INHIBITION OF *E. COLI* RNA POLYMERASE BY POLYADENYLIC ACID

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1. Introduction

Some environmental conditions, such as amino acid starvation of RC^{str} *E. coli* cells, or those occurring after purine deprivation in purine auxotrophs, lead to a generalized inhibition of bacterial gene expression. Under these conditions, the synthesis of several species of RNA (transfer, ribosomal and messenger) is inhibited [1–3]. One of the possible explanations for the phenomenom could be that RNA polymerase becomes inactive.

Recent work indicated that treatment of partially purified preparations of *E. coli* RNA polymerase with ATP and Mg^{2+}, leads to a reversible inactivation of RNA polymerase [4]. It was observed that in the course of the inactivation some adenylic residues became covalently bound to a macromolecule insoluble in trichloroacetic acid. When the inactivation reaction was performed in the presence of ^{32}PαATP, it was shown that the labelled macromolecule cochromatographed with the polymerase in DEAE cellulose. In addition, both the label and the enzyme sedimented together in sucrose gradients. This suggested the existence of a strong degree of association between the enzyme and the radioactive compound.

A portion of this compound, that was recovered in the aqueous phase after phenol extraction, appeared to be polyadenylic acid (poly A) [5] according to the following criteria: 1) resistance to RNAse and DNAse; 2) selective adsorption to nitrocellulose filters using the procedure of Lee et al. [6]; and 3) lability to alkaline treatment (1 N NaOH; 37°). Therefore, it seems that under the conditions for the polymerase inactivation, partially purified enzyme preparations are able to carry out poly A synthesis. In fact, these preparations contain besides RNA polymerase an ATPase [7] and polynucleotide phosphorylase which enable the synthesis of poly A from ATP.

For this reason, the effect of this polynucleotide on the RNA polymerase catalized reaction was further explored.

2. Materials and methods

RNA polymerase was purified from *E. coli* A 19 by the method of Babinet [8] up to the step S_I. DEAE column chromatography of this fraction was carried out according to Avila et al. [9]. After this step the polymerase preparation was further purified by P-cellulose column chromatography [10]. The enzyme thus obtained was free from any polynucleotide phosphorylase contamination.

All experiments were carried out in the presence of sigma factor [11]; the ratio of transcription between native calf thymus and T_4 DNA was 0.71.

Enzyme assay: the enzyme protein (4 μg) and variable amounts of poly A were preincubated for 10 min at 0° in the presence of 170 mM KCl, 20 mM NaCl, 0.06 mM dithiothreitol, 0.06 mM EDTA, 30 mM Tris-HCl (pH 7.9), and 3% glycerol (v:v). The

Table 1
Kinetic parameters of the inhibition of *E. coli* RNA polymerase by different polynucleotides.

Polynucleotide	Inhibition by 1.4 μg/ml of polynucleotide		$I_{0,5}$	
	"Low Salt"	"High Salt"	"Low Salt"	"High Salt"
	(%)	(%)	(μg/ml)	(μg/ml)
Poly A	75	71	0.6	0.6
Poly U	66	1	0.9	15
Poly C	40	0	1.0	–
tRNA	49	27.5	1.4	6

The results correspond to the determinations carried out either in the presence of 74 mM ("low salt") of 200 mM of KCl ("high salt"). $I_{0,5}$: concentration of the polynucleotide that gives one half of the maximum inhibition of polymerase activity.

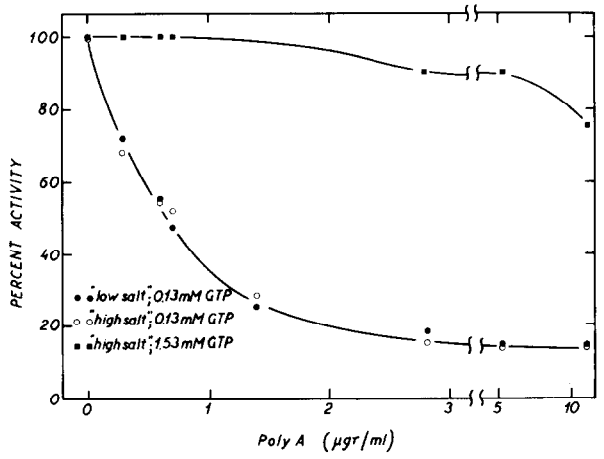

Fig. 1. RNA polymerase activity as a function of poly A concentration. The reactions were carried out at "low" (●) or "high" (○, ■) salt concentration in the presence of 0.133 (●, ○) or 1.53 mM GTP (■). Other conditions were as those indicated under Materials and methods. One hundred percent corresponds to enzyme activity assayed at 0.133 mM nucleoside triphosphates, and in the absence of poly A (10 nmoles per min per mg protein).

