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The affinity of the Klenow fragment of *E. coli* DNA-polymerase 1 to primers containing bases noncomplementary to the template and hairpin-like elements

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The K_m and V_{max} values for a set of primers: $d(pT)_n$ (pC) $(pT)_m$ (n=3–9, m=0–7) and $d(pT)_4$ $(pCpG)_k$ $(pT)_4$ (k=1–5) have been estimated. Poly(dA) was used as a template. The number of complementary bases from the 3′ end to a noncomplementary ones was shown to determine the efficiency of interaction of $d(pT)_n$ (pC) $(pT)_m$ with the Klenow fragment. Oligonucleotides $d(pT)_4$ $(pCpG)_k$ $(pT)_4$, in solution forming duplexes containing hairpin-like elements, show a higher affinity to the enzyme than control $d(pT)_4$, $d(pT)_n$ and $d(pT)_n$ (pC) $(pT)_m$ primers. For example, the K_m value (1.1 nM) for $d(pT)_4$ $(pCpG)_5$ $(pT)_4$ is about 14,000 and 200 times lower than those for $d(pT)_4$ and $d(pT)_n$, respectively. Possible reasons for such an abnormally high affinity of the above primers are discussed.

Klenow fragment; Primer; Abnormally high affinity

1. INTRODUCTION

The mechanism of binding and elongation of homo- and hetero-oligoprimers in the case of FK and other polymerases has been investigated [1–8]. The enhancement of the homo- and hetero-oligonucleotide primers' affinity for the enzyme due to one Watson–Crick hydrogen bond between complementary template and primer is by a factor of about 1.35. This allows for the calculation of the K_m values for primers of various structure and length up to 10 units [8]. The objective laws of the K_m and V_{max} values changing for primers containing 11–25 nucleotides were analyzed.

A comparison of the K_m and V_{max} values of the primers completely complementary to the template or containing non-complementary bases at different positions from the 3′ end in the polymerization reaction catalyzed by DNA-polymerase 1 from *E. coli*, human DNA-polymerase α, DNA-polymerase from *Sulfolobus acidocaldarius* and AMV reverse transcriptase was carried out [4,9,10]. The number of complementary bases from the 3′ end to a noncomplementary nucleotide was shown to determine the efficiency of the interaction of such primers with the enzyme. This paper presents data on the affinity and rate of elongation of $d(pT)_n$, $d(pT)_n$ (pC) $(pT)_m$ primers in comparison with oligonucleotides $d(pT)_4$ $(pCpG)_k$ $(pT)_4$ which, in solution, form duplexes containing hairpin-like elements.

Abbreviations: FK, Klenow fragment of *E. coli* DNA-polymerase 1; V_o, V_{max}, initial and maximum rates of polymerization reaction, respectively.

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2. MATERIALS AND METHODS

Electrophoretically homogeneous FK with a specific activity of 6×10^4 U/mg was obtained according to [11]. poly(dA), dTTP were from NIKTI BAV (USSR), BSA from Koch Light, $MgCl_2$ from Merck, [^{3}H]dTTP from Izotop (USSR). Other compounds were of analytical grade.

The synthesis, characterization and methods of purification of the oligonucleotides have been reported in [1–4].

The polymerase activity of FK was determined at 30°C [8]. The reaction mixture (50–100 μl) contained 50 mM Tris-HCl buffer (pH 7.5), 0.3 mg/ml BSA, 30 μM EDTA, 6 mM NaF, 0.4 A_{260}/ml poly(dA), 15 μM [^{3}H]dTTP ($2–20 \times 10^{14}$ Bq/mol), 2.5 mM $MgCl_2$, 3 mM KCl, and one of the primers. The reaction was started by adding 0.5–100 U/ml FK. The reaction was further carried out [illegible].

The K_m and V_{max} values were determined according to Eisenthal and Cornish-Bowden [12]. Errors in K_m and V_{max} were within 20–40%.

