THE STIMULATIVE EFFECT OF POLYETHYLENIMINE ON THE REACTION OF RNA POLYMERASE FROM CAULIFLOWER

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SUMMARY

The in vitro RNA synthesis and poly(A) synthesis catalyzed by cauliflower RNA polymerase are stimulated by an addition of polyethylenimine (PEI) at a low concentration to the reaction medium. Evidence is presented that PEI exerts its stimulative effect on a reaction coexisting of enzyme, template, and substrate, and not on the template or enzyme alone.

INTRODUCTION

Fractionation of the crude extract with polyethylenimine to purify the DNA-dependent RNA polymerase from Escherichia coli and wheat germ has been reported to result in higher yields and the complete removal of nucleic acids (1-3). We used PEI for the purification of cauliflower RNA polymerase, and have noticed that PEI might bring about an enhancement of RNA synthesis. Experiments were designed to study the effect of various concentrations of PEI on in vitro RNA and purine-homopolymer, poly(A) and poly(G), synthesis catalyzed by DNA-dependent RNA polymerase. This report shows that cauliflower RNA polymerase I is stimulated by low concentrations of PEI in the reaction medium and that stimulation take place only in the coexistence of enzyme, template and substrate, probably during chain elongation.

MATERIALS AND METHODS

Cauliflower RNA polymerase was prepared essentially according to the procedure described previously (4, 5). Enzyme I was obtained after chromatography on DEAE-cellulose DE-52 or DEAE-Sephadex A-25, and further

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Abbreviation : PEI, polyethylenimine

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separated into two subfractions by chromatography on CM-Sephadex C-25, the IA fraction being eluted at 0.075 M ammonium sulfate in TGMED-buffer (50 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 25 % glycerol)and the IB fraction at 0.15 M. The IA fraction was mainly used in this experiment. The activity for RNA synthesis was about 39 pmoles ^{3}H-UTP incorporation/mg protein/10 min. E. coli RNA polymerase was prepared according to Chamberlin and Berg (6) and was purified to the level of their fraction IV.

Assays of enzyme activity were performed as described previously (5) in the following two standard reaction mixtures in a total volume of 0.125 ml. 1) The reaction mixture for RNA polymerase activity contained : 40 mM Tris-HCl pH 7.5 at 20°C, 1 mM $MnCl_2$, 2 mM $MgCl_2$, 12 mM 2-mercaptoethanol, 0.4 mM each of ATP, GTP, CTP and 0.2 mM [^{3}H]-UTP (40 µCi/µmole, 14 cpm per pmole), 40 µg/ml denatured calf-thymus DNA and the enzyme solution (50 µl). 2) The reaction mixture for poly(A) polymerase or poly(G) polymerase activity contained : 40 mM Tris-HCl pH 7.5 at 20°C, 5 mM $MnCl_2$, 15 mM $MgCl_2$, 12 mM 2-mercaptoethanol, 0.4 mM [^{3}H]-ATP for poly(A) polymerase activity or [^{3}H]-GTP for poly(G) polymerase activity (each 20 µCi/µmole), 40 µg/ml denatured calf-thymus DNA or synthetic poly(U) or poly(C) and the enzyme solution (50 µl). After incubation for 10 or 20 min at 40° C for the RNA polymerase and poly(G) polymerase assay, and at 30° C for the poly(A) polymerase assay, 100 µl of the incubation mixture was assayed using the paper-disc method (Whatman 3-MM, 24 mm) described in a previous paper (4). Enzyme activity is expressed as the cpm incorporated under the given assay conditions.

RESULTS

Experiments with cauliflower RNA polymerase showed that the presence of PEI at a low concentration in the RNA synthesizing medium stimulated the incorporation of labeled UTP when denatured DNA was used as the template (Fig. 1A). Maximum stimulation was reached at a concentration of 2×10^{-3} % PEI. Additions of PEI at concentrations higher than 4×10^{-3} % resulted

