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Role of nucleoside components and internucleotide phosphate groups of oligodeoxyribonucleotide template in its binding to human DNA polymerase α

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Affinity labelling of human placenta DNA polymerase α (EC 2.7.7.7) with the reactive oligodeoxyribonucleotide $d(pT)_2pC[Pt^{2+}(NH_3)_2OH](pT)_7$ was used for quantitative analysis of enzyme interaction with oligodeoxyribonucleotides as templates. Dissociation constants and Gibb's energy values for different oligothymidylates $d(pT)_nT$ where $n = 1$–14 have been evaluated by competitive experiments of these ligands with Pt^{2+} reagent. The data obtained prove the formation of one Me^{2+}-dependent electrostatic contact and a hydrogen bond between the enzyme and one phosphate of these templates. One may suppose that the hydrophobic interaction of any other monomeric link of oligodeoxyribonucleotides with the enzyme template site takes place.

DNA polymerase α; Affinity labeling; Oligodeoxyribonucleotide template; (Human placenta)

1. INTRODUCTION

Affinity modification has been used for the first time for evaluation of the affinity and type of binding of oligodeoxyribonucleotide templates to the template site of DNA polymerase in [1,2]. The oligonucleotide $d(pT)_2pC[Pt^{2+}(NH_3)_2OH](pT)_7$, which modifies selectively the template site of human placenta DNA polymerase α, was suggested as an affinity reagent. Using the latter the dissociation constants of complexes of the enzyme template site with oligodeoxyribonucleotides can be found from dependences of the enzyme inactivation rates on concentrations of the affinity reagent and the corresponding competitive oligodeoxyribonucleotides protecting the enzyme against inactivation.

Here, the above approach was applied to determine the affinity of oligodeoxythymidylates of different length and that of their analogs, ethylated via internucleotide phosphates, to the template site of DNA polymerase α from human placenta. The type of binding of oligodeoxyribonucleotides to the template site of DNA polymerase has been established.

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2. MATERIALS AND METHODS

Preparations of DNA polymerase α from human placenta (spec. act. of 8.5×10^3 U/mg) were obtained as in [1]. One unit corresponds to 1 nmol [^{3}H]dNTP incorporated into acid-insoluble materials in 1 h at 37°C. DNA from salmon sperm, dNTP, poly(dA) and poly(dT) were from Niktibav (USSR), $MgCl_2$ and $MnCl_2$ from Merck and bovine serum albumin (BSA) from Koch Light. [^{3}H]dTTP and [^{3}H]dATP with specific activities of 22×10^3 Ci/mol were from Izotop (USSR). The other reagents used were analytical grade.

Synthesis, identification of structures, deter-

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mination of purity and molar absorption coefficients of all oligodeoxyribonucleotides were as described in [1,3,4]. $d(pT)_2pC[Pt^{2+}(NH_3)_2OH](pT)_7$ was synthesized by treating $d(pT)_2pC(pT)_7$ with *cis*-aquohydroxydiamminoplatinum [1].

In experiments on inactivation of DNA polymerase α its activity was determined as in [1,2] at 30°C. The mixture (50 μl) contained 50 mM Tris-HCl buffer, pH 8.0, 2 mM $MgCl_2$, 0.3 mg/ml BSA, 2 A_{260}/ml nuclease activated DNA and 20 μM dNTP: ddATP, dCTP, dGTP, dTTP (dATP with specific activity equal to 1×10^3 Ci/mol). The reaction was started by adding 0.5–1.0 units enzyme.

DNA polymerase α was modified by $d(pT)_2pC[Pt^{2+}(NH_3)_2OH](pT)_7$ at 30°C by the known procedure [1,2]. The mixture (100 μl) contained 50 mM Tris-acetate buffer, pH 7.4, 0.12 mM $MnCl_2$, 0.5 mg/ml BSA and 10–15 units enzyme. The reaction was started by adding the affinity reagent. The reagent concentration was varied over a wide range. Aliquots of these mixtures were added to the mixture used for enzyme activity measurements. In experiments on determination of dissociation constants for competitive ligands (K_Y) the reagent concentration was 1 μM.

Dissociation constants of the complexes of the affinity reagent (K_X) and competitive unreactive ligands (oligodeoxyribonucleotides) (K_Y) with DNA polymerase were determined from the following affinity modification scheme:

$$E + X \rightleftharpoons EX \ (K_X)$$

$$EX \longrightarrow EZ \ (k)$$

$$E + Y \rightleftharpoons EY \ (K_Y),$$

where EX is the enzyme-affinity reagent complex; EZ is the product of modification; k is the rate constant of EX to EZ transformation; and EY is the enzyme-competitive ligand complex. For the treatment of experimental data we used a graphical plot of the form:

$$\frac{1}{k_{app}} = \frac{1}{k_{app}} + \frac{K_X}{x_0}\left(1 + \frac{y_0}{K_Y}\right) \text{ [5]}$$

where $k_{app} = k/(1 + (K_X/x_0))$, and x_0 and y_0 are initial concentrations of the affinity reagent and competitor.

