The algorithm of estimation of the K_m values for primers in DNA synthesis catalyzed by human DNA polymerase α

Tat'yana I. Kolocheva, Galiya A. Maksakova, Ol'ga D. Zakharova, Georgy A. Nevinsky*

Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, Lavrentyeva Ave, 8, Novosibirsk 630090, Russian Federation

Received 2 September 1996; revised version received 16 October 1996

Abstract DNA synthesis with various deoxyribo homo- and heterooligoprimers in the presence of complementary templates was investigated. The lengthening of $d(pN)_n$ primers ($n = 1–10$) by a unit resulted in an increase of the primer affinity and a maximal rate of polymerization. The coefficient of the affinity enhancement of primers due to formation of one hydrogen bond between primer and template was found to be 1.35. The dependence of the primer affinity and polymerization rate on template-primer structure in solution was analyzed and the objective laws of the changes of the K_M and V_{max} values were revealed.

Key words: DNA polymerase α; Human placenta; Kinetics; Thermodynamics

1. Introduction

The mechanism of binding and elongation of homooligoprimers in cases of KF, HDPA, human DNA polymerase β and DNA polymerases from archaebacteria as well as AMV and HIV reverse transcriptases has been investigated [1–15]. dNMP, NMP and dNTP were shown to be the minimal primers of all DNA polymerases. HDPA like all the other DNA polymerases [1–15] interacts only with the 3'-terminal nucleotide of the primer due to formation of several contacts. All other nucleotide units of the primer interact only with the template by base-pairing. The dependence of $-\log K_M(-\Delta G°)$ vs. the number of the primer nucleotide units (n) was linear up to $n = 9–10$. In the present work we have investigated the relationship of K_M and V_{max} values for homoprimers and heterooligonucleotides in human DNA polymerase α. A common calculation algorithm of the K_M values for primers of various structure and length was found. The dependence of the primer efficiency on the structure of the template-primer duplex in solution was revealed.

2. Materials and methods

Nucleotides, polynucleotides and salts were from Sigma or Pharmacia. The synthesis of homogeneous ODNs was done according to [1]. Electrophoretically homogeneous HDPA from human placenta was obtained as in [16].

The HDPA activity was determined at 30°C in optimal conditions. The reaction mixture (50–150 μl) contained 50 mM Tris-HCl, pH 7.5, 0.3 mg/ml BSA and 10–50 μM EDTA. Other conditions are listed in Table 1. The reaction was started by adding HDPA. Further treatment of the mixture was done as in [8].

The K_M and V_{max} values were determined according to Eisenthal and Cornish-Bowden [17]. Errors in K_M and V_{max} were within 10–30%.

3. Results and discussion

The optimal conditions for copying various templates were found (Table 1). Activated DNA was shown to be the best substrate for HDPA. $MgCl_2$ was the optimal cofactor for activated DNA and poly(dG)-oligo(dC). The rates of copying poly(dT), poly(dA) and poly(dC) and M13 DNA in the presence of $MnCl_2$ were 4–10 times higher than with $MgCl_2$.

The minimal primers were nucleoside-5'-monophosphates; their K_M values decreased in the order dCMP > dAMP > dTMP ≈ dGMP (Table 2). The dependence of log K_M and relative V_{max} for homo-ODNs vs. the number of nucleotide units (n) is presented in Fig. 1. The coefficient of the primer affinity enhancement (f) with the increase of the ODNs length by a unit ($n = 1–10$) was estimated as the slope of the linear dependence of log K_M and was found to be equal to 1.82 ± 0.01 for $d(pA)_n$ and $d(pT)_n$ and 2.46 ± 0.01 for $d(pC)_n$ and $d(pG)_n$. Taking into account 2 and 3 hydrogen bonds of Watson-Crick base-pairing for A-T and G-C, the increase in the primer affinity due to one hydrogen bond formation was estimated to be a factor of 1.35 ($\sqrt{1.82} = \sqrt[3]{2.46} = 1.35 \pm 0.01$). The K_M change for homoprimers ($n = 1–10$) may be described by two algorithms: (1) $K_M(n) = K_M(1) \times 1.35^{-k}$, where $K_M(1)$ is the K_M value for dNMP and k is a difference in the number of hydrogen bonds formed by template with dNMP and $d(pN)_n$; (2) $K_M(n) = K_M(10) \times 1.35^{p}$, where $K_M(10)$ is the K_M value for $d(pN)_{10}$, p is the difference in the number of hydrogen bonds formed by the template with $d(pN)_{10}$ and the shorter primer $d(pN)_n$. The V_{max} values for dNMP primers are different (Fig. 1, Table 2). The results may be described by a common equation: $V_{max} = V_{max}(1) \times (q)^{n-1}$, where $V_{max}(1)$ is a maximal rate for corresponding dNMP, and q is a factor of the V_{max} enhancement with the increase of the primer length by a unit.