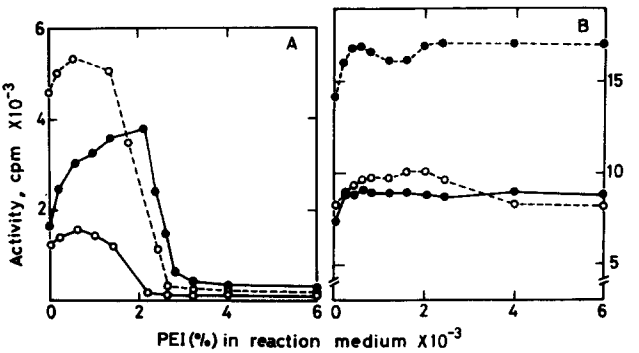


Fig. 1. Effect of PEI on in vitro RNA synthesis and purine-homopolymer synthesis at various concentrations of PEI. A, purified RNA polymerase I from cauliflower was assayed in standard reaction systems with denatured DNA as the template. ●——●, RNA polymerase activity ; O- - -O, poly(A) polymerase activity ; O——O, poly(G) polymerase activity. B, E. coli RNA polymerase was assayed with the reaction medium used for the cauliflower enzyme. ●——●, RNA polymerase activity with denatured DNA as the template ; ●- - -●, RNA polymerase activity with native DNA ; O- - -O, poly(A) polymerase activity with denatured DNA.

in a decrease in incorporation. A non-enzymatic assay showed a negligible incorporation in the low concentration range of PEI. Poly(A) polymerase and poly(G) polymerase activities were also stimulated by low concentrations of PEI, though the rates of increase were less than that for RNA synthesis when denatured DNA was used as the template ; the optimum concentration was 2×10^{-4} and 6×10^{-4} %, respectively. When synthetic poly(U) was used as the template, a more stimulative effect for poly(A) synthesis, as described below, was found than when DNA was used as the template. The optimal concentration was 1.2×10^{-3} %.

Experiments with E. coli RNA polymerase showed a pattern for PEI-stimulation different from that of the cauliflower enzyme (Fig. 1B). RNA polymerase activity and poly(A) polymerase activity were slightly stimulated (the maximum rates were 20 % and 26 %, respectively) over a low concentration range, but a non-inhibitory effect was found at a higher concentration, such as 1×10^{-2} %.

In another series of experiments, the effect of PEI on the three enzyme

Table 1. Effect of PEI on three polymerase activities by cauliflower enzyme I with different templates

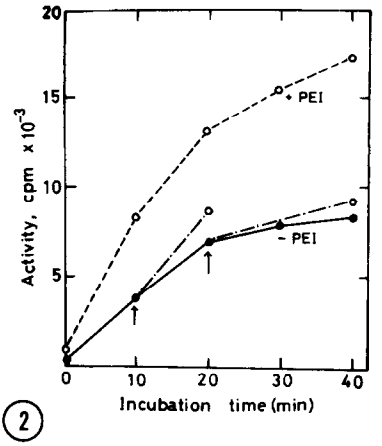
	RNA polymerase		Poly(A) polymerase		Poly(G) polymerase	
	Denatured DNA	Native DNA	Denatured DNA	Poly(U)	Denatured DNA	Poly(C)
- PEI	1824	380	4624	5790	2141	4215
+ PEI*	2989	1131	5960	20272	2710	4527
Increase (%)	164	298	129	355	127	107

* Concentration of PEI added to the reaction medium for RNA polymerase, Poly(A) polymerase, and poly(G) polymerase were 2×10^{-3} %, 2×10^{-4} %, and 6×10^{-4} %, respectively.

activities [RNA polymerase, poly(A) polymerase and poly(G) polymerase] using different templates was investigated at the optimal concentrations for stimulation of respective enzyme. The results are summarized in Table 1. The presence of PEI during RNA synthesis directed by native DNA causes an incorporation of [^{3}H]-UTP about three fold that of the control. Poly(A) polymerase activity is also increased with PEI, especially when the synthetic homopolymer, poly(U), is used for the template. Poly(G) polymerase activity is also stimulated, but no significant increase was found when synthetic poly(C) was used.

The effect of PEI on the time course of the poly(A) synthesizing reaction was tested in an incubation at 30° C. Figure 2 shows that the high stimulative efficiency of PEI is even more apparant after longer reaction times. When PEI is added in the middle of incubation, the extent of incorporation is increased. An addition at 10 min brings about a higher stimulation rate than one at 20 min.