3. RESULTS AND DISCUSSION

As reported in [1] $d(pT)_2pC[Pt^{2+}(NH_3)_2OH](pT)_7$ modifies the template site of DNA polymerase α from human placenta in accordance with the known criteria of affinity modification [5]. For evaluating affinities of oligonucleotides of different length and monomeric ligands to the template site of DNA polymerase, experiments were made on protecting the enzyme against inactivation by these ligands.

Dependences of k_{app} of the enzyme inactivation rate during polymerization on concentrations of $d(Tp)_{14}T$ and $d[Tp(Et)]_{14}T$ at a fixed concentration of $d(pT)_2pC[Pt^{2+}(NH_3)_2OH](pT)_7$ are shown in fig.1. Using similar dependences we have found K_Y of complexes of the enzyme template site with various ligands (see table 1). Fig.2 demonstrates the dependence of log K_Y on the number of mononucleotide links (n) in various ligands. It can be seen that the affinity of oligothymidylates for the enzyme template site is independent of the presence or absence of a 5′-terminal phosphate and 3′-terminal oxy group. An increase in length of oligothymidylate by one link (upon varying n from 2 to 15) leads to the enhancement of its affinity to the enzyme by a factor of 1.72, which corresponds to a -0.32 kcal/mol change in the Gibbs' free energy. This change in binding energy is far smaller than that observed during formation of hydrogen bonds or electrostatic contacts [6], being more comparable with weak hydrophobic interactions. One can assume that 14 of 15 monomeric links of oligothymidylate $d(pT)_{15}$ can interact with the template site of DNA polymerase in a similar manner to hydrophobic binding.

In order to evaluate the contribution of internucleotide phosphates, we examined affinities of oligonucleotides which had been completely or partly ethylated via phosphates. As follows from table 1, both ethylation of one internucleotide phosphate in the case of individual diastereomers $d(Tp)_8Tp'(Et)T$ and $d(Tp)_8Tp''(Et)T$ and ethylation of four of seven phosphates in isomers $d[Tp'](Et)Tp]_3Tp'(Et)T$ and $d[Tp''(Et)Tp]_3Tp''$-(Et)T have a negligible effect on the affinity of oligothymidylates. The values of K_d of completely ethylated $d[Tp(Et)]_7T$ and $d[Tp(Et)]_{14}T$ are 7–9-times higher than those of $d(Tp)_7T$ and $d(Tp)_{14}T$. The affinity of $d(pT)_2pC[Pt^{2+}(NH_3)_2OH](pT)_7$ to

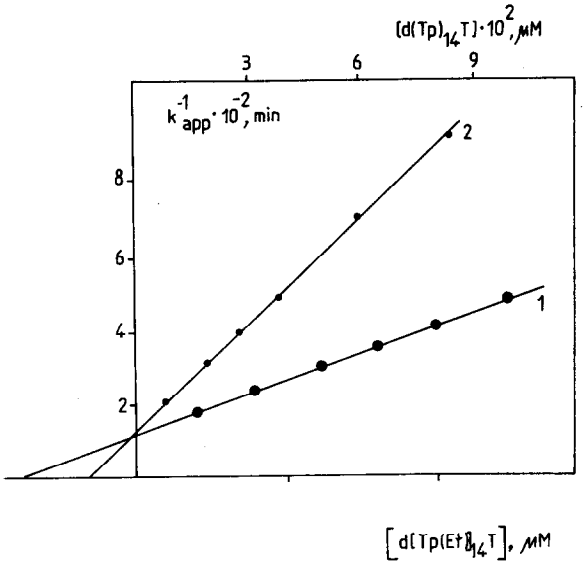

Fig.1. Dependence of k_{app} of DNA polymerase inactivation in the reaction of DNA polymerization on the concentration of $d(Tp)_{14}T$ (1) and $d[Tp(Et)]_{14}T$ (2) at fixed concentration of $d(pT)_2pC[Pt^{2+}(NH_3)_2OH](pT)_7$ equal to 1 μM.

the enzyme in the presence of Mn^{2+} (K_X = 0.5 μM) is nearly 8-times higher than that in their absence (K_X = 4.0 μM). Thus the affinity to the templates decreases by a factor of 7–9 both upon removal of

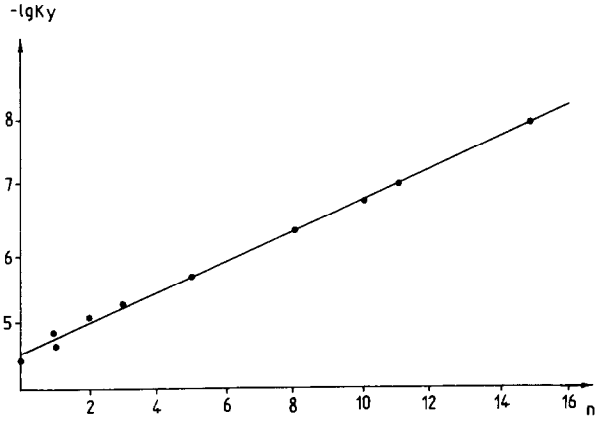

Fig.2. Dependence of log K_Y (where K_Y are dissociation constants for complex formation of oligothymidylates and other ligands with DNA polymerase α) on the number of mononucleotide links in deoxyoligoribonucleotide. At n = 0, K_Y corresponds to KH_2PO_4, at n = 1 d(pT) and d(Tp).