total volume was 0.065 ml. The polymerase reaction was then initiated by the addition of the other components to give the following concentrations: 33 μg/ml T_4 DNA, 0.133 mM CTP, GTP, UTP and ATP, 3.3 mM $MnCl_2$, 50 mM Tris-HCl (pH 7.9), 0.025 mM EDTA, 0.025 mM dithiothreitol, 7 mM mercaptoethanol, 5 mM NaCl, 7 mM NH_4Cl and either 74 mM ("low salt") or 200 mM ("high salt" concentration) of KCl. The total volume was 0.15 ml. In addition, either ^{3}H-CTP or ^{3}H-UTP (specific activity 10 μCi per μmole) were used as the labelled substrate. Incubations were at 37° for 10 min and the reactions were stopped and counted for radioactivity as described previously [4].

Polynucleotide samples were obtained from Miles Laboratories, Inc.

3. Results and discussion

Poly A is a strong and specific inhibitor of the *E. coli* RNA polymerase as suggested by the following evidence:

i) The polynucleotide is a more efficient inhibitor of *E. coli* polymerase than the other RNA species studied. Fig. 1 shows the inhibition curves of the reaction carried out either at "low" or "high salt" concentration in the presence of different amounts of poly A. It can be observed that half maximum inhibition either at "low" or "high salt" concentrations was obtained at 0.6 μg/ml poly A. This value is lower than that of the other RNA species (table 1).

ii) Poly A inhibits the reaction in the presence of 0.2 M KCl. It seems likely that if the inhibition plays a role in the regulation of RNA transcription it must operate at KCl concentrations about 0.2 M [12]. Under this condition, that approaches physiological salt levels [13], transcription of DNA templates is more specific [14, 15]. As it is shown in table, this salt concentration does affect the inhibition by poly

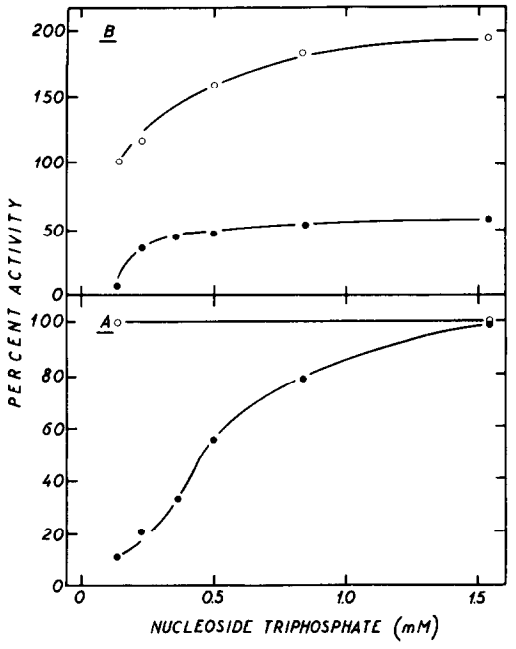

Fig. 2. RNA polymerase activity as a function of GTP (A) or ATP (B) concentration. Reactions were carried out at "high salt" concentration in the absence (○) or presence (●) of poly A (2.8 μg/ml). GTP and ATP were added as Mn^{2+} salts. Other conditions were as those indicated in fig. 1.

Under the same conditions inhibition by the other RNA species is almost reversed.

iii) Poly A inhibition of RNA polymerase is sensitive to GTP concentration. Recent evidence indicated that RNA accumulation in bacterial cells is very sensitive to GTP levels [1, 2]. At 0.2 mM intracellular GTP, the accumulation of RNA is abolished. Fig. 2 shows that in the physiological range of GTP concentrations, increasing levels of the nucleotide diminish the inhibition by poly A. In fact, GTP concentrations about 1.5 mM almost reverse the inhibitory effect (fig. 1). This reversion is observed in the presence of "high salt" but not at "low salt" concentration. On the other hand, increasing concentrations of ATP, the other initiator nucleotide, stimulate polymerase activity but they have not an important effect on the enzyme inhibition by poly A.

Therefore, it seems that the overall inactivation of RNA polymerase at "high salt" concentration, has two requirements: 1) an adequate amount of poly A; and 2) low concentration of GTP.

Previous evidence indicated that amino acid starvation in RC^{str} bacterial strains produces a drastic decrease in the GTP pool and a moderate shrinkage of the ATP pool [1, 2]. In these conditions RNA accumulation is sharply reduced. This inhibition of RNA synthesis cannot be simply accounted for by the level of nucleoside triphosphates available for the RNA polymerase-catalyzed reaction. However, the arrest in RNA synthesis could be explained by accepting that GTP modulates polymerase inhibition by a ligand such as poly A. This control mechanism would also explain the decrease of the RNA content at low bacterial growth rates [16].

The possible existence of poly A in bacterial cells is under study in this laboratory.

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