Various Specificities of the DNA Dependent RNA Polymerase for Synthetic Templates

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The transcription of the homopolymer templates poly dA : dT and poly dG : dC with RNA polymerase is described. In both cases the pyrimidine strands are transcribed preferably, as shown by studies in which only one or both of the triphosphates complementary to the strands were present as substrates. A poly dA : dT dependent poly A synthesis which had reached a plateau could no longer be stimulated by addition of new poly dA : dT. If instead poly dG : dC and GTP were added, the beginning of a poly G synthesis could be observed.

The beginning of the DNA dependent RNA synthesis has a decisive regulatory meaning for the living cell, but the definitive explanation of the question in regard to the composition and the structure of the starting points for the DNA dependent RNA polymerase is still unsettled. Until to the present time several authors have noted that DNA of various origins have a definite number of binding sites for RNA polymerase. It is also well known that pyrimidine clusters occur in various DNA transcription of A-DNA regions rich in AT base pairs were preferred. In the present work we were interested in the modes of reaction of the RNA polymerase on synthetic templates. We found that the previously observed preferred transcription of the pyrimidine strands of poly dG: dC and of poly dTC : dAG were also true in the case of poly dA : dT. Moreover, considerable differences in the strand specificity of the RNA polymerase were shown whether only one or both of the triphosphates complementary to the strands were present as substrates. In comparing the properties of the templates poly dA : dT with those of the poly dG : dC it became evident that a poly A synthesis which has come to a stand still on poly dA : dT can no longer be stimulated by addition of new poly dA : dT. If instead poly dG : dC and GTP are added to the reaction mixture, the beginning of a poly G synthesis is to be observed.

Materials and Methods

ATP, phosphoenolpyruvate and pyruvate kinase were purchased from C. F. Boehringer, Mannheim, Germany; CTP, GTP and UTP were purchased from PL Biochemicals, Inc., Milwaukee, Wis., USA; 14C-ATP, 14C-CTP, 14C-GTP, and 14C-UTP were purchased from Schwarz Bioresearch, Inc., Orangeburg, N. Y., USA.

RNA polymerase (E.C. 2.7.7.6) was purified from E. coli according to the method of Zillig et al. up to the sucrose gradient step. Its specific activity was determined by Zillig's definition and protein by the method of Lowry.

The incubation mixtures contained in all cases: 0.03 M tris-acetate, pH 7.9; 0.12 M NH4Cl; 0.03 M Mg-acetate; 0.02 M phosphoenolpyruvate (Na-salt); 20 μg/ml pyruvate kinase. The concentration of template is given by optical density units (260 μm) per incubation volume. The amount of used triphosphates, templates and enzyme is seen from the text below the figures. The mixtures were preincubated for 10 min. at 37° without enzyme; incubation temperature was 37°.

To follow the progress of polymerisation aliquots of 0.025 ml were taken after certain time intervals and applied to paper strips (2 x 10 cm²) (DEAE-cellulose
Whatman DE 81. After elution with 0.3 M ammonium formate and drying, the polymeric material remaining at the start was counted in a liquid scintillation counter (Tricarb, Mod. 4312).

The templates poly dA:dT and poly dG: dC were prepared with DNA polymerase (E.C. 2.7.7.7), the first one using chemically synthesized d(pA)₉ and d(pT)₁₂ as primer, the second one in the unprimed reaction of that enzyme.

Results

a) Higher specificity of RNA polymerase for the pyrimidine strands in poly dA: dT and poly dG: dC

To decide whether one of the two homopolymer strands of poly dA: dT and poly dG: dC is transcribed preferably, the following experiments were undertaken: 1) The kinetics of RNA polymerase in presence of only one of the triphosphates complementary to the base of the template being transcribed. 2) As in 1) but in presence of both ATP + UTP respectively CTP + GTP. The triphosphates were used in all cases in saturation concentration.

3) With poly dA: dT moreover a template saturation curve was taken (Figs. 1, 2 and 3).