Experimentally determined K_M values for heteroprimers complementary to M13 DNA are given in Table 3 and Fig. 1. The affinity of the 3'-dT unit of the heteroprimer (and of dTMP for the hetero-template) is about 22-fold higher than that for $d(pT)_{10}$, while the experimental and calculated K_M values for hetero-ODNs are the same within the accuracy of their determination. It means that while the efficiency of complementary interactions between templates and primers practically does not depend on the template, the affinity of the 3'-terminal nucleotide primer unit as well as of the minimal primer dTMP to the enzyme and relative V_{max} values are markedly influenced by the template sequence.

*Corresponding author. Fax: (7) (3832) 351665/3459.
E-mail: nev@modul.bioch.nsk.su

Abbreviations: HDPA, human DNA polymerase α; KF, Klenow fragment of *E. coli* DNA polymerase I; ODN, oligodeoxynucleotide; ss and ds, single- and double-stranded, respectively

PII S0014-5793(96)01298-7

Table 1
Optimal concentrations of the components of the mixtures for polymerization catalyzed by human DNA polymerase α

Component	Template-primer complex					
	Poly(dG) $d(pC)_{10}$	Poly(dC) $d(pG)_{10}$	Poly(dA) $d(pT)_{10}$	Poly(dT) $d(pA)_{10}$	Act.DNA	M13 DNA $d(pN)_{10}$
Template, $A_{\lambda\ max}$/ml[a]	0.16	0.20	0.54	0.34	2.0	0.3–0.4
Primer [μM]	3.0	1.6	2.3	3.1	–	0.02
dNTP [μM][b]	50	60	40	50	50	25.0
$MgCl_2$ ($MnCl_2$) [mM]	2.0	(0.7)	(0.15)	(0.2)	2.0	(0.2)
KCl [mM]	40	7.0	0.0	0.0	60	0.0
HDPA [U/ml]	50–200	0.5–2.0	0.1–10	0.1–10	0.05–0.3	0.5–4.0
relative rate (%)	0.7–1.0	80–90	40–50	10–15	100[c]	7–10

[a]The saturated concentrations of templates and primers which do not inhibit the polymerization reaction. The lengths of the templates were: 100–700, 100–500, 50–400 and 50–150 nucleotides for poly(dT), poly(dA), poly(dC), poly(dG). λ_{max} was equal to 266 nM for poly(dT), 260 nM for poly(dA), 271 nM for poly(dC) and 252 nM for poly(dG).
[b][^{3}H]dNTPs had a specific activity of $0.2–20\times10^{14}$ Bq/mol.
[c]Relative activity for activated DNA was taken as 100%.

As one can see from Fig. 1 and was shown earlier for other polymerases [1–14], the optimal primers for all DNA polymerases are decanucleotides; they reside within the binding cleft of various polymerases [1–15]. The further lengthening of primers leads to a decrease of their affinity and conversion rate. We suppose that it may reflect the different efficiency of template-primer interaction within and outside the DNA binding cleft of polymerase [1–15]. All polymerases melt the polymerase-bound region of DNA. ΔG^0 values of A-T and G-C pair formation for the DNA in the complex with the enzyme estimated from f factors ($\Delta G^0 = -RT \ln f$) are equal to −0.35 and −0.53 kcal/mol when the same values for base-pairing in solution are −1.2..−1.3 and −2.0..−2.4 kcal/mol, respectively. It follows that base-pairing interaction in the template-primer duplex within the enzyme is essentially lower than outside the enzyme (in solution). On the one hand, DNA polymerases may adjust the template-primer complex to the optimal conformation by melting it. On the other hand, as we suppose, template-primer interactions outside the enzyme may have an impact on the orientation of the 3′-nucleotide of the primer in the active site of the polymerase, thus influencing the primer's efficiency [15].

