Volume 274, number 1,2, 156-158 FEBS 09093 November 1990

A comparison of the initiating abilities of ribo- and deoxyriboprimers in DNA polymerization catalyzed by AMV reverse transcriptase

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Received 18 September 1990

The difference in optimal conditions for DNA polymerization catalyzed by AMV reverse transcriptase on poly(A) and poly(dA) templates with $d(pT)_{10}$ and $(pU)_{10}$ primers has been found. A comparison of the initiating abilities of $d(pT)_{10}$ and $(pU)_{10}$ primers under optimal conditions for various template·primer complexes has been made. The best template·primer complex was poly(A)·$d(pT)_{10}$ and the worst was poly(A)·$(pU)_{10}$. The lengthening of $d(pT)_n$ primers by a mononucleotide unit ($n=2$–10) increases their affinity by a factor of about 2 and 3 in the case of poly(dA) and poly(A) templates, respectively. The affinities of $d(pT)_{11-25}$ to the enzyme does not change with the primer length.

AMV reverse transcriptase; K_m for primer

1. INTRODUCTION

The mechanism of binding and elongation of homo- and heterooligoprimers of various length by prokaryotic, eukaryotic and archaebacterial DNA polymerases has been investigated [1-8]. dNMP, NMP and dNTP were shown to be the minimal primers of DNA polymerases [3-8]. A common algorithm for calculation of the K_m values for primers of various structure and length has been found [7]. Like other DNA polymerases, reverse transcriptase needs a primer with the 3′-OH end to initiate DNA synthesis. It has been shown that reverse transcriptases could utilize as primers not only specific tRNAs, but also ribo- and deoxyribooligonucleotides [9]. However, details of the specific interaction of primers with this kind of DNA polymerases remain unclear.

Here we present some quantitative characteristics of the interaction of ribo- and deoxyriboprimers with AMV-RT. For the first time, the dependence of the K_m valus of $d(pN)_n$ primers on their length (n) was studied.

2. MATERIALS AND METHODS

Electrophoretically homogeneous $\alpha\beta$ form of AMV-RT ($1-10\times10^{-3}$ U/mg) was obtained according to [10]. Poly(dA) and BSA were from Sigma, poly(A) was Boehringer Mannheim, dTTP was from NIKTI BAV (USSR). $MgCl_2$ was from Merck, [^{3}H]dTTP (25×10^3 Ci/mol) was from Izotop (USSR). Other compounds were of analytical grade.

The synthesis, purification and methods of characterization of oligo- and mononucleotides were described earlier [1,3,8].

The polymerizing activity of AMV-RT was measured at 37°C. The reaction mixture (50–100 μl) contained 50 mM Tris-HCl buffer (pH was optimal for each system investigated), 1 mg/ml BSA and 0.2 units A_{260}/ml poly(A) or poly(dA). Other conditions are listed in Table I.

The reaction was started by adding of AMV-RT (1–5 units). Further treatment of reaction mixtures was done as in [1-8] using the method of acid-insoluble precipitates, ion-exchange chromatography [5,8] and electrophoresis [8].

The K_m and V values were determined according to Eisenthal and Cornish-Bowden [11]. Errors in K_m and V were within 20–40%.

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Abbreviations: AMV-RT, AMV reverse transcriptase; $(pN)_n$, ribooligonucleotides; V, V_{max} of polymerization reaction

3. RESULTS AND DISCUSSION

The optimal conditions for copying of poly(A) and poly(dA) templates in the case of $d(pT)_{10}$ and $(pU)_{10}$ primers are presented in Table I. Figs 1 and 2 show the dependences of the initial rate of polymerization on the pH value of reaction mixtures and the concentration of $MgCl_2$, respectively. The optimal concentration of $MgCl_2$ is seen to decrease depending on the kind of template·primer complexes in the order of poly(dA)·$d(pT)_{10}$ > poly(dA)·$(pU)_{10}$ > poly(A)·$d(pT)_{10}$ > poly(A)·$(pU)_{10}$.

The optimal pH value of DNA polymerization for these template·primers decreases in a reverse manner.

