Interaction between Auxin-binding Protein-I and RNA Polymerase II

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Interactions between auxin-binding protein-I (ABP-I), purified from etiolated mung bean seedlings, and nuclear components from mung bean tissues were investigated. When NaCl-solubilized components of chromatin were put on an affinity column of ABP-I-linked Sepharose 4B, a small amount of the material was retained on the affinity column and was eluted with 1 M NaCl. RNA polymerase II activity was detected in the eluted fraction. Partially purified RNA polymerase II from mung bean nuclei and purified RNA polymerase II from wheat germ also bound to ABP-I. Indole-3-acetic acid was not necessary for the binding of RNA polymerase II to ABP-I. Acid-denatured ABP-I did not bind to RNA polymerase II from wheat germ. The addition of ABP-I to the reaction mixture for RNA synthesis in vitro caused a stimulation of the activity of wheat germ RNA polymerase.

When plants or excised plant tissues are treated with auxin, a specific set of translatable mRNAs is generated within 15 min. 1-4) Several cDNA clones for mRNAs whose levels are regulated by auxin have been isolated and characterized,5-11) and auxin-regulated genes have also been isolated.7,12-15) The modulation by auxin of the expression of specific genes may be explicable in terms of recognition of auxin by a specific receptor, which then initiates a complex interaction among nuclear components, with resultant alterations in transcriptional activity and/or selection of genes to be expressed.2,16-19)

Soluble auxin-binding proteins have been isolated from a variety of plant tissues in efforts to clarify the nature of auxin receptors.17-20) Some of these proteins have a high affinity for auxins and the binding is auxin-specific, reversible, and saturable, characteristics that suggest that these proteins may be auxin receptors. Although these criteria are often used to distinguish real receptors from non-functional binding proteins, it is necessary to clarify the biological function of the binding proteins if they are to be classified as putative receptors. To date, the activity of various soluble auxin-binding proteins has been identified as the stimulation of RNA synthesis in isolated nuclei.21-25)

The auxin-binding proteins (ABP-I and ABP-II) purified from etiolated mung bean seedlings26-28) stimulate the synthesis of mRNA in isolated nuclei.29) In the presence of ABP-I and ABP-II, IAA has no further effect on transcription, in terms of the overall degree of stimulation. However, translation in vitro of polyadenylated RNA [poly(A)+RNA] prepared from such nuclei showed that the level of one specific product of translation, in each case, increased when the nuclei were treated with either ABP-I or ABP-II in the presence of IAA.29) It appeared that ABP-I or ABP-II alone, without IAA, stimulated the synthesis of the same species of mRNA as those synthesized in the control nuclei. The levels of two peptides of the same molecular weights and isoelectric points as the two specifically translated peptides were also elevated in the translation products of poly(A)+RNA from IAA-treated or 2,4-D-treated sections of mung bean hypocotyls.29) We have suggested the possibility that one of the mechanisms of action of auxin may involve a direct interaction between a soluble receptor protein, such that the resultant auxin-receptor complex, possibly together with other protein factors, can subsequently recognize the promoter region of an auxin-regulated gene. To examine this possibility, we investigated the interactions between auxin-binding proteins and nuclear components using ABP-I.

Materials and Methods

Plant material Etiolated seedlings of mung bean (Vigna radiata Wilczek) were grown on an agar-gel bed for 3 days, as previously described.30)

Purification of ABP-I. Auxin-binding protein-I (ABP-I) was purified from etiolated mung bean seedlings as described in a previous paper.26)

Preparation of ABP-I-linked Sepharose 4B. Purified ABP-I (8-10 mg) was coupled to 1 g of CNBr-activated Sepharose 4B under the conditions suggested by the supplier (see below).

Isolation and solubilization of chromatin. All procedures were done at 4°C, and all buffers used for dialysis and chromatography contained 10 mM 2-mercaptoethanol.

