DNA-DEPENDENT POLY (G) SYNTHESIZING ACTIVITIES IN DNA-DEPENDENT RNA POLYMERASE FRACTIONS FROM CAULIFLOWER INFLORESCENCE

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SUMMARY

DNA-dependent RNA polymerase fractions isolated from cauliflower inflorescence were found to be accompanied with poly (A) and poly (G) synthesizing activities in the presence of denatured calf-thymus DNA. The dialysis against Tris-HCl buffer in the absence of Mg^{++}, dithiothreitol (DTT) and EDTA resulted in a great increase of poly (A) polymerase activity while RNA polymerase and poly (G) synthesizing activities decreased.

INTRODUCTION

Poly (A) polymerase activity in the presence of DNA has been reported in the RNA polymerase fraction of procaryotes (1, 2, 3) and of eucaryote (4). There is, however, little poly (G) synthesizing activities (1, 2, 3). When a mixture of synthetic homopolymers of deoxyguanylic and deoxycytidylic acids is used as primer, some amounts of labeled GTP incorporation has been found in Eshcherichia coli RNA polymerase (5). Poly (G) polymerase induced in E. coli by RNA bacteriophage has been observed in the presence of poly (C) as a primer. Isolated nuclei from chick muscle are capable of catalyzing the synthesis of homopolymeric guanylic and adenylic acids (7). Recently, naturally occurring polyadenylate and polyguanylate have been isolated from mouse liver (8, 9). These findings suggest the existence of poly (G) polymerase which requires a natural primer in eucaryotes. The inflorescence of cauliflower (Brassica oleracea var. botrys) exhibits a peculiar hypertrophic differentation with continuous growth, and DNA-dependent RNA polymerase can be easily isolated (10). We have examined a homopolymer synthesizing capacity of the RNA polymerase fractions. High incorporating activity of labeled-ATP and -GTP was observed in DEAE-purified fractions,

and the dialysis against Tris-HCl buffer in the absence of Mg^{++}, DTT and EDTA resulted in a great increase of poly (A) synthesizing activity. Our observations are described here as a preliminary report.

MATERIALS AND METHODS

Chemicals. Radioactive [^{14}C]-ATP, -UTP,-GTP and -CTP were obtained from the Radiochemical Centre of Amersham. Unlabeled nucleoside triphosphates, highly polymerized calf-thymus DNA and yeast RNA (type XI) were purchased from Sigma Chemical Co., yeast transfer RNA from Boehringer and Mannheim Co., pancreatic ribonuclease (3000 units/mg) from Worthington Biochemical Co., and DEAE-cellulose DE-52 from Whatman Co. All other chemicals were reagent grade.

Preparation of RNA polymerase. DNA-dependent RNA polymerase fractions were isolated from apical part of the cauliflower head with an improved procedure of the method described in the previous paper (10). 100 g of frozen tissue were homogenized with TGMED-buffer (50 mM Tris-HCl pH 8.0, 5 mM $MgCl_2$. 0.1 mM EDTA, 1 mM DTT, 25% Glycerin) containing 0.6 M ammonium sulfate and 5 g of Polyclar-AT (polyvinylpyrrolidone). Then 20 g of polyclar-AT was mixed with 200 ml of the homogenate, and centrifuged at 15,000 g for 40 min. The supernatant was centrifuged again at 105,000 g for 60 min. The supernatant was brought to 43% saturation of ammonium sulfate by addition of saturated ammonium sulfate solution. After centrifugation at 15,000 g for 40 min, the supernatant was dialyzed overnight against TGMED-buffer. It was centrifuged at 15,000 g for 40 min to remove turbid material formed during dialysis. The supernatant was loaded on DEAE-cellulose columns and washed with 150 ml of TGMED-buffer. Stepwise elution was carried out with 150 ml of TGMED-buffer containing 0.12 M KCl, followed by a linear gradient of KCl from 0.12 M to 0.4 M in TGMED-buffer.

