

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Abstract. DNA fragments are modified with high efficiency (80-90%) by alkylating derivatives of oligonucleotides bearing 4(N-2-chloroethyl-N-methylamino)benzyl group at the C5 atom of the terminal or internal deoxyuridine residue. Target modification at a single point is achieved.

Reactive oligonucleotide derivatives complementary to some sites of the target nucleic acids can modify them site-specifically and probably will be used as therapeutic drugs. In the reagent-target complex the reactive group is close to some nucleotide residues of the target and results in their chemical modification. The reagents bearing different groups attached to terminal phosphate or ribose residue are the most frequently used[1-4]. New publications concerning the oligonucleotide derivatives, with reactive moieties in a heterocyclic base of one of the nucleosides, have also appeared. Thus, reagents carrying bromo- or iodoacetamide groups attached through different spacers to the C5 position of deoxyuridine were suggested. Such groups are spatially close to the target and can efficiently strike it at definite point[5,6].

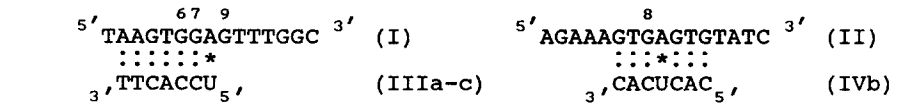
The most complete information about the sequence-specific modification was obtained for the alkylating reagents with 4(N-2-chloroethyl-N-methylamino)benzyl (RCl) group attached to 5'-phosphate (5'-reagents) or to the 2',3'-cis-diol group of ribose (3'-reagents)[1,4]. The sites and the efficiency of modification depend on the place of the alkylating group attachment. In the case of 5'-reagents, the target base adjacent to the RCl group which is not involved in duplex formation, is preferentially modified. Mostly the third

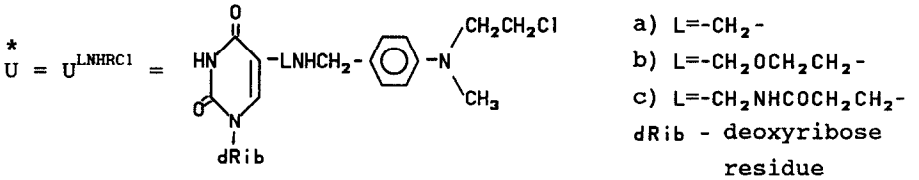
Only deoxyderivatives are used in this report, therefore the prefix "d" in the symbols of deoxyribonucleosides and oligonucleotides is omitted.

nucleotide of the target across from the reagent 3′-end which is not involved in complex formation is subjected to modification with 3′-reagents. As a rule, some neighboring bases are also modified in addition to the above mentioned main sites of the target[7-9].

Previously[10] we suggested a method of synthesis for alkylating oligonucleotide derivatives bearing the RCl group attached to the C5 atom of deoxyuridine through spacers of various lengths. This method provides the possibility of obtaining an alkylating reagent with a reactive group in any position of the oligonucleotide chain. For this purpose, oligonucleotides, containing an aliphatic amino group at the C5 atom of deoxyuridine residue, were synthesized preliminarily. The RCl group was attached to the amino spacer afterwards. Such reagents were expected to effectively modify the target at a determined point. It was shown[10] that the presence of the RCl group in a 5′-terminal U residue did not change the oligonucleotide ability for duplex formation.

In this communication the site-specific modification of DNA-fragments (I) and (II) by oligonucleotide derivatives containing modified deoxyuridine residue (U^{LNHRCl}) either on 5′-end (reagents IIIa-c) or in the middle of a chain (reagent IVb) was studied.





The modification of ^{32}P-labelled targets (I) and (II) was carried out at 22°C for 48 hours (this time was sufficient to practically complete hydrolysis of C-Cl bond in RCl[11]). As shown earlier[10], the melting temperature of duplexes formed by oligonucleotide (I) and reagents (IIIa-c), was 24-26°C. The reaction mixtures were analyzed by gel electrophoresis (8 M urea, 0.05 M Tris-borat, pH 8.5, 1 mM EDTA). Autoradiograms were quantified with Ultroscan XL densitometer (LKB Bromma, Sweden). The dependence of the efficiency and the points of modification on spacer length (L) was studied using reagents (IIIa-c) in various ratios to target (I) (1:1 and 10:1). Molar extinction coefficients of oligonucleotides (ε_{260}) were estimated using the values

of ε for dinucleotides[12]. For compounds (IIIa-c) and (IVb) the extinction coefficients were calculated as the sum of ε_{260} values for RCl residue ($14.7 \cdot 10^3$ $M^{-1}sm^{-1}$)[13] and unmodified oligonucleotides.