The K_m values for dTTP are nearly the same for the first three complexes, being about 2-fold higher only in the case of poly(A)·$(pU)_{10}$. The K_m values of primers strongly depend on the template. If the K_m value is a measure of affinity, then $d(pT)_{10}$ (K_m = 8 nM) has a maximal affinity to the enzyme with poly(A) template

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

Table I

Optimal concentrations of the components of the reaction mixtures for AMV-RT-catalyzed DNA polymerization

Other components	Template · primer			
	poly(dA)·d(pT)$_{10}$ (I)	poly(dA)·(pU)$_{10}$ (II)	poly(A)·d(pT)$_{10}$ (III)	poly(A)·(pU)$_{10}$ (IV)
pH at 50 mM Tris-HCl buffer	7.2	7.8	8.0	8.3
$MgCl_2$ (mM)	18	8	5	2.5
KCl (mM)	0 (0-60)[a]	0	50 (40-200)[a]	0 (0-10)[a]
[^{3}H]dTTP (μM)	60 (K_m = 20 μM)	60 (K_m = 20 μM)	60 (K_m = 20 μM)	132 (K_m = 44 μM)
primer (μM)	110 (K_m = 22 μM)	70 (K_m = 14 μM)	0.04 (K_m = 0.008 μM)	220 (K_m = 44 μM)

[a]Given in parentheses is the range of KCl concentration not influencing the initial rate

and a 2.8×10^3 times lower affinity in the presence of poly(dA). In contrast to deoxyriboprimers, (pU)$_{10}$ has a low affinity both with poly(A) and poly(dA) templates.

It should be mentioned that in the case of poly(A) · (pU)$_{10}$, both the lowest affinity to the enzyme of dTTP and (pU)$_{10}$ and the minimal rate of the polymerization reaction are observed (see Table II). Table II demonstrates the dependence of the initial polymerization rate on the composition of the reaction mixture. In all cases, using of non-optimal conditions for the template · primer complex under study leads to a significant decrease in the polymerization rate. It is interesting that the decrease in the rate of polymerization was more pronounced for the preparations of AMV-RT with a high specific activity ($\sim 1 \times 10^4$ U/mg) than for those with a low activity (1×10^3 U/mg). For the enzyme preparations with a high specific activity, a noticeable change of the optimal pH value for the investigated template · primer complexes is observed.

As was shown earlier [12], a minimal primer for AMV-RT is dNTP (primer-independent synthesis of DNA). The level of polymerization with poly(A) · (pU)$_{10}$ and poly(dA) · (pU)$_{10}$ under optimal conditions for I–III and I, IV template · primer complexes, respectively, is comparable with the level of polymeriza-

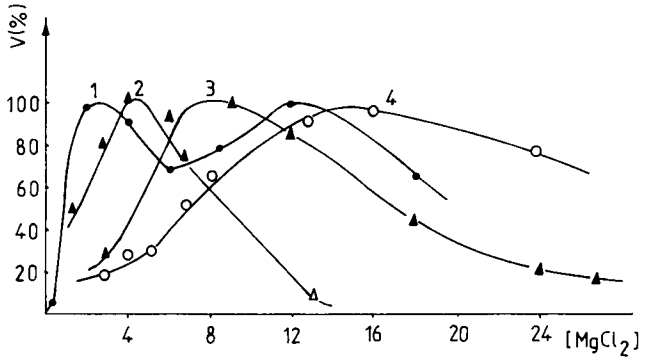

Fig. 1. Dependence of the relative initial rates of polymerization reaction, catalyzed by AMV reverse transcriptase, on the concentration of $MgCl_2$ in the case of: poly(A)·(pU)$_{10}$ (1); poly(A)·d(pT)$_{10}$ (2); poly(dA)·(pU)$_{10}$ (3); and poly(dA)·d(pT)$_{10}$ (4).

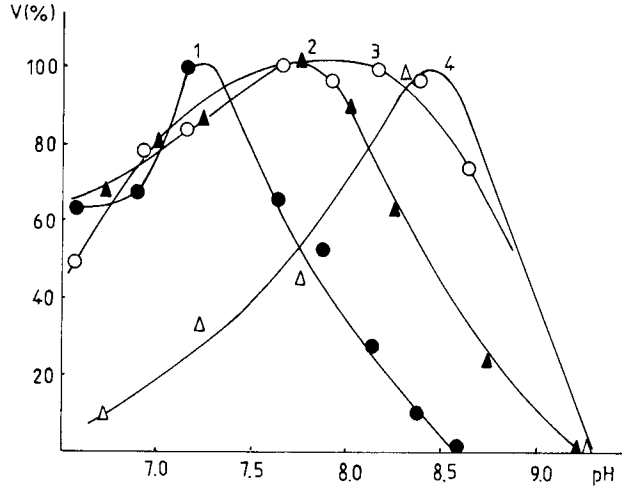

Fig. 2. Dependence of the relative initial rates of polymerization reaction, catalyzed by AMV reverse transcriptase, on the pH value in the case of: poly(dA) · d(pT)$_{10}$ (1); poly(dA) · (pU)$_{10}$ (2); poly(A) · d(pT)$_{10}$ (3); and poly(A) · (pU)$_{10}$ (4).

