THE EFFECT OF INDOLE ACETIC ACID ON RNA POLYMERASE *IN VITRO*.

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Summary: Indole acetic acid (IAA) added in RNA polymerase system isolated from the chromatin of coconut nuclei stimulates RNA synthesis only in presence of another protein fraction. This seems to act as an acceptor protein for IAA. The IAA-acceptor protein complex interacts with DNA but not with RNA polymerase or initiation factor (factor B) *per se*. That additional species of RNA is synthesized in presence of IAA-acceptor protein has been documented.

That Indole acetic acid (IAA) can influence RNA synthesis in plant cells has been reported from a number of laboratories (1 - 8). Mathysse and Phillips (9) demonstrated that an acceptor protein for auxin can interact with the chromatin to derepress the genome. In their system, the hormone and acceptor protein do not affect the rate of RNA synthesis if pure DNA is used as template. However, the actual mechanism of IAA stimulation on RNA synthesis by isolated RNA polymerase system is yet to be elucidated.

Recently, RNA polymerase and factors for initiation and termination have been isolated and purified from chromatin of coconut nuclei in our laboratory (10 - 12). This, in fact, facilitates the study of IAA action on the transcription process *in vitro* in a completely homologous system. Some of our findings will be described in the present paper.

MATERIALS AND METHODS

2-^{14}C-Indole acetic acid and ^{3}H-UTP were purchased from Radiochemical Centre, Amersham, Bucks, England. ATP, GTP, CTP and IAA were obtained from Sigma Chemical Co., St.Louis, Mo., U.S.A. Aquacide was obtained from CalBiochem, Lowengraben, Switzerland. DNA was isolated from coconut endosperm by the method of Marmur (13).

Assay for in vitro RNA synthesis: RNA polymerase CI and initiation factor (factor B) were isolated and purified from coconut (Cocos nucifera) endosperm nuclei by the method described earlier (10, 11). The assay procedure was the same as described.

RESULTS AND DISCUSSION

IAA added to an in vitro RNA synthesizing system containing RNA polymerase CI, initiation factor (factor B) and DNA, all from coconut endosperm nuclei, did not have any effect. But RNA synthesis by intact nuclei isolated from the same tissue was stimulated by IAA. It was assumed that some component(s) in the nuclei was also necessary for RNA synthesis stimulation, in addition to IAA. Subsequently, a protein was purified from nucleoplasmic fraction by DEAE-cellulose, ammonium sulphate and CM-cellulose fractionation (Fig. 1). The protein eluted from CM-cellulose column at 0.5 M KCl (peak II in Fig. 1) stimulated RNA synthesis in vitro by two to three folds in presence of IAA (Table I). Both the initiation of RNA synthesis as studied by the incorporation of β,γ-^{32}P-ATP as well as elongation of RNA chain as studied by the incorporation of ^{3}H-UTP were similarly affected. However, the acceptor protein alone seemed to be slightly inhibitory, the reason for which is not understood at present.

