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# L, D-Polydeoxyribonucleotides to provide an essential inhibitory effect on DNA polymerase β of human myeloid leukemia HL60 cells

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ABSTRACT

The inhibitory effect of D and L-polynucleotides of a given length (40-50n) on the catalytic activity of DNA polymerase β isolated from chromatin cells of acute myeloid leukemia HL-60 was evaluated. The synthesized L enantiomer was found to have a higher inhibitory activity than the synthesized and isolated D enantiomers of polynucleotides. The work also proposes a biophysical model that describes this effect.

## 1. Introduction

DNA polymerase β (DNA Polβ) belongs to the family of X-polymerase and plays a key role in base excision repair (BER) [1,2]. It should be noted as well that DNA polymerase shows hyperexpression in malignant tumors, thus holding a promise as a target in antitumor therapy [3–7].

Our earlier studies demonstrated the efficient inhibition of catalytic activity of Polβ extracted from the cell line of promyelocytic leukemia HL-60 by aptamers of various compositions, both taken from HL-60 cells ($ssDNA_{40\text{-}50}$) and synthetic ones, for example those containing repetitive D-Nucleosides Thymidine or Adenosine [8].

We found that, for all of the examined D-polynucleotides suppressing the DNA Polβ catalytic activity, their length emerges as the critical parameter responsible for the inhibition. Notably, efficient inhibition of the DNA polymerase β is observed for aptamers with lengths in the 50–100 nm bracket [8]. The effect is explained by non-specific interactions between ferment and substrate, which are mainly due to reversible van der Waals forces proportional to substrate length. Clearly, the non-specific character of the interaction between D-polynucleotide and DNA polymerase β has to be presumed considering the low sensitivity of the catalytic activity inhibition process to aptamer composition. In other words, sufficiently long substrate molecules stick to the ferments of wrap around them, insulating the active centers. The DNA Polβ having two active centers, the inhibition takes two such substrate molecules, as fully confirmed in experiment. D-polynucleotides longer than 100 nm tentatively compactify over themselves, which makes efficient blocking of the active center impossible. A similar effect occurs in the case of short molecules of the substrate as their lengths fail to provide for sufficiently strong bonding with the ferment under the conditions of thermally activated dissociation [8].

However, such polynucleotides in real live systems may be subject to impacts from various types of nuclease and lose their inhibitive capacity due to length degradation [9]. Naturally, the process may reduce the bioavailability of potential medications, and, therefore, their efficiencies.

It should be noted that under low physiological temperatures the effect exerted on ligands by all ferments without exception is based on the molecular complementarity principle, which functions according to the lock-and-key scheme [10]. Underlying this lock-and-key interaction, which is fundamental across biology, is the matching of the chiral domains of ligand and ferment. The above follows from the so-called homochirality of nature, which, in its turn, makes chirality the parameter critical to the catalytic effect of the corresponding ferment on ligand. Switching the ligand chirality sign should cause the fermentative activity to vanish due to the impossibility of brining the ligand to coincide with the ferment's chiral reaction domain or volume via any rotations or translations [16]. It is obvious that the same pertains equally to the nuclease discussed above [9,15].

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The chirality of nucleotides and, in particular, D-polynucleotides in organisms being defined by D-sugars, it is clear that, owing to the above symmetry regards stemming from chirality, replacing the latter with L-sugar should neutralize the impact of nuclease.

Still, the issue of the inhibitive activity as applied to DNA polymerase β of such L-polynucleotides remains open. As a result, the objective of the present paper has been set to estimate the inhibitive activity of DNA-Polβ by L-polynucleotides (Poly (L-dT)$_{50}$) and to compare it against the inhibitive activity due to D-polynucleotides extracted from HL-60 cells (ssDNA$_{40\text{-}50}$) and to synthetic D-polynucleotides (Poly(A)$_{50}$, Poly (dT)$_{50}$) having the length ≈50*n*.

## 2. Materials and methods

### 2.1. Cell culture

The HL-60 human myeloid leukemia cell line has been purchased from the Hungarian Cell Bank, Pasteur Institute of Hungary, Szeged, NCBI Code C427. Cells were maintained in suspension culture at +37 °C under 5% CO2/air in RPMI 1640 (Gibco, UK) supplemented with 10% FCS and antibiotics: 100 U/mL Penicillin and 100 μg/mL Streptomycin. The cells were subcultured three times weekly, ATRA (Sigma, USA). The passage procedure and monitoring of cell viability were carried out according to the method [11] in modifications [12].

### 2.2. Ligands

Poly (dT)$_{50}$ (ThermoFisher, USA), Poly(A)$_{50}$ (IBC RAS, Russia); Poly (L-dT)$_{50}$ (ICBFM SB RAS, Russia), ssDNA$_{40\text{-}50}$ from cells HL-60 (RNRMU, Russia).