The effect of poly(U) concentration as the template in the PEI-stimulation of poly(A) synthesis was examined in a standard reaction system with an optimum concentration of PEI. As shown in Figure 3, at a low concentration



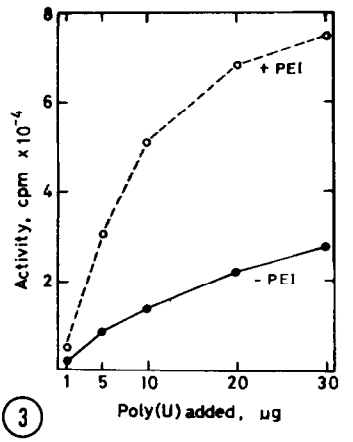


Fig. 2 (left). Time course of poly(A) polymerase activity stimulated with PEI. Cauliflower enzyme I was assayed with synthetic poly(U) as the template. The arrow indicates an addition of PEI to the PEI-free medium.

Fig. 3 (right). Dependence of poly(A) polymerase activity on the poly(U) template. Cauliflower enzyme I was assayed with the standard reaction medium, using various concentrations of poly(U) as the template.

range, below 10 µg in the assay system, the stimulation rate by PEI increases with the increment in poly(U) concentration, but the rate decreases at a higher concentration. This decrease is assumed to be due to a too great incorporation of ^{3}H-labeled nucleotide in the paper disc method.

Models showing the mechanism of the PEI-effect may be divided into three general classes, according to whether PEI modifies the enzyme, template or substrate. Thus, PEI was pre-incubated with the enzyme or the template, followed by incubation for poly(A) synthesis. Table 2 shows that the poly(A) synthesizing activity directed by the pre-incubated enzyme or template has decreased.

DISCUSSION

These results demonstrate that PEI is able to stimulate the synthesis of RNA and poly(A) catalyzed by cauliflower RNA polymerase *in vitro* at a low concentration of PEI, and to inhibit synthesis at high concentrations. The pattern of stimulation with various concentrations of PEI differs from that

Table 2. Effect of PEI on the incorporation of ^{3}H-ATP during PEI-preincubation with the enzyme or template

Exp.	Preincubation	Incubation for poly(A) synthesis	Incorporation cpm
1	Enzyme	poly(U) + ^{3}H-ATP	2039
	Enzyme	poly(U) + ^{3}H-ATP + PEI	11616
	Enzyme + PEI	poly(U) + ^{3}H-ATP	8891
	No enzyme	poly(U) + ^{3}H-ATP + PEI	50
2	poly(U)	Enzyme + ^{3}H-ATP	2783
	poly(U)	Enzyme + ^{3}H-ATP + PEI	13828
	poly(U) + PEI	Enzyme + ^{3}H-ATP	8995
3	Enzyme + poly(U)	^{3}H-ATP + PEI	9135
	Enzyme + poly(U) + PEI	^{3}H-ATP	5468
4	^{3}H-ATP	poly(U) + PEI + Enzyme	13192
	^{3}H-ATP + PEI	poly(U) + Enzyme	14427

Poly(A) polymerase activity by cauliflower enzyme I was assayed with the poly(U) template. Preincubation was at 24° C for 20 min, followed by incubation for the poly(A) synthesizing system at 30° C for 10 min.

of E. coli RNA polymerase (Fig. 1). No inhibition at a high level of concentration was found in the experiment with the E. coli enzyme. Probably, the E. coli enzyme is resistant to such a high cencentration of PEI. As shown in Figure 2, the stimulative effect of PEI seems to more marked when the PEI is added early in the incubation rather than at a late point. This means that the stimulative incorporation of labeled substrate can not be attributed to a non-enzymatic artifact (e. g. that PEI would support such small nucleotide-chain products synthesized that is not measureable by the present paper-disc method). Since pre-incubation of PEI with the enzyme or template does not result in an increase in stimulation (Table 2), it is unlikely that PEI acts to change enzyme or template properties for stimulation. The increase takes place only with the co-existence of the enzyme, template and substrate. Therefore PEI may act mainly on chain elongation.

However, the RNase included in the enzyme fraction should be inhibited by PEI, but since the crude enzyme, before separation by column chromatography, also showed a pattern of stimulation similar to that of the purified enzyme fraction, the possibility of RNase inhibition is negligible.

PEI is a synthetic compound and does not exist in vitro. However, the marked increase in RNA and poly(A) syntheses during chain elongation may prove useful in the study of artificial RNA synthesis.

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