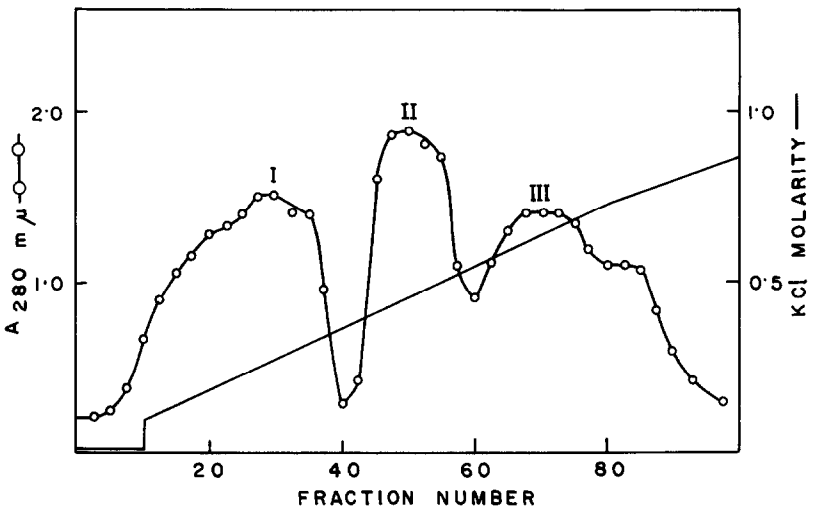

Fig. 1 - Purification of acceptor protein. IAA acceptor protein was isolated and purified from the nucleoplasm by the method of Mathysee and Phillips (9) with slight modification. Coconut endosperm nuclei (10) were homogenized with Potter-Elvjem homoginizer fitted with a tight teflon pestle in presence of TME buffer (Tris HCl, pH 8.0, 0.01 M; 2-mercaptoethanol, 1 mM; and EDTA, 0.1 mM). The slurry was centrifuged at 3000 x g for 15 min. The resultant supernatant was centrifuged at 100,000 x g for 1 hour to free it from subcellular particles, and treated with equal volume of 50% slurry of DEAE cellulose previously equillibrated with TME buffer, pH 8.0, and finally filtered through glass wool. The filtrate was made 40% saturated with ammonium sulphate and was kept in ice for one hour. The resultant ppt. collected by centrifugation was dissolved in TMME buffer (Tris-maleic acid-NaOH, pH 6.4, 0.01 M; 2-mercaptoethanol, 1 mM; EDTA, 0.1mM;) and dialysed overnight against the same buffer. The protein solution was layered on CM-cellulose column (20 x 2 cm), previously equillibrated with TMME buffer, and eluted with a linear gradient of KCl in the above buffer. The protein eluted at 0.5 M KCl (peak II) had the activity and was concentrated by aquacide and dialysed overnight against TMEG buffer (TME buffer containing 5% glycerol) and stored at -12°.

On electrophoresis in polyacrylamide gel (14), the protein gave a single band indicating that it might be homogeneous. The protein was tested for IAA binding capacity by equilibrium dialysis. It was noted that the protein can bind ^{14}C-IAA whereas other proteins in its place can not (Table 2). The binding of ^{14}C-IAA with this acceptor protein was also confirmed by polyacrylamide gel electrophoresis (results not presented). This protein might

Table 1. Stimulation of RNA synthesis by IAA and acceptor protein in an in vitro system.

Incubation system	β,γ-^{32}P-ATP incorporated (pmoles/mg of enzyme)	^{3}H-UMP incorporated (nmoles/mg of enzyme)
Complete	60	6.3
Complete + Acceptor protein	35	4.2
Complete + Acceptor protein + IAA	108	14.8

The complete system (0.25 ml) contained Tris-HCl, pH 8.0, 0.04 M; EDTA, 0.2 mM; 2-mercaptoethanol, 5 mM; $MnCl_2$, 2 mM; K_2HPO_4, 0.4 mM; KCl, 0.16 M; coconut DNA, 10 µg; ATP, GTP, CTP and UTP, 0.15 mM each of which either UTP was ^{3}H-labelled (specific activity 4 x 10^6 cpm/µmole) or ATP was β,γ-^{32}P-labelled (2 x 10^8 cpm/µmole) for detection of initiation (12). 10 µg of RNA polymerase CI, 1 µg of factor B, 2 µg of acceptor protein and 10^{-6}M IAA were used in appropriate cases. Aliquots from the incubation mixture were soaked in whatman 3 MM filter paper discs and dried. The discs were treated with 10% TCA, washed with 5% TCA containing 0.25 M sodium pyrophosphate, ethanol and finally with ether. The discs were then dried and counted with toluene liquifluor in Beckman LS-100 counter.

Table 2. Binding of ^{14}C-IAA with acceptor protein.