As we have shown earlier [8], the 15-fold decrease of affinity of the 3′-unit of riboprimers to HDPA in comparison with deoxyprimers is attended by an about 10-fold increase of the polymerization rate. The enhanced mobility of the 3′-terminus of the d[$(pT)_9$(pRibose)] primer with an abasic unit at the 3′-end led to an increase of the V_{max} by a factor of 20 without a marked decrease in primer affinity [3,8,15]. Though one might expect an inverse relationship between substrate affinity and conversion rate, typical for catalytic processes, in the case of HDPA and other polymerases [1–15] there is no strong inverse correlation between K_M and V_{max} values for template-primers.

In principle, all known DNA polymerases are structurally related. The maximal rate of polymerization, catalyzed by HDPA (like other polymerases), depends on many factors: template-primer, orientation of primer 3′-terminal nucleotide, pH, ionic strength, different divalent cation requirements [12–15]. The enzymatic characteristics of HDPA with various template-primers are similar to those for FK, both enzymes cannot utilize polyribotemplates [1–15] and with different template-primers all the parameters of polymerization reaction vary drastically. Taken together, the data discussed indicate that the rate of polymerization reaction depends on various factors and there is no good correlation between all of them. To date there are no quantitative data concerning the role of DNA adaptation in the catalysis of its conversion. From our point of view it is possible to find a good explanation for the experimental facts only taking into account the initial conformation of polynucleotide duplexes in solution and their capability to be adapted to the optimal conformation after complex formation with each specific polymerase.

Table 2
The K_M and relative V_{max} values for homo-oligoprimers of various structure and length complementary to corresponding homodeoxypolynucleotide templates[a]

Oligonucleotide	K_M [μM]/V_{max} (%)[b]			
	$d(pT)_n$	$d(pA)_n$	$d(pC)_n$	$d(pG)_n$
dNMP	45.0/6.9	71.0/6.5	360.0/30.0	43.0/19.0
$d(pN)_2$	23.0/9.5	35.0/9.6	148.0/41.0	17.5/23.0
$d(pN)_3$	15.0/12.6	–	60.0/46.0	7.1/28.0
$d(pN)_4$	8.0/17.5	12.3/19.0	24.6/51.0	2.9/33.0
$d(pN)_5$	4.2/22.0	–	10.0/55.0	1.2/40.0
$d(pN)_6$	2.5/30.0	4.3/27.6	–	–
$d(pN)_7$	1.4/41.0	–	2.0/ 68.0	0.2/58.0
$d(pN)_8$	0.65/58.0	1.0/58.0	–	–
$d(pN)_9$	0.45/75.0	0.56/76.0	0.35/75.0	–
$d(pN)_{10}$	0.23/100.0	0.31/100	0.14/100.0	0.013/100.0
$d(pN)_{11}$	0.16/95.0	0.35/97.0	–	–
$d(pN)_{12}$	0.18/75.0	0.18/80.0	–	–
$d(pN)_{15}$	0.10/54.0	0.06/74.0	0.58/55.0	0.022/60

[a]For the conditions used see Table 1.
[b]V_{max} for every $d(pN)_{10}$ was taken as 100%.

Table 3
The K_M and V_{max} values for heterooligo-primers of various length (n) complementary to M13 phage DNA

Primer (3′-5′)[a]	n	K_M [nM]		V_{max} (%)
		Experimental	Calculated[b]	Experimental
dTMP	1	2200.0	2000.0	65
d(TTG)	3	420.0	445.0	75
d(TTGC)	4	130.0	180.0	80
d(TTGCA)	5	100.0	99.0	83
d(TTGCAG)	6	42.0	40.0	90
d(TTGCAGC)	7	20.0	16.0	91
d(TTGCAGCA	8	10.0	9.0	93
d(TTGCAGCAC)	9	4.0	3.6	95
d(TTGCAGCACT)	10	2.0	–	100
d(TTGCAGCACTGA)	12	2.2	2.5[c]	90
d(TTGCAGCACTGACC)	14	3.5	2.8	87
d(TTGCAGCACTGACCC)	15	3.8	3.2	85

[a]The primers were complementary to the following region of M13 phage DNA: 5′-CGTTTTACAACGTCGTCGTGACTGGG-3′.
[b]Calculated using the equation: $K_M(n) = 2.0\ \text{nM} \times (1.35)^p$, where 2.0 nM is the experimental K_M value for the decanucleotide, and p is the difference in the number of hydrogen bonds formed by DNA with the decanucleotide and with one of the analyzed d(pN)$_n$ primers.
[c]Calculated using the equation: $K_M(n) = 1.1\ \text{nM} \times (1.13)^{n-10}$.

