A higher affinity of AMV reverse transcriptase for template-primers correlates with a lower rate of DNA synthesis

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The kinetics of copying of poly (A)–oligo (dT) and poly (C)–oligo (dG) by reverse transcriptase from avian myeloblastosis virus have been studied, and binding affinity of enzyme for template-primer and primer alone have been determined separately. Although the maximal rate of DNA synthesis obtained with poly (C)–oligo (dG) is higher than that for poly (A)–oligo (dT), the binding affinity of the enzyme for poly (C)–oligo (dG) or oligo (dG) is considerably lower than that for poly (A)–oligo (dT) or oligo (dT). Hence, for the more efficient template, poly (C)–oligo (dG), both template-primer and primer bind less tightly to the enzyme.

Reverse transcriptase *Template binding* *DNA synthesis*

1. INTRODUCTION

Native and synthetic double-stranded DNAs, DNA–RNA hybrid templates and RNAs can be used by reverse transcriptase as templates for the synthesis of complementary DNA. Comparison of reaction rates using various synthetic polynucleotides has shown that ribopolymers are in general more efficient templates than deoxyribopolymers for reverse transcriptase. A definite preference has been observed for cytidylate-containing and, to a lesser extent, adenylate-containing hybrid templates for the enzyme from AMV and other mammalian retroviruses [1–4]. The reasons for this preference are not clearly understood. In order to obtain maximum copying of template it is necessary to optimize several reaction conditions such as the type (Mg^{2+} or Mn^{2+}) and concentration of divalent cation, pH, temperature and optimum annealing of template to primer [2,5]. However, variations resulting from these factors do not significantly alter the general trends observed with regard to the efficiency of utilization of different templates by the enzyme. In order to examine possible factors that might contribute to the observed differences, we have carried out detailed studies on the kinetics of DNA synthesis by AMV reverse transcriptase using two template-primers, poly (A)–oligo (dT) and poly (C)–oligo (dG). The results of our findings are presented here.

Abbreviation: AMV, avian myeloblasblastosis virus

2. MATERIALS AND METHODS

Unlabelled deoxynucleoside triphosphates, synthetic templates and primers were obtained from PL Biochemicals. Radioactive deoxynucleoside triphosphates, [*methyl*-^{3}H]TTP (56 Ci/mmol) and [^{3}H] dGTP (8 Ci/mmol) were purchased from Amersham-Searle. Purified AMV reverse transcriptase was purchased from Life Sciences. Enzyme assays were carried out as in [6,7]. Each reaction mixture contained 0.1 mM dGTP or TTP (spec. act. 100 cpm/pmol) and variable amounts of primer and template as described below. All reactions were carried out at 23°C in order to minimize dissociation of template-primer. Template-primers (with different molar ratios in terms of dNMP concentrations) were freshly annealed under optimum conditions prior to use [8,9]. For annealing poly

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(A) and oligo (dT), the required amounts of the components were mixed in 0.2 M NaCl, 0.01 M Tris–HCl (pH 7.4) heated at 55%C for 15 min and cooled slowly to room temperature before use. Poly (C) was annealed to oligo (dG) by mixing the required amounts in 0.2 NaCl, 0.01 M Tris–HCl (pH 7.4), keeping the mixture at 85°C for 5 min and letting the sample cool slowly to room temperature.

3. RESULTS

3.1. Rates of template copying

The rates of poly (A) oligo (dT)instructed and poly (C)–oligo (dG)instructed DNA synthesis by reverse transcriptase were compared at saturating concentrations of precursor dNTP and template-primer at 23°C. The V_m with poly (C)–oligo (dG) is approximately 15-fold higher than the V_m with poly (A)–oligo (dT) (see table 1). Lower values are obtained, particularly with poly (C)–oligo (dG) if the template-primer is not freshly annealed before use.