3. RESULTS AND DISCUSSION

The optimal conditions for the copying of the poly(dA) template and for NaF as a selective inhibitor of the 3′-5′-exonuclease activity of FK were used [8]. According to [13,14], the K_m values for primers determined using initial rates of the polymerization reaction under the above described conditions negligibly differ from K_d. This allows for using the K_m values as a measure of the affinity of primers to FK. The dependence of $-\log K_m$ ($-\Delta G^\circ$) vs. the number of the $d(pT)_n$ primer nucleotides (n) is linear up to n=9–10:

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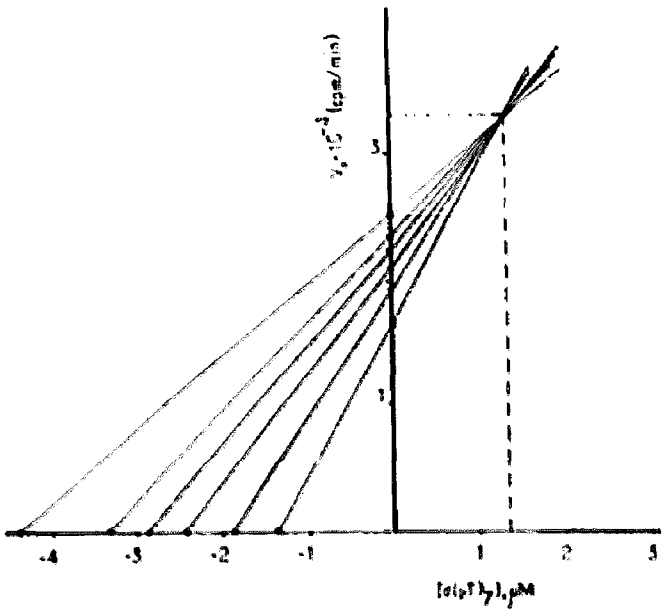


Fig. 1. The dependencies of initial rates of the polymerization reaction as a function of concentration of d(pT)$_7$ (determination of K_m and V_{max} values)

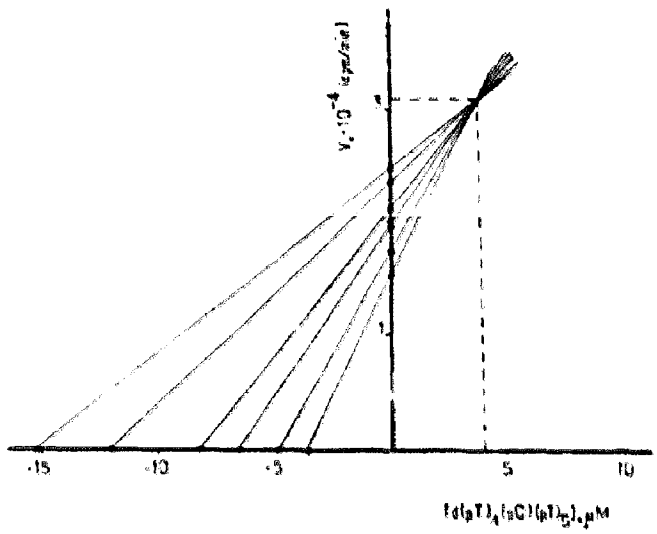


Fig. 2. The dependencies of initial rates of the polymerization reaction as a function of concentration of d(pT)$_4$ (pC) (pT)$_2$.

$$K_m(n) = {}^{*}{}_{m}(1) \times 1.82^{1-n}$$

where K_m (1) is K_m for dTMP (n=1) and 1.82 is a factor of the affinity enhancement with the increase of primer length by one nucleotide unit [2,5–8]. The dependencies of the initial rates of the polymerization reaction as a function of concentration of d(pT)$_7$ and d(pT)$_4$ (pC) (pT)$_2$ primers are shown in Fig. 1. The K_m and V_{max} values for all the primers investigated are given in Table I. In all cases the K_m and V_{max} values for inexact d(pT)$_n$ (pC) (pT)$_m$ primers depend on the position of a noncomplementary nucleotide in the primer chain. The K_m values for d(pT)$_4$ (pC) (pT) and d[(pT)$_2$ (pC)]$_2$ (pT) are approximately the same as for d(pT)$_7$ (pC) (pT)$_2$ and (dpT)$_7$. A similar situation is observed for the V_{max} values. The affinities of d(pT)$_4$ (pC) (pT)$_5$ and d(pT)$_2$ (pC) (pT)$_7$ do not differ from d(pT)$_5$ and d(pT)$_7$, respectively. These data correlate well with analogous results for human DNA-polymerase α, archaebacterial DNA-polymerases and AMV reverse transcriptase [4,9,10]. The primer's unit noncomplementary to the template obviously disturbs the interaction of the primer's 5′ end part with the template.