To analyze this phenomenon the following factors should be considered. The duplexes with a G-C pair were found to be drastically polymorphic [18]. In contrast, some A-T polymers were found to be in B-form, with some variations within the B-genus [18]. In an earlier publication [3] we supposed from kinetic and thermodynamic analyses that all template-primers adopt B-form DNA in the case of FK. This suggestion was confirmed by the X-ray crystallography of FK complexed with DNA, showing that the enzyme accommodates ds B-DNA [19]. Even the binding by FK of the ribonucleotide template, which is known to adopt the A-conformation in solution, leads to its transition to the B-form [20]. RNA-RNA complexes (A-conformation) as well as hybrid DNA-RNA complexes (the ribo strand is in a more A-like and the deoxyribo strand in a B-like conformation [18,20–22]) can be adapted to B-DNA by FK. B-DNA was shown to be the best substrate for pro- and eukaryotic polymerases, including FK and HDPA [1–14]. The kinetic analysis of the retroviral HIV, AMV [13,14] and DNA polymerase β [12–14], which is very similar to retroviral reverse transcriptases, strongly suggests that in the case of a ribo template and deoxyribo primers, when the template is in an A-like and the primer in a B-like conformation in solution, an optimal adaptation to the enzymes providing the greatest affinity and translocation rate takes place. This may explain differences of optimal template-primer for reverse transcriptases and DNA polymerase β as compared with FK and HDPA. X-ray analysis data strongly support this idea. The section of the ds DNA that is removed from the active site of DNA polymerase β and protrudes into solution has characteristics of B-DNA [23], while near the active center it has typical A-DNA characteristics. In complex with HIV reverse transcriptase the template primer has the A-form (near the active site) and B-form regions (near the RNase H site) separated by a significant bend (40–45°) [24,25]. Thus, the adaptation of DNA conformation to the optimal one for the enzyme may be a very fine process.

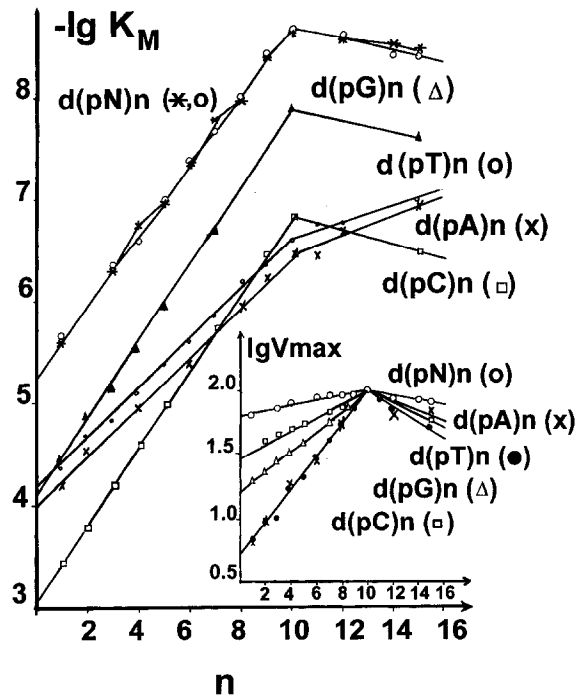

Fig. 1. The dependence of −log K_M and log of relative values of V_{max} on the length (n) of various primers in the presence of corresponding complementary templates. Sequences of d(pN)$_n$ primers complementary to M13 phage DNA are presented in Table 2: ○ and *, experimental and calculated K_M values, respectively.

This led us to believe that an essential factor determining the template-primer affinity to DNA polymerase as well as the rate of polymerization is the possibility of ds nucleic acid to adopt the optimal conformation in complex with the enzyme. The variable optimal conditions of polymerization – pH, concentration of different salts etc. (Table 1) with a particular template-primer complex – correspond to the required changes of these factors, providing optimal conditions for the template primer adaptation.

Acknowledgements: This work was supported by a grant from the Russian Foundation of Basic Investigations (95-04-12949), a grant from the Siberian Division of the Russian Academy of Sciences and grants NP J000 and NP J300 from the International Science Foundation.