### 2.3. Enzyme samples

PAGE-homogenous beta-like monomeric, 66.5 kDa DNA polymerases (EC 2.7.7.7) purified from chromatine of HL-60 acute myeloid leukemia cells [13]. Thus, to purify these chromatine-associated DNA repair programming enzymes, the following [13]—promoted technique has been used. First, the chromatin fraction of the cell lysate was obtained. For this purpose, the protein containing crude nuclei phenol-chloroform extracts [13] were mixed with 10 vol of an ice-cold acetone and kept at +4 °C overnight. An acetone-insoluble material was precipitated at 20,000 rpm, 20 min, +4 °C. The pellets were extensively re-washed with acetone using the same procedure and then dissolved in 5–6 vol (w/v) of 25 mM potassium-phosphate (pH 6.30)/0.5% NaCl/1.5 mM EDTA/0.01% glutathione/0.05% heparin/1.0% 2-mercaptoethanol/80–100 U/mL nuclease S followed by 40 min incubation at +37 °C. Post-incubation mixture were treated by sonication at 80 KHz, 30 min, +60 °C, under a non-stop extensive shaking. Then this mixture was submitted to a scalar fractionation path reaching the 30%–70% ammonium sulfate saturation, consequently. The precipitate obtained were collected at 10,000 rpm, 20 min, and dissolved in 15 mM potassium phosphate buffer (pH 6.0)/0.2% NaCl (10 vol, w/v). The solution was subjected to dialysis against 20 mM potassium phosphate buffer (pH 6.0) and lyophilized. The lyophilized powder was first dissolved in 15 mM potassium phosphate (pH 6.30)/5.0 mM MgCl2/1.5 mM EDTA/0.0001% sodium azide and passed through the fiberglass filters with 0.3–0.4 μ pore diameter (Millipore 5R, Millipore, France). The transparent solution was subjected to ultrafiltration on membranes with the molecular size exclusion limit of 5.0 kDa at 800 p.s.i. (Diaflo Y5.0 25 mm membranes, Amicon BV, The Netherlands). The membrane-retained material was then extracted with 10 mM Tris-HCl (pH 8.0)/1.0% 2-mercaptoethanol (v/v), 5.0 mL per a razor-disintegrated membrane, +30 °C, 12 h, with a following concentration in a rotor evaporizer.

The 1.5–2.5 mL samples were then applied onto a 1.5 × 50 cm (V = 98 mL) column packed with the TOYOPEARL HW 55F gel and equilibrated by the eluent buffer consisting of 15 mM potassium phosphate (pH 6.30)/5.0 mM MgCl2/0.0001% sodium azide. Elution rate: 0.8 mL/min (room temperature). In each one of the consequently eluted 1.5 mL fractions, the DNA polymerase activity has been measured according to Ref. [13].

### 2.4. Enzyme activity measurements

DNA-Polβ catalytic activity values were expressed in amounts of [3H]dTTP incorporated into nascent DNA chains in 1 min of incubation at optimal conditions corrected per 1.0 mg of pure enzyme protein ([3H] DNAcpm/mg protein) as described in Refs. [13]. The enzyme catalytic activity was measured in 0.15 mL incubation mixtures consisting of 50 mM Tris-HCl (pH 8.0)/8.0 mM dithiothreitol/15 mM MgCl2/15% glycerol (v/v)/27 2 μg act DNA, calf thymus/50 μg each of dATP, dCTP, dTTP, dGTP/0.25 μmole [Methyl-1,2–3H]dTTP (90–120 Ci/mmol, NET520A, NEN)/150 mM NaCl. The tritium-labeled nucleotide was purchased from New England Nuclear, USA. These compound concentration values were first pre-optimized within both pH 6.0–9.0 and 5.0 mM–50.0 mM $MgCl_2$ ranges. These mixture samples were first pre-incubated at +37 °C for 60 min. Then 5.0–7.5 μg of pure enzyme was added to each one of these running samples and they were incubated at +37 °C for 60 min longer. The ice cold incubation samples (0 °C, 60 °C after pre-incubation) as well as the trypsin treated samples (20 μg/mL trypsin, Merck GmbH, Germany, +37 °C, 60 °C) were taken for controls. The post-incubation mixtures were subjected to a quantitative extraction of the DNA ultramicro-amounts using an AccuPrep Genomic DNA Extraction Kit (Bioneer Corp., Korea).