Chromatin was isolated from etiolated mung bean seedlings (300 g fresh wt.) by the method of Murray and Key.31) The chromatin pellet was suspended in 10 mM Tris-HCl buffer (pH 7.6) that contained 2 mM EDTA-Na2, and homogenized with five gentle strokes of a Teflon homogenizer. The homogenate was centrifuged at 12,000 x g for 20 min and the supernatant was decanted and saved. This washing procedure was repeated once and the supernatants were combined (washing solution). The chromatin pellet was then suspended in 10 mM Tris-HCl buffer (pH 7.6) that contained 2 mM NaCl and the mixture was stirred for 15 hr. The solubilized chromatin fraction was centrifuged at 18,000 x g for 30 min and the supernatant obtained was dialyzed against 10 mM Tris-HCl buffer (pH 7.6) for 15 hr with several changes of the buffer. The precipitate formed during the dialysis was collected by centrifugation at 18,000 x g for 30 min, and was solubilized in 10 mM Tris-HCl buffer (pH 7.6) that contained 0.1% (v/v) Triton X-100 (insoluble fraction). The supernatant was retained as the NaCl-solubilized fraction. The three fractions obtained in this way from chromatin were passed separately through a column of Sepharose 4B (2 cm i.d. x 4 cm) which had been equilibrated with 10 mM Tris-HCl buffer (pH 7.6) or the same buffer

Abbreviations: ABP, auxin-binding protein; poly(A)+RNA, polyadenylated RNA.
plus 0.1% Triton X-100. Each fraction, after passage through this column, was put on an affinity column of ABP-I-linked Sepharose 4B (2 cm i.d. x 4 cm) which had been equilibrated, and was eluted with the same buffer.

Partial purification of RNA polymerase II from mung bean nuclei. The nuclei were extracted from hypocotyls collected from 3-day-old seedlings (150 g fresh wt.) and purified by discontinuous sucrose density-gradient centrifugation, as described in a previous paper.

The purified nuclei were suspended in 50 mm Tris-Cl buffer (pH 7.9) that contained 0.5 m (NH₄)₂SO₄, 0.1 mM EDTA-Na₂, 5 mm MgCl₂, and 25% (v/v) glycerol (buffer A) and homogenized with a Teflon homogenizer. The homogenate was centrifuged at 27,000 x g for 30 min. Solid (NH₄)₂SO₄ was added to the supernatant to 100% saturation, and the resultant precipitate was collected by centrifugation at 27,000 x g for 30 min. The precipitate was dissolved in 50 mm Tris-Cl buffer (pH 7.9) that contained 50 mm (NH₄)₂SO₄, 0.1 mM EDTA-Na₂, 0.5 mm MgCl₂, and 25% (v/v) glycerol (buffer B), and the solution was dialyzed against the same buffer for 18 hr with several changes of the buffer. Any precipitate formed during dialysis was removed by centrifugation at 27,000 x g for 30 min.

A portion of the resulting supernatant (about 4 mg protein) was put onto a column of Mono Q HR 5/5 which had been equilibrated with buffer B. The column was washed with the same buffer and proteins were eluted with a linear gradient from 50 mm to 0.5 m (NH₄)₂SO₄ in buffer B by HPLC (LKB-Produkter, Bromma, Sweden). In the case of chromatography of NaCl-solubilized chromatin proteins, proteins were eluted with a concave gradient (File No. 15 of LKB 2155 HPLC Controller) from 50 mm to 1 m (NH₄)₂SO₄ in 10 mm Tris-Cl buffer (pH 7.6).

Assay of RNA polymerase activity. RNA polymerase activity was assayed with 50 μl of each preparation in 0.5 ml of a reaction mixture that contained 50 mm Tris-Cl buffer (pH 8.0), 10 mm dithiothreitol, 5 mm MgCl₂, 1 mm MnCl₂, 50 mm (NH₄)₂SO₄, 5% (v/v) glycerol, 0.4 mm each of ATP, GTP, and CTP, 0.02 mm (155 kbq) [5,6-H]UTP, and 40 μg of heat-denatured calf thymus DNA. Reactions were incubated for 15 min at 30°C and stopped by the addition of 2 ml of 10% (w/v) trichloroacetic acid that contained 8 mm sodium pyrophosphate. The precipitate was collected by centrifugation at 24,000 x g for 30 min. The pellet was suspended in 1 ml of 10% trichloroacetic acid that contained 8 mm sodium pyrophosphate and the suspension was centrifuged again. This washing procedure was repeated twice more. The precipitate was dissolved in 0.5 ml of 0.1 m KOH, the pH was adjusted with 0.5 ml of 0.1 m Tris-Cl buffer (pH 7.6), and radioactivity of the solution was measured with a liquid scintillation counter.

Affinity chromatography of RNA polymerase II on a column of ABP-I-linked Sepharose 4B. The partially purified RNA polymerase II from mung bean and the purified RNA polymerase II from wheat germ were separately dialyzed against buffer B, passed through a column of Sepharose 4B (2 cm i.d. x 4 cm) and put on the affinity column (1 cm i.d. x 4 cm) which had been equilibrated with buffer B. The column was washed with buffer B and eluted with buffer B that contained 0.3 m instead of 50 mm (NH₄)₂SO₄.

