Complementary addressed modification of oligonucleotide d(pGpGpCpGpGpA) with platinum derivative of oligonucleotide d(pTpCpCpGpCpCpTpTpT)

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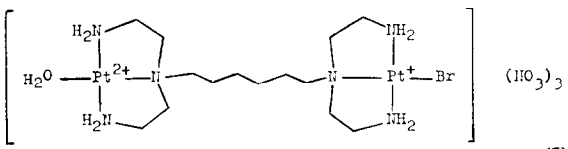
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New heterobifunctional reagent $[BrPt(dien)-(CH_2)_6-(dien)Pt(H_2O)]^{3+}$ (I) (–dien = diethylenetriamine residue) is proposed for preparation of complementary addressed reagents, reactive derivatives of oligonucleotides and polynucleotides. Two reactive groupings of (I) bind to oligonucleotides at different rates due to the higher reactivity of $-[(dien)Pt(H_2O)]^{2+}$ as compared to $-[(dien)Pt\ Br]^{+}$. Using (I), bromodiethylenetriaminoplatinum group was attached to the oligonucleotide d(pTpCpCpGpCpCpTpT-pT) (II) by rapid reaction of aquatriaminoplatinum group of (I) with guanosine residue of (II). The reactive oligonucleotide derivative III thus obtained was shown to modify predominantly 5′-terminal guanosine residue in the complementary oligonucleotide d(pGpGpCpGpGpA) (IV).

Affinity modification *Platinum reagent* *Cross-linked oligonucleotide*

1. INTRODUCTION

Directed modification of single-stranded nucleic acids with reactive derivatives of oligonucleotides (complementary-addressed modification [1,2]) is a promising approach for derivatization of certain sequences in nucleic acids. The most efficient methods of preparation of these reactive derivatives consist in modification of oligonucleotides and polynucleotides with heterobifunctional reagents having two chemical functions which are different in specificity or in reactivity [3–7]. Here, we report the use of new heterobifunctional reagent $[BrPt(dien)-(CH_2)_6-(dien)Pt(H_2O)](NO_3)_3$ (I):



(I)

for attachment of reactive platinum group to oligonucleotide:

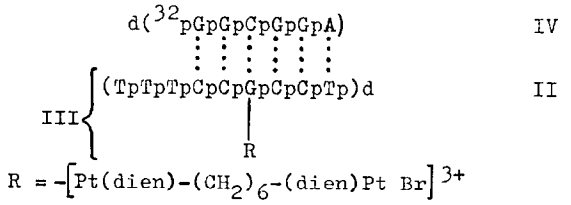
d(pTpCpCpGpCpCpTpTpT) (II)

by modification of guanosine residue in oligonucleotide II (reaction A). The obtained:

[bromo-*N,N,N′,N′*-tetrakis-(2-aminoethyl)-1,6-hexamethylenediamine]diplatinum derivative (III)

was used for modification (reaction B) of the complementary oligonucleotide:

d(pGpGpCpGpGpA) (IV)



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2. MATERIALS AND METHODS

Oligonucleotides II and IV were synthesized as in [8,9] and purified by column chromatography on Nucleosil 5 C18 from Macherey-Nagel (Düren). 5′-^{32}P-Labelling of oligonucleotides was done by polynucleotide kinase exchange reaction [10]. Concentrations of oligonucleotide solutions were determined spectrophotometrically at pH 7 at ionic strength 0.1 M. Calculated according to [11], molar extinction coefficients at 260 nm were $\epsilon_{II} = 6.54 \times 10^4\ M^{-1}.cm^{-1}$ and $\epsilon_{IV} = 6.08 \times 10^4\ M^{-1}.cm^{-1}$.

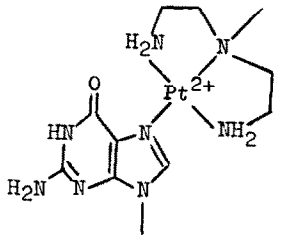
A solution of 1 mM reagent I was prepared as in [7]. Platinum derivative III was prepared by incubation of equal volumes of 100 μM oligonucleotide II solution and 80 μM reagent I solution in water at 20°C (reaction A). At time intervals aliquots of reaction A mixture were analysed by DEAE-cellulose micro-column chromatography [13] in a linear gradient of 0.1–0.3 M KBr containing 7 M urea, 10 mM CH_3COOH-NaOH (pH 5.5). In the cross linking experiments (reaction B) 1.5-fold excess of 150 μM ^{32}P-labelled oligonucleotide IV was added directly to the reaction A mixture incubated for 1 min. The reaction B solution containing 0.1 M $NaClO_4$, 10 mM HEPES (pH 7.1) was incubated at 20°C.