Table 1

Values of the dissociation constants (K_X) for complex formation of different ligands with the template binding site of human placenta DNA polymerase α

Ligand	K_Y (μM)
$PO(OC_2H_5)_3$	600
KH_2PO_4	53
d(Tp)	25
d(pT)	15
$d(pT)_3$	8.3
$d(pT)_5$	2.2
$d(Tp)_7T$	0.73
d[Tp'(Et)Tp]$_3$Tp'(Et)T	0.53
d[Tp''(Et)Tp]$_3$Tp''(Et)T	0.43
$d[Tp(Et)]_7T$	5.1
$d(Tp)_9T$	0.15
$d(pT)_9p(ddT)$	0.15
$d(pT)_7pC(pT)_7$	0.15
$d(pT)_2pC[Pt^{2+}(NH_3)_2OH](pT)_7$	0.5
$d(Tp)_8Tp'(Et)T$	0.14
$d(Tp)_8Tp''(Et)T$	0.14
$d(Tp)_{10}T$	0.077
$d(Tp)_{14}T$	0.0091
$d[Tp(Et)]_{14}T$	0.081

All values were determined in the presence of Mn^{2+}. The estimation error was no more than 30%. The designations p' and p'' correspond to tetrahedral enantiomeric atoms of phosphorus from the triester derivatives. The initial isomers (trythyl)Tp(Et)T(levulinyl) are designated according to the order of elution from silica gel (first p' then p''). The designations are preserved in the case of the longer ethyl derivatives made on the base individual diastereomers of dinucleotides [2]

Mn^{2+} and upon ethylation of all internucleotide phosphates.

On the other hand, when passing from phosphate to d(pT) and further from d(pT) to $d(Tp)_{14}T$ one observes a monotonic change in K_Y according to a decreasing geometric progression, i.e. 53 μM × $(1.72^{-1})^n$, where 53 μM is the value of K_Y for the phosphate and n equals the number of internucleotide links in the ligand. These data lead us to conclude that a single internucleotide phosphate of the template forms one, most probably, Me^{2+}-dependent electrostatic contact with the enzyme ($\Delta G \approx -1.1$ to -1.2 kcal/mol).

Complete ethylation of KH_2PO_4 results in triethyl phosphate production and in a decrease of the ligand affinity by a factor of 11.3 (see table 1), that is almost the same as in the case of ethylation of all internucleotide phosphates of oligonucleotides. Triethyl phosphate seems to form a hydrogen bond with the enzyme by the oxygen atom of the P=O group. From K_Y for triethyl phosphate (600 μM) the Gibbs' free energy of this interaction has been estimated (−4.4 to −4.5 kcal/mol).

The value of the Gibbs' free energy of phosphate binding (6 kcal/mol as estimated from K_Y = 53 μM) is the sum of contributions of binding energy of triethyl phosphate and of the electrostatic interaction assumed here. Most probably, both interactions are provided by the same phosphate, which is also valid for the internucleotide phosphate of oligonucleotides of different length.

Thus, our approach has allowed us to show that oligodeoxyribonucleotides are involved in the interaction with the template site of DNA polymerase α due to Me^{2+}-dependent electrostatic contact and a hydrogen bond of one of the internucleotide phosphates. Other monomeric links of the template form only the hydrophobic type contacts with the enzyme.

REFERENCES

[1] Nevinsky, G.A., Podust, V.N., Levina, A.S., Khalabuda, O.V. and Lavrik, O.I. (1987) Bioorg. Khim. 13, 357–368.
[2] Nevinsky, G.A., Levina, A.S., Podust, V.N. and Lavrik, O.I. (1987) Bioorg. Khim. 13,
[3] Levina, A.S., Nevinsky, G.A. and Lavrik, O.I. (1985) Bioorg. Khim. 11, 358–369.
[4] Nevinsky, G.A., Frolova, E.I., Levina, A.S., Podust, V.N. and Lebedev, A.V. (1987) Bioorg. Khim. 13,
[5] Gorshkova, I.I. and Chimitova, T.A. (1983) in: Affinity Modification of Biopolymers (Knorre, D.G. ed.) pp.56–81, Nauka, Siberian Division, Novosibirsk.
[6] Fersht, A. (1977) in: Enzyme Structure and Mechanism, Freeman, San Francisco.