![Graph](Image)

Fig. 1. Incorporation of AMP and UMP with RNA polymerase on poly dA: dT template. ××× AMP incorporation in presence of ATP, △△△△ AMP incorporation in presence of ATP + UTP, ○○○○ UMP incorporation in presence of UTP, •••• UMP incorporation in presence of UTP + ATP. The incubation mixtures (1.0 ml) contained besides the essential salts (cf. Materials and Methods): RNA polymerase (specific activity 470 U/mg protein), 90 μg protein; poly dA: dT, 1.4 O.D. units; triphosphates: 1) 2 mM ¹⁴C-ATP (specific activity 0.125 mCi/mM) (××××); 2) 1 mM ¹⁴C-ATP (specific activity 0.25 mCi/mM) + 1 mM UTP (△△△△); 3) 2 mM ¹⁴C-UTP (specific activity 0.25 mCi/mM) + 1 mM ATP (××××); 4) 1 mM ¹⁴C-UTP (specific activity 0.575 mCi/mM) + 1 mM ATP (○○○○).

![Graph](Image)

Fig. 2. Incorporation of CMP and GMP with RNA polymerase on poly dG: dC template. ×××× GMP incorporation in presence of GTP, △△△△ GMP incorporation in presence of GTP + CTP, ○○○○ CMP incorporation in presence of CTP, •••• CMP incorporation in presence of CTP + GTP. The incubation mixtures (1.0 ml) contained besides the essential salts (cf. Materials and Methods): RNA polymerase (specific activity 200 U/mg protein), 120 μg protein; poly dG: dC, 1.28 O.D. units; triphosphates: 1) 2 mM ¹⁴C-GTP (specific activity 0.39 mCi/mM) (××××); 2) 1 mM ¹⁴C-GTP (specific activity 0.78 mCi/mM) + 1 mM CTP (△△△△); 3) 2 mM ¹⁴C-CTP (specific activity 0.31 mCi/mM) (○○○○); 4) 1 mM ¹⁴C-CTP (specific activity 0.62 mCi/mM) + 1 mM GTP (●●●●).

From Figs. 1 and 2 it is evident that in comparison with the pyrimidine strands of the used templates RNA polymerase has a higher specificity than with the purine strands. Moreover a change in the strand specificity in presence of the second complementary triphosphate is noticeable. If one plots a Lineweaver-Burk plot like Fig. 3, one then notices that v_max for the incorporation of AMP is about five times as large as v_max for the incorporation of UMP under the given conditions.

14 A. LEZIUS, Göttingen, unpublished results.
Rate of Incorporation of AMP and UMP

The enzyme per se could take place in the same period.

Fig. 3. Rate of incorporation of AMP (×××) or UMP (○○○) on increasing poly dA:dT template concentration. The incubation mixtures (0.5 ml) contained besides the essential salts (cf. Materials and Methods): RNA polymerase (specific activity 300 U/mg protein), 64 μg protein; increasing amounts of poly dA:dT; 2 mM ¹⁴C-ATP (spec. activity 0.16 mC/mM) (×××) respectively 2 mM ¹⁴C-UTP (spec. activity 0.29 mC/mM ○○○). After incubation for 15 min. at 37°, the reaction mixture was cooled by an ice bath and the reaction stopped by addition of 0.1 ml of an aqueous solution of 2.5 mg/ml bovine serum albumine, 1.0 ml water and 1.6 ml of trichloracetic acid (10% solution). The acid insoluble material was filtered on membrane filters (MF 50, Sartorius Membranfiltergesellschaft Göttingen) and repeatedly washed with ice cold TCA (5% solution). After drying the radioactive material was counted in a liquid scintillation counter.

b) Various template behaviour of poly dA : dT and poly dG : dC against RNA polymerase

Under similar conditions sometimes poly A was synthesized on poly dA : dT and sometimes poly G on poly dG : dC. Whereas the poly A synthesis reaches a plateau after about 60 min., the poly G synthesis continues during the period of observation (Fig. 4).