### 2.5. Synthesis of L-polynucleotide (50n)

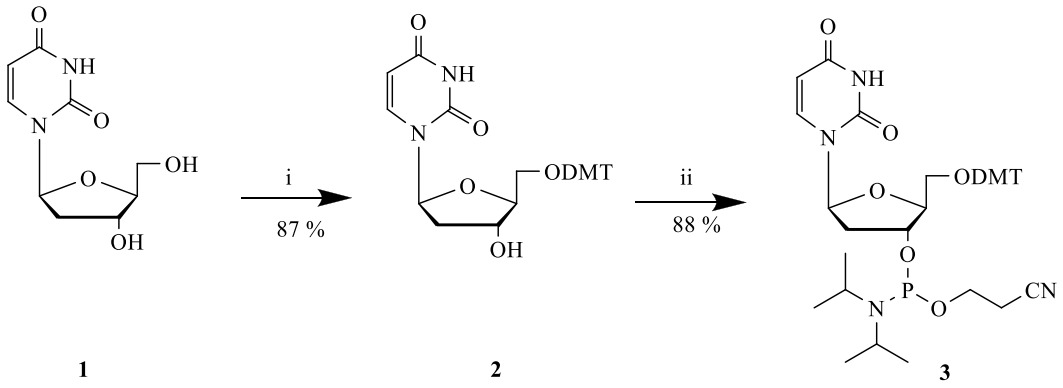
Compounds **2**–**3** was synthesized according to Ref. [14].

#### 2.5.1. 5′-O-(4,4′-Dimethoxytrityl)-L-thymidine (2)

L-Thymidine (500 mg; 2.06 mmol, Fluorochem, UK) was coevaporated three times with anhydrous pyridine, dissolved in dry pyridine (4 mL), 4,4′-dimethoxytrityl chloride (698 mg; 2.06 mmol) was added in portions in 1 h. The reaction mixture was stirred at ambient temperature and the progress of the reaction was monitored by TLC. After complete consumption of the nucleoside (2 h) the reaction mixture was diluted with $CH_2CL_2$ and the organic phase was washed with saturated aqueous $NaHCO_3$ solution and dried over $Na_2SO_4$. After filtration the solvent was evaporated and the residue was purified by column chromatography (0–30% acetone in $CH_2CL_2$ containing 0.1% pyridine v/v). Fractions containing the product were combined and evaporated in vacuo. The residue was then dissolved in dichloromethane (2 mL) and the product was precipitated with a ten-fold volume of hexane to give **2** (1.11 g; 87%). TLC ($CH_2Cl_2$/MeOH 9:1; UV). $R_f$ 0.47. $^1$H NMR (acetone-d6): 1.45 (s, 3H, $CH_3$); 2.26–2.42 (m, 2H, H(2′)); 3.35 (d, 2H, 2H(5′), *J* = 3.4 Hz); 3.78 (s, 6H, $OCH_3$); 4.03 (m, 1H, H(4′)); 4.58 (m, 1H, H(3′)); 6.36 (t, 1H, H(1′), *J* = 5.9 Hz); 6.84–6.92 (m, 4H, Ph); 7.20–7.51 (m, 9H, Ph); 7.61 (s, 1H, H(6)).

#### 2.5.2. 5′-O-(4,4′-dimethoxytrityl)-L-thymidine 3′-O-(2-cyanoethyl-N,N, diisopropyl)phosphoramidite (3)

Compound 2 (545 mg, 0.88 mmol) was dissolved in a freshly distilled $CH_2Cl2$ (4 mL), diisopropylammonium ethylthiotetrazolide (102 mg, 0.44 mmol) was added, followed by addition of *N,N,N′,N′*-tetraisopropyl-(2-cyano)ethyl phosphodiamidite (0.42 mL, 1.32 mmol). The reaction was monitored by TLC ($CH_2Cl_2$/acetone 4:1; UV). After 2 h, the reaction mixture was evaporated, the residue was treated with hexane (30 mL), and the mixture was kept overnight at −20 °C. Hexane was then decanted, and the residue was purified by chromatography on silica gel column with 1% $Et_3N$ in hexane:$CH_2Cl_2$ (1:1). Elution with 1% $Et_3N$ in $CH_2Cl_2$ gave the target fractions. Fractions containing the product were combined and evaporated in vacuo. The residue was then dissolved




i: DMTrCl, Py, 2h
ii: 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphodiamidite,