The modification resulted in the formation of cross-linked reagent-target products with mobilties lower than the mobility of the initial unmodified target (Fig.1). The extent and points of modification (Fig.2) were determined by the data of gel electrophoresis after the cleavage of the target at the alkylated purine residues (10% aqueous piperidine, 100°C, 30 min). When the reagent-target ratio was 1:1, the extent of modification was about 60% and reached 80-90% at 10 fold excess of reagents (IIIa-c). In the latter case, the ratio of extents of modification of G6:G7:G9 was approximately 2:1:3 and 1:2:1 for reagents (IIIa) and (IIIb), respectively. For compound (IIIc) the alkylation occured mainly at G6 residue.

Fig.1. Autoradiogram of the gel. Lanes 1-3 represent the reactions of target [5'-^{32}P]pTAAGTGGAGTTTGGC (I) with reagents (IIIa-c); lane 4, target (I). The concentration was $1 \cdot 10^{-6}$M for the target and $1 \cdot 10^{-5}$M for the reagents; buffer, 0.16 M NaCl/ 0.02 M Na_2HPO_4/ 0.1 mM EDTA, pH 7.4; 22°C; 48 h.

The modification of DNA fragments, by alkylating reagent with the RCl group attached to U residue in the middle of the oligonucleotide chain, was investigated using target (II) and reagent (IVb). The extent of modification determined after piperidine treatment of the reaction mixture was 83% with the G8 base being alkylated predominantly. Only weak modification (<5%) of the other G residues, located near the alkylating group, was observed (Fig.3). Similar results were previously obtained[5] where the alkylation of the target, using a reagent with iodoacetamide group in the middle of the oligonucleotide chain, occured with high yield at a single G residue.

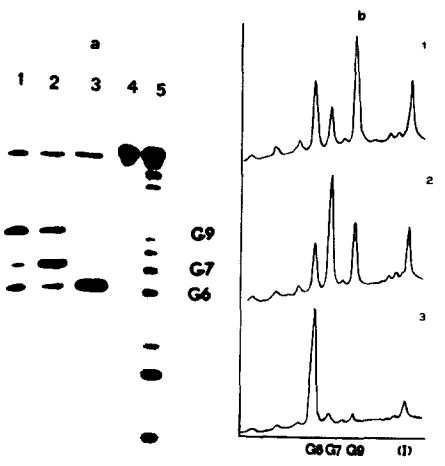


Fig.2. Autoradiogram of the gel (a) and its densitograms (b). Lanes 1-3 represent the reactions of target (I) with reagents (IIIa-c), respectively, after piperidine treatment of the reaction mixtures; lane 4, target (I); lane 5, A+G sequencing reaction. Modification conditions are given in the legend to Fig.1.

Comparing the data obtained by the modification of targets (I) and (II) by reagents (IIIb) and (IVb) having alkylating groups with the same spacer (Fig.2, lane 2 and Fig.3, lane 2), it can be concluded that deoxyguanosine residue adjacent to the RCl group near the 5′-end of the target is modified more. In the case of reagent (IVb), where the RCl group is attached in the middle of the oligonucleotide chain, such G residue is the main point of modification. When the spacer is longer (derivative IIIc), the second G base towards the 5′-end of the target relative to U^{LNHRCl} residue (G6 in target I) is preferentially alkylated.

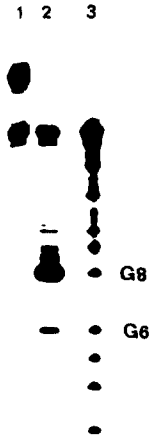


Fig.3. Autoradiogram of the gel. Lanes 1 and 2 represent the reaction of target [5′-^{32}P]pAGAAAGTGAGTGTATC (II) with reagent (IVb) before and after piperidine treatment, respectively; lane 3, A+G sequencing reaction. The reaction conditions are given in the legend to Fig.1.

The extent of modification of the target does not depend on a combination of the length of the spacer (L) and the position of the U^{LNHRCl} residue. This feature of alkylating reagents makes them different from the photoreagents with the perfluoroarylazido group[14]. Thus, the extent of modification of the target (I) by the reagents U^{LNHQ}CCACTT (spacers L are the same as for RCl reagents, Q=-CO-C_6F_5-N_3) is only 35% for L=-CH_2- and 70% for the two others L. Oligonucleotide reagents, with the photoactive group in the middle of the chain, modify the target with lower efficiency, in contrast to derivatives with such group at the end of the chain. Besides, in the case of target (II) and reagent CACU^{LNHQ}CAC both G8 and G10 residues are modified[14].

The results obtained lead to a conclusion that alkylating oligonucleotide derivatives, carrying RCl group at deoxyuridine residue, can be effective for sequence-specific nucleic acid modifications. These reagents can provide highly effective modification of the target in a definite point. This is not usually the case for reagents with RCl residue attached to 5′-phosphate or 2′,3′-cis-diol group of the ribose. The method of introducing an alkylating group into oligonucleotide yields to obtain efficient and accurate reagents with the alkylating group in the middle of the chain.

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