Only the 3′ end of primers interacts with DNA-polymerases [2,5–8]. All other nucleotide units of the primer interact only with the template. The K_m value for d(pT)$_{10}$ (pC) is higher by a factor of 60 than that for d(pT)$_{11}$. Such a decrease in the primer affinity was supposed to take place not only due to the disturbance of the complementary interaction of the incorrect 3′-terminal nucleotide with the template, but also due to a decrease in the efficiency of formation of an electrostatic contact and hydrogen bonds between the 3′ end and the enzyme [4,9,10]. Obviously, the above contacts are not completely destroyed because without

Table I

The K_m and V_{max} values for various primers

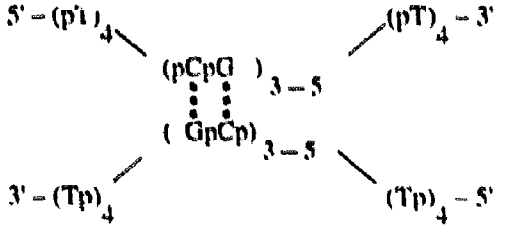
Primer (5′ → 3′)	K_m* (μM)	Relative values of V_{max}* (%)
d(pT)$_7$	22.0	40
d(pT)$_4$ (pC) (pT)	18.0	38
d[(pT)$_2$ (pC)]$_2$ (pT)	18.0	30
d(pT)$_4$ (pC) (pT)$_2$	16.0	35
d(pT)$_5$	7.5	2
d$_9$pT$_1$	4.1	64
d(pT)$_4$ (pC) (pT)$_2$	5.0	78
d(pT)$_6$	1.3	50
d(pT)$_8$	0.5	90
d(pC) (pT)$_7$	1.3	90
d(pC)$_2$ (pT)$_6$	8.0	97
d(pT)$_2$ (pC) (pT)$_6$	1.3	90
d(pT)$_{10}$**	0.22	100
d(pT)$_{11}$	0.20	100
d(pT)$_{10}$ (pC)	12.0	184
d(pT)$_3$ (pCpG) (pT)$_4$	13.3	39
d(pT)$_4$ (pCpG)$_2$ (pT)$_3$	0.69	0.47
d(pT)$_4$ (pCpG)$_3$ (pT)$_4$	0.027	0.43
d(pT)$_3$ (pCpG)$_4$ (pT)$_4$	0.0062	0.51
d(pCpG)$_4$ (pT)$_4$	0.0035	0.54
d(pT)$_3$ (pCpG)$_5$ (pT)$_4$	0.0011	0.54

*The K_m and V_{max} values were determined 3–4 times. Errors in K_m and V_{max} were within 20–40 %.

**The initial rate for poly(dA)-d(pT)$_{10}$ was taken as 100% (6.7 × 10^3 cpm/min).

them the primer affinity must decrease by approximately five orders of magnitude [4,9,10].

Similarly to inexact $d(pT)_n$ (pC) $(pT)_m$ primers, the affinity of $d(pT)_4$ $(pGpG)_k$ $(pT)_4$ primers to FK could be significantly reduced. However, in contrast to the former primers, the K_m values for $(pCpG)_{3-5}$-containing oligonucleotides are lower by a factor of 20–450 than that for $d(pT)_8$. A comparison of the affinity of $d(pCpG)_4(pT)_4$ and $d(pT)_4(pCpG)_4(pT)_4$ for FK serves as evidence for the absence of the enzyme interaction with the 5'-end $d(pT)_4$-unit of the latter oligonucleotides. Self-complementary oligonucleotides $d(pT)_4$ $(pCpG)_{3-5}$ $(pT)_4$ form in solution duplexes with hairpin-like elements.



The melting points of such duplexes [15,16] are higher than the temperature (30°C) at which the polymerization reaction was carried out. Taking into account this fact, it could be expected that open hairpins of such duplexes form additional contacts with the enzyme. The formation of the above contacts must lead not only to an increase in the primer affinity but also to a decrease in the maximum rate of the polymerization reaction. Therefore a decrease in the V_{max} values for $(pCpG)_{3-5}$-containing oligonucleotides by a factor of 830–1250 in comparison with $d(pT)_8$ also speaks in favour of the above suggestion.

Thus, for the first time we have shown a possibility of priming the DNA synthesis by oligonucleotides containing hairpin-like elements. The self-complementary oligonucleotides investigated have an extremely high affinity to FK.

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