^{32}P-Labelled oligonucleotides and platinum oligonucleotide derivatives were analysed by gel-electrophoresis on polyacrylamide gels (20% acrylamide + 0.5% bis-acrylamide) containing 7 M urea, 50 mM Tris-borate (pH 8.3), 2.5 mM EDTA and were isolated from gels by electroelution on the DEAE-papers Whatman DE 81 from Whatman (Maidstone). Purine-specific partial fragmentation of oligonucleotides was done by treatment with 2% diphenylamine in 66% formic acid for 2 h at 20°C [12].

Melting of the complex of 25 μM oligonucleotides II and IV in 0.1 M $NaClO_4$, 10 mM HEPES (pH 7.1) was studied with a Beckman DU-8 spectrophotometer using a temperature-controlled cell.

3. RESULTS AND DISCUSSION

Bis-platinum compound I is a heterobifunctional reagent for the H_2O molecule and the bromide ions coordinated to platinum differ in their ability to participate in nucleophilic substitution reactions [7]. The chelating diethylenetriamine residue does not participate in exchange reactions. It is known that the acidodiethylenetriaminoplatinum groups attack primarily the N7 atom of guanine residues in nucleic acids [14]. This platination of guanine residue does not interfere with its ability for complementary interactions [15].



It was found that reagent I modifies oligonucleotide II at pH 5 (reaction A) with its high reactive group $-[(dien)Pt(H_2O)]^{2+}$ and converts oligonucleotide II with 95% yield to the platinum derivative III having negative charge −6 due to the positively charged platinum grouping $-[Pt(dien)-(CH_2)_6-(dien)Pt\ Br]^{3+}$ (fig.1). Fig.2a shows the kinetic curve of reaction A. The

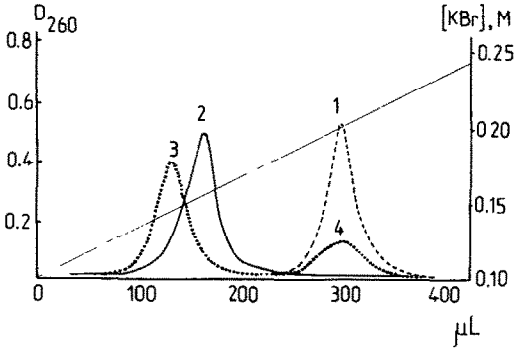


Fig.1. DEAE-cellulose microcolumn chromatography of: nonanucleotide II, d(pTpCpCpGpCpCpTpTpT) (1); hexanucleotide IV, d(pGpGpCpGpGpA) (2); and reaction A mixture of $[BrPt(dien)-(CH_2)_6-(dien)Pt-(H_2O)]^{3+}$ (I) with nonanucleotide II after 1 min incubation in water (pH 5) at 20°C (3,4) (3, platinum oligonucleotide derivative III; 4, unreacted nonanucleotide II). Conditions of chromatography: linear gradient 0.1–0.3 M KBr, 7 M urea, 10 mM CH_3COOH–NaOH (pH 5.5).

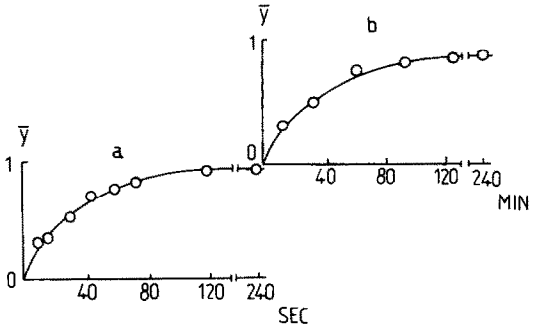


Fig.2. Kinetics of reactions of the heterobifunctional reagent I, $[BrPt(dien)-(CH_2)_6-(dien)Pt(H_2O)]^{3+}$, with oligonucleotides: (a) the extent of monofunctional attachment of the reagent I (40 μM), to the oligonucleotide II, d(pTpCpCpGpCpCpTpTpT) (50 μM), in water (pH 5) at 20°C (reaction A) measured by absorbance at 260 nm of the peak 3 (fig.1) after ion-exchange chromatography as function of time; (b) the extent of crosslinking between the platinum oligonucleotide derivative III (25 μM) and oligonucleotide IV, d(^{32}pGpGpCpGpGpA) (37.5 μM), in 0.1 M $NaClO_4$, 10 mM HEPES (pH 7.1) at 20°C (reaction B) estimated from radioactivity of the product V band (fig.3 (2)) after gel-electrophoresis as function of time; $\bar{y}$, quotas of reacted aqua- (a) and bromo- (b) diethylenetriaminoplatinum groups of reagent I.

oligonucleotide derivative III is stable under conditions of partial purine-specific splitting in accordance with the G-specificity of reaction A and with the known fact that platination of guanosine at N7 stabilizes its glycosyl linkage [14].