3.2. Binding of template-primer

In order to examine how the binding affinity of a template-primer to the enzyme affects the efficiency of template copying, binding constants were determined for poly (A)–oligo (dT) and poly (C)–oligo (dG). Using reciprocal plots of initial velocity *vs* template-primer concentrations binding constants for the templates were determined. The results presented in table 1 clearly indicate that there is stronger binding between the enzyme and the less efficient template-primer, poly (A)–oligo (dT). Additional evidence for higher affinity of the enzyme for poly (A)–oligo (dT) was obtained from template competition studies. In the first series of experiments, poly (C)–oligo (dG) was added to reactions in which poly (A)–oligo (dT)-instructed DNA synthesis was monitored. It was observed that the copying of poly (A)–oligo (dT) remained unaffected even at high concentrations of poly (C)–oligo (dG). However, when poly (A)–oligo (dT) was added to reactions in which poly (C)–oligo (dG)-instructed DNA synthesis was monitored, it was observed that poly (A)–oligo (dT) could effectively lower the copying of poly (C)–oligo (dG) (fig. 1A,B).

Table 1

Kinetic constants for template-primers[a]

Template-primer	V_m	K_m
	(pmol.unit^{-1}.min^{-1})	(μg/ml)
Poly (A)–oligo (dT)	55.6	8.7
Poly (C)–oligo (dG)	860	17.0

[a] Enzyme reactions were carried out at template-primer concentrations of 0.8–80 μg/ml as described in section 2. Kinetic constants were obtained from reciprocal plots of initial velocity *vs* template-primer concentration

3.3. Binding of primer

We have also examined the effect of differences in the enzyme-primer binding constants on the efficiency of DNA synthesis with a view to find out how chain initiation events taking place at the primer termini affect the efficiency. In order to determine the binding constants for primer

Table 2

Kinetic constants for primers[a]

	V_m (pmol.unit^{-1}.min^{-1})		K_m (μg/ml)	
	5 μg/ml (T)[b]	10 μg/ml (T)	5 μg/ml (T)	10 μg/ml (T)
Poly (A)–oligo (dT)	27.4	46.5	0.013	0.043
Poly (C)–oligo (dG)	80	176	0.360	1.24

[a] Enzyme reactions were carried out at template-primer ratios of 1:0.002 to 1:0.5 at the template concentrations specified above. Samples were assayed as described in section 2 and kinetic constants were obtained from reciprocal plots of initial velocity *vs* primer concentration