In order to explain the reason for the plateau in the case of the poly A synthesis in one case new poly dA:dT and ATP were added to the reaction, which had come to a stand still, and in the other case poly dG : dC and GTP.

From Figs. 5 and 6 it is evident that the poly A synthesis which has come to a stand still cannot again be stimulated by addition of new poly dA : dT. Addition of poly dG : dC and GTP in this place leads on the contrary to the beginning of a poly G synthesis. Through the control experiments, which originally contained no poly dA : dT, one can see clearly that it is impossible that an inactivation of
Fig. 6. Incorporation of AMP (XXX) on poly dA:dT template and after 90 min. of GMP (OOO) on poly dG:dC template. Control: Incorporation of AMP (VVV) in absence of poly dA:dT and after 90 min. of GMP (●●●) on poly dG: dC template. The incubation mixtures (0.5 ml) contained besides the essential salts (cf. Materials and Methods): RNA polymerase (spec. activity 400 U/mg protein), 10 μg protein; poly dA:dT, 0.83 O.D. units; 1 mM 14C-ATP (spec. activity 0.16 mC/mM). Work up as in Fig. 5. After 90 min. 0.25 ml of a solution of the essential salts, which contained 0.86 O.D. units of poly dG:dC and 1 μmole 14C-GTP (spec. activity 0.58 mC/mM) were added.

Discussion

The results of the experiments described under a) are a further hint that RNA polymerase begins the transcription of a DNA template on pyrimidine clusters. Analogous to the results of MAITRA and HURWITZ, the poly A synthesis, which is preferred to the poly U synthesis, should have its reason for being in that on the dT strand of the poly dA : dT more chains are initiated that on the dA strand. Thus, the enzyme has a higher affinity to the dT strand than to the dA strand or — in general — a higher affinity to the pyrimidine strand than to the purine strand.

The apparently inhibiting influence of a triphosphate on the incorporation of a second triphosphate, here complementary, is as yet relatively little understood. BERG and CHAMBERLIN already described this phenomenon by the use of poly dAT as template for RNA polymerase, and our own experiments showed that ATP was also able to inhibit the incorporation of GMP on poly dG : dC. To what extent the inhibition of the used triphosphates depends on the character of the triphosphate must be shown by further research.

The comparison of the poly dA : dT dependent poly A synthesis with the poly dG : dC dependent poly G synthesis shows that the initial rate of the poly A synthesis is about twice as great as that of the poly G synthesis. In a relatively short time the AMP incorporation reaches a plateau, whereas the GMP incorporation continues. In the case of the poly A synthesis the rise of the plateau is, according to RILEY et al., explainable by the formation of a triple strand between poly dA :dT and poly rA, on which the enzyme is arrested, whereas poly dG : dC can form no corresponding triple strand with poly rG.

On the basis of this result the following two experiments were made possible. In the first one (Fig. 5) it is evident that the poly dA : dT dependent poly A synthesis, which has come to a stand still, can no longer be stimulated by addition of new poly dA : dT. This result confirms that of Bremer and Konrad, which they discovered on native DNA. According to their experiments the DNA dependent RNA synthesis which had reached a plateau could no longer be stimulated by addition of new DNA. On the other hand a resumption of polymerisation takes place with the addition of poly dG : dC and GTP to the poly A synthesizing system, which had come to a stand still. This new synthesis cannot be explained by the fact that the affinity of RNA polymerase to poly dG : dC is higher than to poly dA : dT. For the enzyme which becomes free in the equilibrium from the complex poly dA : dT-enzyme should equally well begin a new synthesis on the new poly dA : dT as on the poly dG : dC. The results could be explained with the supposition of two enzyme species or two binding sites on the enzyme, from which the one for poly dA : dT, the other for poly dG : dC is specified.

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16 H. MAITRA and F. Cramer, Göttingen, unpublished results.
17 M. RILEY et al., J. molecular Biol. 28, 359 [1966].