Platinum derivative III was used for modification of oligonucleotide IV (reaction B). This reaction was carried out at 20°C under conditions where the complementary complex of oligonucleotides II and IV melts at 28°C. It was found that the platinum derivative III reacts with oligonucleotide IV with formation of the single oligonucleotide crosslinked product V (85% yield) which can be isolated by gel-electrophoresis under denaturing conditions (fig.3 (2)). The kinetic curve of reaction B is shown in fig.2b. In the first experiments modification of oligonucleotide II was done with the purified platinum derivative III. It was purified by DEAE-cellulose column chromatography in the presence of KBr preserving the reactive bromotriaminoplatinum group [7] and desalted by gel filtration. However, it was found that this purification step can be omitted. Direct treatment of the oligonucleotide IV with the mixture of reaction A products containing the platinum derivative III resulted in the same extent of oligonucleotide crosslinking.

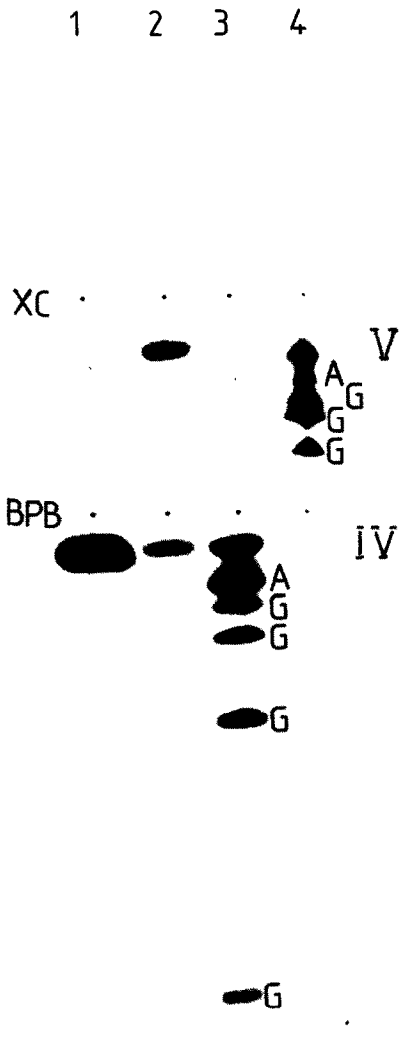


Fig.3. Autoradiograph of the polyacrylamide gel of oligonucleotide analysis experiments: (1) d(^{32}pGpGpCpGpGpA) (IV); (2) reaction B mixture of the platinum oligonucleotide derivative III with 1.5-fold excess of d(^{32}pGpGpCpGpGpA) (IV) after 2 h incubation; (3) purine-specific partial splitting of d(^{32}pGpGpCpGpGpA) (IV); (4) purine-specific partial splitting of the oligonucleotide crosslinked product V containing d(^{32}pGpGpCpGpGpA) (IV); XC and BPB mark the positions of xylene cyanol and bromphenol blue, respectively.

To identify positions of platinated guanosines in the oligonucleotide IV modified with platinum derivative III, the product of crosslinking was subjected to purine-specific splitting by formic acid/diphenylamine treatment. Purine-specific splitting of d(^{32}pGpGpCpGpGpA) (IV) gave the corresponding radioactively labelled oligonucleotide fragments which were analysed by gel-electrophoresis (fig.3 (3)). These fragments did not appear in the case of splitting of product V under similar conditions (fig.3 (4)). This indicates that the predominant platination site in the oligonucleotide IV is the 5′-terminal guanosine residue (G1) in accordance with the stability of platinated guanosine residue under this splitting procedure. The ethanol precipitation steps used in the procedure do not guarantee the recovery of ^{32}P which could appear in the case of splitting of oligonucleotide IV at G1. Therefore one can not draw definite conclusions about platination of G2. Analysis with the CPK-atomic models showed that both guanosine residues G1 and G2 are available for the reactive platinum group in the B-structured complementary complex of the oligonucleotide IV with the oligonucleotide derivative III. G3 and G4 are poorly available for the platinum reactive group in this complex.

The observed site-specificity of platination of oligonucleotide IV with the platinum derivative III is consistent with the intracomplex character of reaction B. It was found that the platinum derivative III does not react with d(^{32}pTpCpCpG-pCpCpTpTpT) (II) taken in 5-fold excess under the similar reaction B conditions. This demonstrates that intermolecular reaction of reactive oligonucleotide derivative with non-complementary oligonucleotide is slow or inefficient.

The results obtained show that the bis-platinum compound I is a promising heterobifunctional reagent for preparation of reactive derivatives of oligo- and polynucleotides. Procedure for preparation of oligonucleotide derivatives is simple and the efficiency of the complementary addressed modification with the platinum oligonucleotide derivative is high. It is worth noting that DNA modified with diethylenetriaminoplatinum group can not be repaired in vivo [16]. Therefore, oligonucleotide derivatives carrying reactive platinum groups of this type can be particularly useful for directed damage of certain sequences in nucleic acids in vivo.

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