Gel-electrophoresis. Gel-electrophoresis of the purified RNA polymerase II from wheat germ was done in a 5% gel by the method of Davis. SDS-polyacrylamide gel electrophoresis was done using a 7.5—20% gradient gel by the method of Laemmli.

Protein measurement. Protein was measured by the method of Lowry et al. Bovine serum albumin was used as a standard protein.

The purified DNA from mung bean hypocotyls. DNA was purified from mung bean hypocotyls by the method of Paszkowski et al. The purified DNA was used as a template instead of calf thymus DNA to examine effects of ABP-I on the activity of RNA polymerase II from wheat germ.
Table I. Analysis of RNA Polymerase Activity in Mono Q-Separated Fractions

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Conditions</th>
<th>[\textsuperscript{3}H]-UMP incorporated (dpm)</th>
<th>[\textsuperscript{3}H]-UMP incorporated (pmol)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Complete</td>
<td>2,590 ± 185</td>
<td>2.08</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>- DNA</td>
<td>176 ± 20</td>
<td>0.14</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>- ATP, GTP, CTP</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>- Mg\textsuperscript{2+}, Mn\textsuperscript{2+}</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Complete</td>
<td>2,690 ± 160</td>
<td>2.14</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>+ \textgamma-amanitin (5 \mu g/ml)</td>
<td>994 ± 90</td>
<td>0.78</td>
<td>36</td>
</tr>
</tbody>
</table>

* NaCl-solubilized chromatin proteins retained on a column of ABP-I-linked Sepharose 4B were separated on mono Q. Protein fractions eluted at 0.22 M (NH₄)₂SO₄ were used for the assay. The assay was done in triplicate and the mean values ± standard error are presented.

Fig. 3. Partial Purification of RNA Polymerase II from NaCl-solubilized Nuclear Proteins.
Open circles, [\textsuperscript{3}H]-UMP incorporated; closed circles, [\textsuperscript{3}H]-UMP incorporated in the presence of \textgamma-amanitin (0.1 \mu g/ml). Fractions 47–52 were pooled as partially purified mung bean RNA polymerase II.

put on a column of Mono Q, it was separated into three peaks with absorbance at 280 nm (Fig. 2). RNA polymerase activity was detected in the second peak, which was eluted at 0.22 M (NH₄)₂SO₄. The large peak, eluted last, had an absorption maximum at 260 nm and was considered to contain nucleic acids. The RNA polymerase activity detected in the second peak was dependent on the addition of DNA, NTP, and Mg\textsuperscript{2+} plus Mn\textsuperscript{2+}, and \textgamma-amanitin inhibited the activity (Table I). From these results it was considered that ABP-I had the ability to bind to RNA polymerase II itself or to a complex of the polymerase with other components. Since, in this experiment, nucleolar components were solubilized in the absence of glycerol, EDTA-Na₂, and Mg\textsuperscript{2+}, which are required for stabilization of RNA polymerase II, it was considered that the RNA polymerase II was partially denatured. Therefore, we next examined the interaction using RNA polymerase II that had been extracted from nuclei under mild conditions.

Interaction of ABP-I and RNA polymerase II
Solubilized proteins from isolated mung bean nuclei were separated by HPLC on a column of Mono Q. RNA polymerase I, which is insensitive to \textgamma-amanitin, and RNA polymerase II, which is sensitive to this drug, were eluted at 0.1 and 0.3 M (NH₄)₂SO₄, respectively (Fig. 3). Since, the activity of RNA polymerase II from mung bean was very labile and could not be purified, the fractions that contained RNA polymerase II were put directly on the column of ABP-I-linked Sepharose 4B to investigate the interaction. As shown in Fig. 4, all the RNA polymerase activity was retained on the affinity column and was completely eluted by 0.3 M (NH₄)₂SO₄. Inclusion of 10 \mu M IAA did not affect the retention of RNA polymerase II activity by the column. Thus, it seemed likely that ABP-I had the ability to bind to RNA polymerase II. However, since only partially purified RNA polymerase II had been used in this experiment, we next examined this possible affinity using purified RNA polymerase II from wheat germ (Table II and Fig. 5). The subunit structure of
the purified RNA polymerase II is almost the same as reported by Jendrisak and Burgess. The addition of α-amanitin (0.1 μg/ml) inhibited the activity almost completely (data not shown).