**Fig. 1.** Scheme of the synthesis of monomers.

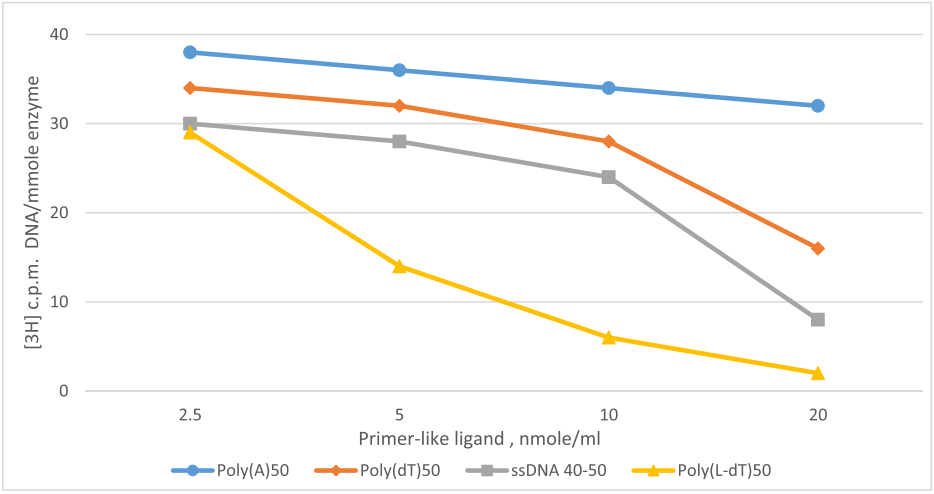



**Fig. 2.** The catalytic activity of DNA polymerase β in the presence of the ligands studied.

in dichloromethane (2 mL) and the product was precipitated with a ten-fold volume of hexane to give **3** (625 mg; 88%) as a white powder. TLC ($CH_2Cl_2$/acetone 4:1; UV). $R_f$ 0.56, 0.62. Mixture of diastereoisomers: $^1$H NMR (acetone-d6): 1.02–1.20 (m, 12H, $(CH_3)_2CH$); 1.46 (m, 3H, $CH_3$); 2.36–2.50 (m, 2H, H (2′)); 2.57–2.78 (m, 2H, $CH_2CN$); 3.32–3.47 (m, 2H, 2H (5′)); 3.53–3.92 (m, 10H, $CH_3O$, $CH_2O$, $(CH_3)_2CH$); 4.09–4.21 (m, 1H, H(4′)); 4.73 (m, 1H, H(3′)); 6.35 (m, 1H, H(1′)); 6.84–6.94 (m, 4H, Ph); 7.20–7.52 (m, 9H, Ph); 7.61 (d, 1H, H(6), $J = 8.4$ Hz). $^{31}$P NMR (acetone-d6): 149.06, 149.13.

Polynucleotide synthesis was performed on an ASM-800 DNA/RNA synthesizer (Biosset) on a 0,1 μmolar scale. Oligonucleotide (50-mer) was synthesized using phosphoramidite **3** (0.05 M solution in $CH_3CN$). Oligonucleotide was deprotected and removed from the solid support using conc. $NH_3$ (aq). Purification was accomplished using RP-Cartridge (ChemGenes Inc.) according to standard manufacturer's protocols (see Fig. 1).

## 3. Results and discussion

Fig. 2 illustrates the comparison between the inhibiting activities due to D-polynucleotides and L-polynucleotides of length 50n for DNA polymerase β. A D-RNA-oligomer of same length, for which the inhibitive activity is absent due to self-aggregation [8], was involved in the probe within the same experiment as a verification measure. As it can be seen in Fig. 2, the L-polynucleotide exhibits the highest inhibiting activity, which exceeds that of the (Poly $(dT)_{50}$) D-enantiomer by a factor of 8, of $ssDNA_{40\text{-}50}$ - by a factor of 4, and of Poly$(A)_{50}$ - by a factor of 16 (at c = 20 nmoL/ml).

Thus, the established phenomenon makes it possible to employ L-polynucleotides as efficient non-redundant inhibitors of the catalytic activity of polymerase β.

The effect identified can be briefly explained as follows. As discussed above, the interactions between D-polynucleotide and Polβ is of non-specific character and is not complementary in the lock-and-key sense. However, complementarity may manifest itself at the level of monomer chiral domains rather than at the macromolecular level [17]. Such effects have been explored in our studies of the self-assembly of supramolecular strings in various solvents with trifluoroacetylated α-aminoalcohols [18,19]. It was demonstrated experimentally that molecular complementarity pertinent to the domain of a chiral trifluoroacetylated α-aminoalcohols carbon atom can be arranged both for L-L (D-D) and L-D, albeit with a disparity in efficiencies. The complementarity choice between the two options is likely determined by an entire ensemble of van der Waals interactions over all monomers of the interacting chiral domains of the active center and ligand.

As a result, stronger interaction in the complementary pair of monomers translates into a more intense sticking effect and more efficient inhibiting of beta polymerase. This is likely to be the effect

observed in the case of L-polynucleotides.

Importantly, the non-specific character of the inhibiting is indicative of the germane role of long-range van der Waals interactions, as the coincidence of the extremely anisotropic short-range hydrogen bonds under the conditions of variable array of polynucleotides is extremely unlikely.

## 4. Conclusions

In all likelihood, the achieved effect of inhibiting the beta polymerase ferment by L-polynucleotides, which do not occur naturally in living organisms and, for this reason, are stable against the degradation of nuclease, opens novel opportunities for resolving the problem of biostability of the studied inhibitors of DNA polymerase β with the aim of treating oncological disease.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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