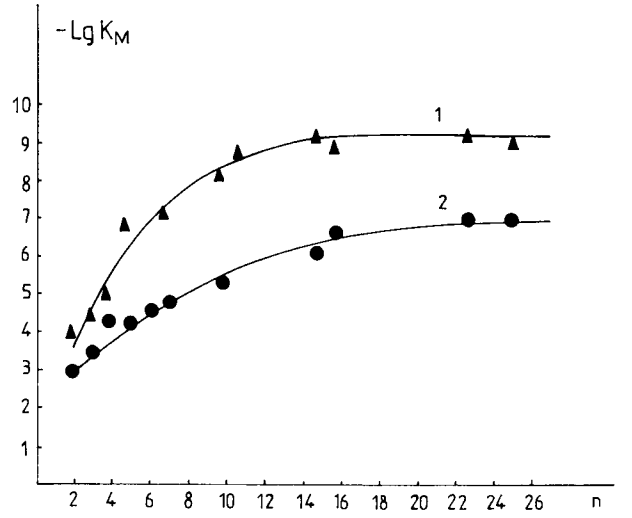

Fig. 3. Dependence of the logarithm of K_m on the length of d(pT)$_n$ primers for DNA synthesis, catalyzed by AMV reverse transcriptase, in the presence of poly(A) (1) and poly(dA) templates (2).

Table II

Relative values of initial rates of polymerization reaction for various template·primer complexes under optimal conditions for poly(A)·d(pT)$_{10}$, poly(A)·(pU)$_{10}$, poly(dA)·d(pT)$_{10}$ and poly(dA)·(pU)$_{10}$ complexes

	Relative values of the initial rate of polymerization for template·primer complexes (%)			
	poly(dA)·d(pT)$_{10}$	poly(dA)·(pU)$_{10}$	poly(A)·d(pT)$_{10}$	poly(A)·(pU)$_{10}$
poly(dA)·d(pT)$_{10}$	34	~25	13	3.3
poly(dA)·(pU)$_{10}$	~0[b]	6.7	3.1	~0[b]
poly(A)·d(pT)$_{10}$	4.7	40	100[a]	33
poly(A)·(pU)$_{10}$	~0[b]	~0[b]	~0[b]	2.7

[a] The initial rate for poly(A)·d(pT)$_{10}$ was taken as 100% (4.8×10^5 cpm/min)

[b] The rate of polymerization was equal to that for a system with a template of [^{3}H]dTTP in the absence of decanucleotide primer

tion in the presence of [^{3}H]dTTP only. For poly(dA)·(pU)$_{10}$, poly(A)·(pU)$_{10}$ and poly(dA)·d(pT)$_{10}$, a more optimal adaptation of these duplexes to the active site of the enzyme may be necessary. Therefore, poly(A)·d(pT)$_{10}$ is an optimal template·primer for AMV-RT.

In solution, ribo- and deoxyribonucleotide chains of poly(A)·poly(dT) take A-like and B-like conformations, respectively [13]. The duplexes with the dT·dA pair were found to be in B-forms [14] and poly(A)·poly(U) - in A-forms [13,14]. Based on the above experimental data, one could assume that the hybrid form of poly(A)·d(pT)$_{10}$ is optimal for reverse transcriptase. At the same time, a similar analysis showed that DNA-dependent DNA polymerases accommodate double-stranded B-DNA [7].

The K_m and V values for d(pT)$_n$ primers were determined using the initial rates of polymerization. The V values for primers of various length were nearly the same. The dependences of $-\log K_m$ for d(pT)$_n$ on the number of nucleotide units (n) are given in Fig. 3. In contrast to DNA polymerases from prokaryotes, eukaryotes and archaebacteria [3-8], for AMV-RT these dependences are not linear at n = 2-10. The coefficients of the primer affinity enhancement with the increase of d(pT)$_n$ length by one unit were found to be equal to about 2 and 3 for poly(dA) and poly(A) templates, respectively. This implies different efficiencies of rA·dT and dA·dT pairing on the enzyme.

The results obtained demonstrate that general regularities of the interaction of AMV-RT with primers do not differ significantly from those of mesophilic eubacterial, pro- or eukaryotic DNA polymerases.

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