As shown in Fig. 6a, all the RNA polymerase II activity was also retained on the column of ABP-I-linked Sepharose 4B and was completely eluted by 0.3 M (NH₄)₂SO₄. A small amount of protein passed through the affinity column and was considered to be denatured RNA polymerase II. No RNA polymerase II was retained both on a affinity column of acid denatured, ABP-I-linked Sepharose 4B (Fig. 6b) and on a column of bovine serum albumin-linked

Table III. Effects of ABP-I on the Activity of RNA Polymerase II from Wheat Germ

<table>
<thead>
<tr>
<th>Additions</th>
<th>[³H]-UMP incorporation (dpm) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>9.750 ± 360 100</td>
</tr>
<tr>
<td>+ABP-I (19 μg)</td>
<td>19.900 ± 510 204</td>
</tr>
<tr>
<td>+ABP-I and IAA (10 μM)</td>
<td>19.800 ± 380 203</td>
</tr>
<tr>
<td>+Boiled ABP-I</td>
<td>9.880 ± 290 101</td>
</tr>
</tbody>
</table>

* RNA polymerase II (1.7 μg) purified from wheat germ and mung bean DNA (25 μg) were used in the standard reaction mixture. The assay was done in triplicate and the standard ± standard error are presented. ABP-I used in this experiment had no RNA polymerase activity. The boiled ABP-I was prepared by heating it in boiling water for 3 min.

Fig. 5. Polyacrylamide Gel Electrophoresis (a) and SDS-Polyacrylamide Gel Electrophoresis (b) of the Purified Wheat Germ RNA Polymerase II. Standard proteins of known molecular weight were phosphorylase b, 94,000; bovine serum albumin, 67,000; ovalbumin, 43,000, cytochrome c, 12,400.

Fig. 6. Affinity Chromatography of the RNA Polymerase II Purified from Wheat Germ on the Column of ABP-I-linked Sepharose 4B.

(a) Control. (b) The purified RNA polymerase II was put on a column of acid-denatured, ABP-I-linked Sepharose 4B. The column of ABP-I-linked Sepharose 4B was initially treated with 0.1 M HCl for 10 min at 0°C and washed successively with distilled water and the same buffer as used for the affinity chromatography. Elution with 0.3 M (NH₄)₂SO₄ was begun at fractions indicated by arrows.

**Effects of ABP-I on RNA polymerase II activity**

The addition of ABP-I to the reaction mixture for RNA synthesis in vitro caused the stimulation of the activity of RNA polymerase II from wheat germ (Table III). The stimulation of RNA synthesis by ABP-I was not enhanced by the inclusion of 10 μM IAA in the reaction. The stimulatory effect of ABP-I on the synthesis of RNA was completely destroyed by heating it in boiling water for 3 min. Bovine serum albumin (20 μg) had no effect on the activity of RNA polymerase II (data not shown).

**Discussion**

ABP-I, isolated from etiolated mung bean seedlings on an affinity column of 2,4-D-linked Sepharose 4B and purified by gel filtration, has the ability to bind to RNA polymerase II and stimulates its activity. Since ABP-I itself has no RNA polymerase activity, the stimulation is considered to be caused by the interaction of RNA polymerase II with ABP-I. Stimulation of RNA polymerase II activity by proteins retained on an affinity column of 2,4-D-linked Sepharose 4B was reported by Venis and Rizzo et al. However, the proteins isolated had no auxin-binding activity. By contrast, ABP-I used in this experiment has a relatively high affinity for auxins and stimulates RNA polymerase II activity. The binding of RNA polymerase II to ABP-I (Fig. 4a) and the activation of RNA polymerase II by ABP-I (Table III) occurred in the absence of IAA. Venis also reported that addition of auxin was not necessary for activity of the protein.

In a previous paper we reported that ABP-I stimulated the synthesis of mRNA in isolated nuclei and that IAA had no further effect on transcription, in terms of the overall degree of stimulation. However, translation in vitro of poly(A)⁺RNA prepared from these nuclei showed that the level of one specific product of translation increased when the nuclei were treated with ABP-I in the presence of IAA. A possible mechanism by which ABP-I controls the expression of specific genes may involve recognition of each specific gene by IAA-bound ABP-I, which is converted by the binding of IAA to an active configuration. However, ABP-I alone may bind to other genes with lower specificity. The binding of RNA polymerase II to ABP-I may allow transcription of a particular gene(s).

Recently, Köhler et al. reported that a nuclear
protein prepared from tobacco seedlings binds to promoter regions of auxin-regulated genes. In this context, the relationship between an auxin-binding protein and a nuclear protein that binds to the promoter regions of auxin-regulated genes is interesting.

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References