References

[1] Levina, A.S., Nevinsky, G.A. and Lavrik, O.I. (1985) Bioorg. Khim. 11, 358–362.
[2] Nevinsky, G.A., Frolova, E.I., Levina, A.S., Podust, V.N. and Lebedev, A.V. (1978) Bioorg. Chim. 13, 45–57.
[3] Veniaminova, A.G., Levina, A.S., Nevinsky, G.A. and Podust, V.N. (1987) Mol. Biol. 21, 1378–1385.
[4] Nevinsky, G.A., Levina, A.S., Frolova, E.I. and Podust, V.N. (1987) Mol. Biol. 21, 1193–1200.
[5] Buchrashvili, I.Sh., Chinchiladze, D.Z., Lavrik, O.I., Levina, A.S., Nevinsky, G.A. and Prangishvili, D.A. (1989) Biochim. Biophys. Acta 1008, 102–107.
[6] Knorre, D.G., Lavrik, O.I. and Nevinsky, G.A. (1988) Biochimie 70, 655–661.
[7] Nevinsky, G.A., Nemudraya, A.V., Levina, A.S. and Khomov, V.V. (1989) FEBS Lett. 259, 166–170.
[8] Nevinsky, G.A., Veniaminova, A.G., Levina, A.S., Podust, V.N., Lavrik, O.I. and Holler, E. (1990) Biochemistry 20, 1200–1207.
[9] Nevinsky, G.A., Podust, V.N., Levina, A.S., Khalabuda, O.V. and Lavrik, O.I. (1985) Bioorg. Khim. 13, 357–369.
[10] Podust, V.N., Korobeinicheva, T.O., Nevinsky, G.A., Levina, A.S. and Lavrik, O.I. (1990) Mol. Biol. Rep. 14, 247–249.
[11] Ljach, M.V., Kolocheva, T.I., Gorn, V.V., Levina, A.S. and Nevinsky, G.A. (1992) FEBS Lett. 300, 18–20.
[12] Kolocheva, T.I. and Nevinsky, G.A. (1993) Mol. Biol. 27, 860–867.
[13] Lokhova, I.A., Nevinsky, G.A., Bulichev, N.A., Gorn, V.V., Levina, A.S., Rudenko, N.K., Kavsan, V.M. and Lavrik, O.I. (1990) Mol. Biol. 24, 396–407.
[14] Nevinsky, G.A., Andreola, M.L., Yamkovoy, V.I., Levina, A.S., Barr, Ph., Tarrago-Litvak, L., Tharaud, D. and Litvak S. (1992) Eur. J. Biochem. 207, 351–358.
[15] Nevinsky, G.A. (1995) Mol. Biol. 29, 6–19.
[16] Lavrik, O.I., Nevinsky, G.A., Podust, V.N. and Khalabuda O.V. (1989) Mol. Biol. 23, 387–399.
[17] Cornish-Bowden, E. (1978) in: Principles of Enzyme Kinetics, pp. 260–266, Mir, Moscow.
[18] Katahira, M., Nishimura, Y., Tsuboy, M., Sato, T., Mitsui, Y. and Iikaka, Y. (1986) Biochim. Biophys. Acta 867, 256–367.
[19] Joyce, C.M. and Steitz, T. (1987) Trends Biochem. Sci. 12, 288–292.
[20] Ferrin, L.J. and Mildvan, A.S. (1986) Biochemistry 25, 5131–5145.
[21] Reid, R.S., Stuart, A.L., Gupta, S.V., Latimer, L.J.P. and Lee, J.S. (1987) Nucleic Acids Res. 15, 4325–4335.
[22] Tinoko, I.Ir., Davis, P.W., Hardin, C.C., Puglishi, J.D., Walker, G.-T. and Whyatt, J. (1987) Cold Spring Harbor Symp. Quant. Biol. 52, 135–146.
[23] Pelletier, H., Sawaya, M.R., Kumar, A., Wilson, S.H. and Kraut, J. (1994) Science 264, 1891–1903.
[24] Jacobo-Molina, A., Ding, J., Nanni, R.G., Clark, A.D., Lu, X., Tantillo, Ch., Williams, R.L., Kamer, G., Ferris, A.L., Clark, P., Hizi, A., Hughes, S.H. and Arnold, E. (1993) Proc. Natl. Acad. Sci. USA 90, 6320–6324.
[25] Patel, P.H., Jacobo-Molina, A., Ding, J., Tantillo, Ch., Clark, A.D., Raag, R., Nanni, R.G., Hughes, S.H. and Arnold, E. (1995) Biochemistry 34, 5351–5363.