Protein sample	External Radioactivity (cpm/ml)	Internal Radioactivity (cpm/ml)
Acceptor protein (peak II)	14,230	38,830
Bovine Serum Albumin	12,330	15,420
Peak I protein	13,800	12,320
Peak III protein	10,560	18,130

Binding of ^{14}C-IAA with acceptor protein was studied by equilibrium dialysis. 0.2 ml of acceptor protein (10 mg/ml) obtained as in Fig.1 was placed in a dialysis sac and dialyzed for 18 hrs against 20 ml of TME buffer containing 10^{-6}M ^{14}C-IAA (specific activity, 8 x 10^5 cpm/mµmole). 0.1 ml aliquots from inside and outside the dialysis sac were counted with Dioxan-based liquifluor. Equal amount of bovine serum albumin, peak I and III proteins of CM-cellulose column were also dialyzed as above and counted.

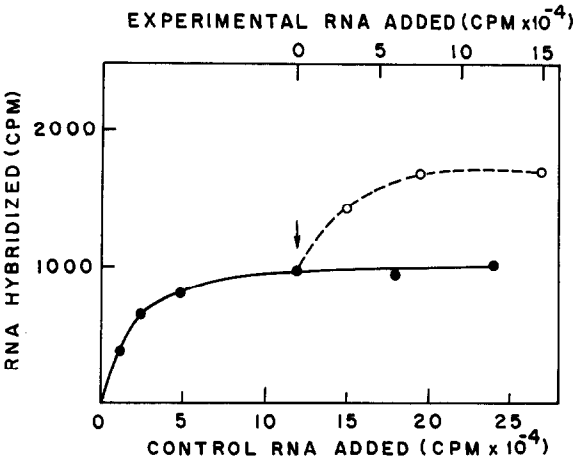

Fig. 2 - Hybridization of *in vitro* synthesized RNA with DNA. ^{3}H-RNA was synthesized by RNA polymerase CI and factor B either in presence (experimental) or absence (control) of acceptor protein and IAA as described in Table 1. Liquid hybridization technique of Attardi *et al.* (15) was used. Varying amounts of labelled RNA (specific activity 2.4 x 10^3 cpm/µg in case of control and and 2 x 10^3 cpm/µg in case of experimental RNA) were incubated with 5 µg of heat-denatured coconut DNA in 2 ml of 2 x SSC buffer (0.15 M NaCl, 0.015 M trisodium citrate) at 70°C for 10 hours. After slow cooling to room temperature the mixture was treated with 10 µg of pancreatic RNase (freed from DNase by heating at 80°C for 15 min.). After incubation with RNase for 15 min. at 21°C, the mixture was passed through Millipore HAWP filters. The filters were washed with 100 ml of 2 x SSC and counted with 10 ml of dioxanbased liquifluor and 1 ml of water, in which the filters completely dissolved giving a uniform phase. —●—●—, Hybridization of varying amounts of control RNA synthesized in absence of IAA; -O-O-O- Hybridization of varying amounts of experimental RNA synthesized in presence of IAA when added on top of saturating amount (12 x 10^4 cpm) of control RNA.

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be analogous to the auxin-acceptor protein reported earlier (9).

Increased RNA synthesis in presence of IAA and acceptor protein might be due to the synthesis of the same types of RNA more efficiently than that in absence of IAA acceptor protein or the synthesis of additional new species of RNA. These possibilities were tested by DNA-RNA hybridization. Varying amounts of labelled RNA synthesized in absence of IAA acceptor protein were hybridized with denatured coconut DNA to obtain a saturation

level. At this point, different amounts of RNA synthesized in presence of IAA acceptor protein were added in order to see whether any additional RNA was hybridized. From Fig. 2, it is apparent that the extent of hybridization is doubled in the latter case. This indicates that RNA synthesized in presence of IAA hybridizes at additional sites of DNA, which are not complimentary to RNA made in absence of IAA.

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