Assay for polymerase activity. The standard reaction mixture for RNA polymerase contained in 0.125 ml : 5 μmoles of Tris-HCl pH 8.0, 0.125 u

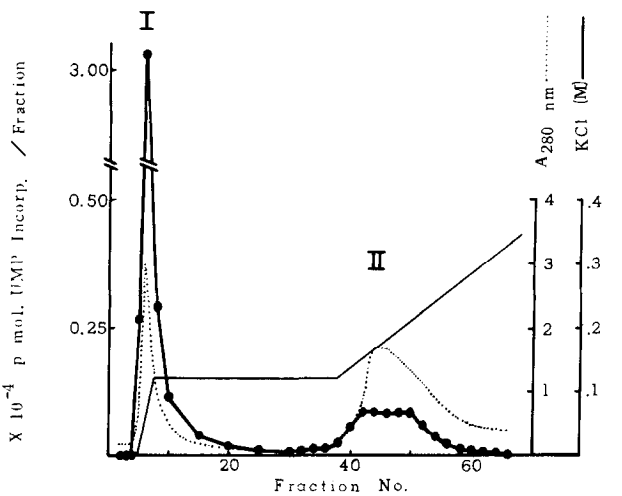

Fig. 1. DEAE-cellulose chromatography of cauliflower RNA polymerase.

A 180-mg sample of protein from the 43 % ammonium sulfate fraction was applied to a 1.5 x 15 cm DEAE-cellulose column. The enzymes were eluted with the conditions described in text. 5-ml fractions were collected. RNA polymerase activity (●——●) was assayed in standard reaction mixture.

moles of $MnCl_2$, 1.0 μmole of $MgCl_2$, 1.5 μmoles of 2-mercaptoethanol, 50 nmoles of each of ATP, GTP, CTP and 25 nmoles of [^{14}C]-UTP (8 μCi/μmole), 5 μg of heat-denatured calf-thymus DNA (single strand was defined from melting profile), and 75 μl of enzyme solution. The reaction mixture for the homopolymer synthesizing system was the same as that for RNA polymerase activity except that [^{14}C]-UTP was replaced by an equal amount of appropriate [^{14}C]-labeled nucleoside triphosphate in the absence of the other three cold substances. Incubation lasted for 10 min at 37°C, and 100 μl of the reaction mixture was pipetted onto a Whatman 3-MM filter paper (2.4 cm disc). After 30 seconds, the discs were dropped into 5 % CCl_3COOH containing 10 mM sodium pyrophosphate (5 ml/disc), and washed 5 times with the same acid solution, followed by ethanol washes 3 times, and air-dried for 15 min. The radioactivity of the disc was measured with a scintillation counter. Enzyme activity was expressed as p moles of nucleotides incorporated in 10 min at 37°C under the assay conditions. Corrections of the activity values were made for zero-time controls.

RESULTS AND DISCUSSION

Chromatography of the RNA polymerase fraction. Chromatography on DEAE-cellulose using stepwise elution followed by a gradient elution with increasing concentration of KCl resolved the enzyme into at least two peaks of polymerase activity (Fig. 1). The first activity peak (DEAE-polymerase I) was eluted with 0.12 M KCl-stepwise elution, and the second peak (DEAE-polymerase II) was eluted with 0.15 M to 0.20 M KCl. The activity of the first peak was insensitive to α-amanitin, and probably corresponded to the nucleolar RNA polymerase I or A (11, 12). The much higher activity than that of the second peak is a problem to study which is now in progress.

Poly (A) and poly (G) synthesizing activities. Even with crude extracts, single nuclotide incorporation with ^{14}C-labeled ribonucleoside triphosphate as a substrate into acid-insoluble material has been found in the present assay conditions (Table 1). Fractionation by ammonium sulfate precipitation (43 % saturation) resulted in increase in their specific activities, especially in poly (A) and poly (G) synthesizing activities. On DEAE-cellulose column chromatography, the first peak has a higher specific activity of poly (A) and poly (G) formation, as well as RNA polymerase activity, than poly (U) and poly (C) forming activities. The leading part of peak II gave also high specific activity of poly (A) and poly (G) formation. It was recognized that the peak of poly (A) polymerase activity had been eluted in somewhat ealier fractions than that of RNA polymerase activity in either peak I and II, suggesting the presence of some different enzymatic entities between poly (A) and RNA polymerases.

Effect of dialysis on the specific activity of poly (A) and poly (G) formation.

As seen in Table 2, dialysis against Tris-HCl buffer in the absence of Mg^{++}, DTT and EDTA resulted in more than 2-fold increases of poly (A) synthesizing activity, while poly (G) and RNA synthesizing activities were somewhat decreased. It may be suggested that poly (A) and poly (G) polymerase could not act as purine polymerase, but act as functional sites for synthe-

Table 1. Homopolymer synthesizing activities during the purification of DNA-dependent RNA polymerase

Fraction	Total protein mg	RNA-polymerase activity	Homopolymer synthesizing activity			
			poly(A)	poly(U)	poly(G)	poly(C)
Crude extract	819.7	52.9	36.8	23.3	16.1	23.2
Ammonium sulfate	241.5	110.4	123.6	50.6	68.1	39.7
DEAE-polymerase I	20.3	1,535.7	1,995.8	411.2	1,199.2	338.3
DEAE-polymerase I (Omit DNA)		(177.8)	(341.7)	(311.5)	(84.7)	(249.8)
DEAE-polymerase II	1.2	736.7	863.1	0	367.7	0

The assay conditions are described in Materials and Methods. Values of total protein are given for 100 g of frozen tissues. The activity was expressed as p moles of ^{14}C-labeled nucleotides incorporated per mg of protein.