[b] Concentration of template

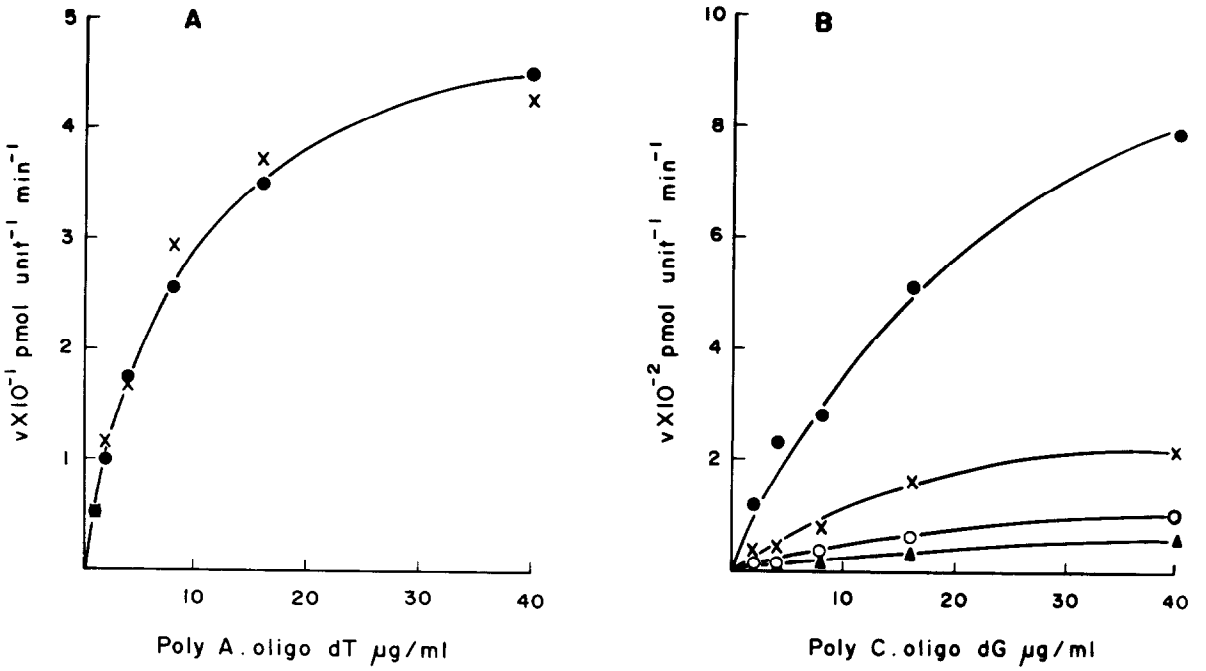

Fig.1. Template exchange experiments with poly (A)–oligo (dT) and poly (C)–oligo (dG). (A) Enzyme samples (0.4 units/25 μl) were preincubated with poly (C)–oligo (dG) (40 μg/ml) for 10 min at 23°C and then assayed for poly (A)–oligo (dT) copying (0.8–40 μg/ml). Activity was determined after 30 min at 23°C as described previously. (●---●) No preincubation with poly (C)–oligo (dG); (×---×) preincubated with 40 μg/ml of poly (C)–oligo (dG). (B) Enzyme samples (0.1 units/25 μl) were preincubated with poly (A)–oligo (dT) (8–40 μg/ml) for 10 min at 23°C and then assayed for poly (C)–oligo (dG) copying (0.8–40 μg/ml) Activity was determined after 30 min at 23°C as described previously (●---●) No preincubation with poly (A)–oligo (dT); (×---×) preincubated with 8 μg/ml poly (A)–oligo (dT); (○---○) preincubated with 20 μg/ml poly (A)–oligo (dT); (▲---▲) preincubated with 40 μg/ml poly (A)–oligo (dT).

molecules, poly (A)–oligo (dT) and poly (C)–oligo (dG) samples with different template-primer ratios but at fixed template concentrations were used for rate determinations. In these experiments the concentrations of template used were below saturation level so that variations in rates with increasing primer concentrations were in the linear range. From table 2 it can be seen that the K_m of the enzyme for oligo (dT) is almost 30 times lower than that for oligo (dG) at the two different concentrations of template employed. A small increase in the value of the primer-binding constants as a function of template concentration is observed for both the template-primers.

4. DISCUSSION

We have here examined the role of template-primer and primer-binding affinity to reverse transcriptase in determining the rates of DNA synthesis. Our results demonstrate a reverse correlation between the affinity of template-primer or primer and rates of DNA synthesis. For the two template-primers studied, poly (A)–oligo (dT) and poly (C)–oligo (dG), binding constants obtained from enzyme activity measurements indicate a tighter binding of the enzyme to the less efficient template-primer, poly (A)–oligo (dT). This trend is clearly more pronounced for the binding of primer to the enzyme where it is observed that oligo (dT) binds almost 30 times tighter than oligo (dG) to the enzyme. Comparative studies could not be carried out with the deoxyribopolymers, poly (dC)–oligo (dG) and poly (dA)–oligo (dT), because of the extremely low efficiency of poly (dA)–oligo (dT) copying, probably due to the for-

mation of triple-helical structures of the polymer in solution [10].

We suggest that a correlation between higher efficiency of template copying and a lower affinity for the polymerase may be expected to be a general phenomenon. For the recognition of a template and for DNA synthesis to proceed a threshold binding affinity would be essential. An increase in the binding affinity would make the rate of synthesis reach an optimal level beyond which any increase in the binding could slow down the rate of synthesis as a result of the slowing down of the movement of the polymerase on the template.

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