Table 2. Effect of dialysis on homopolymer synthesizing activity in the DNA-dependent RNA polymerase fractions

Fraction	RNA polymerase activity	Homopolymer synthesizing activity			
		poly(A)	poly(U)	poly(G)	poly(C)
DEAE-polymerase I (Before dialysis)	2,032.2	1,230.0	56.4	1,023.2	106.5
(After dialysis)	1,350.5	2,356.3	29.0	809.5	64.3
DEAE-polymerase II (Before dialysis)	601.1	861.4	0	385.8	0
(After dialysis)	577.0	1,935.3	0	392.3	0

The activity was expressed as p moles of ^{14}C-labeled nucleotides incorporated per mg protein. DEAE-polymerase I and II were dialyzed for 16 h against 10 mM Tris-HCl buffer containing 5% glycerin in the absence of Mg^{++}, DTT and EDTA. Visking tubes used had been boiled for 30 min in 1% sodium bicarbonate, boiled for 30 min in distilled water, and stored in distilled water at 4°C. Washing with EDTA-solution was omitted.

sizing each homopolymer in the RNA polymerase fractions, and that some structural changes occurred in the sites during the dialysis.

Preliminary characterization of poly (A) and poly (G) synthesizing activity.

The general characteristics of poly (A) and poly (G) polymerizing reaction in DEAE-polymerase I are presented in Table 3. The incorporation

Table 3. Characteristics of poly (A) and poly (G) synthesizing activity

Reaction system	Synthesizing activity			
	poly (A)		poly (G)	
	p moles	%	p moles	%
Complete	149.9	100	92.9	100
- DNA	20.4	13.6	6.6	7.1
+ UTP, 50 nmoles	38.7	25.8	27.6	29.7
+ CTP, 50 nmoles	36.3	24.2	28.7	30.9
+ GTP, 50 nmoles	31.4	20.9	-	-
+ ATP, 50 nmoles	-	-	14.7	15.8
- DNA + yeast RNA, 10 μg	11.3	7.5	16.3	17.5
- DNA + sRNA, 10 μg	59.4	39.6	12.4	13.3
+ RNase, 10 μg	87.0	58.0	91.1	98.1
+ Na_3PO_4, 0.125 μmoles	86.7	57.8	87.4	94.1
Native DNA[1)]	42.9	28.6	13.7	14.7
Heated enzyme[2)]	0.4	0.3	0	0
Product hydrolyzed[3)]	0	0	0	0

DEAE-polymerase I fraction (each 106 μg of protein) was used in this assay system. 1) Native calf-thymus DNA was used instead of denatured DNA. 2) Pre-heated at 50°C for 60 min. 3) Product synthesized was hydrolyzed with 0.3 N KOH at 37°C for 18 h.

of [^{14}C]-labeled ATP or GTP into acid-insoluble material was denatured-DNA-dependent, and native DNA or RNA could not replace it. Addition of other cold nucleoside triphosphate to the homopolymer forming system reduced the incorporation of [^{14}C]-labeled adenylate or guanylate in either case. Pancreatic ribonuclease and inorganic phsphate included in the assay system hardly inhibited the homopolymer formation, especially of guanylate. This may attest to the absence of contributing nucleotide polymerization by RNA polymerase (13) and polynucleotide phosphorylase (14) to the radioactive product. The incorporated radioactivity in the acid-insoluble product was released by alkali-hydrolysis. These results suggest the possible existence of poly (A) and poly (G) synthesizing enzyme in DNA-dependent RNA polymerase. The E. coli poly (A) polymerase fraction has been identified as a subunit in

RNA polymerase (15). It had been, however, isolated by heat-treatment at 50° C and DNA-independent. The present enzyme activity for poly (A) and poly (G) formation is DNA-dependent and highly sensitive to heat-treatment at 50° C. The interrelationships between DNA-independent poly (A) polymerase or poly (C)-dependent poly (G) polymerase in E. coli and the present DNA-dependent poly (A) or poly (G) synthesizing activity should be clarified, respectively, in view of the structure and biological functions of DNA-dependent RNA polymerase in eucaryote.

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