Enhanced Activity of the Soluble Ribonucleic Acid Polymerase from 2,4-Dichlorophenoxyacetic Acid-treated Maize Seedlings

Received for publication December 21, 1971

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There is a large body of literature which suggests a role for plant hormones in nucleic acid synthesis although the precise nature of this role remains obscure (4). The addition of auxins such as 2,4-D to intact seedlings results in a rather large accumulation of nucleic acid and protein. Cranberry bean plants treated with 2,4-D, even into the herbicidal range, contained almost double the amount of nucleic acids of control plants (7). Similarly, mesocotyl sections from maize seedlings sprayed with 2,4-D concentrations up to 8 g/l contained more than double the amount of nucleic acid and protein of control tissue (8, 12).

O’Brien et al. (6) have reported increased activity of the chromatin-bound RNA polymerase (nucleoside triphosphate; RNA nucleotidyl transferase EC 2.7.7.6) from hypocotyl tissue subsequent to the application of 2,4-D to soybean seedlings. Chromatin from both control and 2,4-D-treated seedlings exhibited similar potential for RNA synthesis when saturated with Escherichia coli RNA polymerase. The RNA products synthesized by chromatin from control and treated seedlings were also different (2, 6). Enhanced RNA polymerase activity of chromatin from IAA-treated cucumber seedlings has also been reported (3).

The results reported here demonstrate the enhanced activity of the soluble RNA polymerase of maize seedlings subsequent to the application of 2,4-D to seedlings. The purification and characterization of this enzyme has been previously reported (9, 10).

MATERIALS AND METHODS

Maize grain (Zea mays L.) was soaked in running tap water for 6 hr prior to being placed on sterile, moist filter paper in porcelain trays (180 seeds/tray). The seedlings were grown in a dark incubator at 25°C. Ten ml of appropriate concentrations of 2,4-D, neutralized with NaOH, were applied to the seedlings of each tray with an atomizer at the times indicated in Figure 1 prior to harvest. All seedlings were harvested 5 days after soaking was begun. The shoots were harvested into liquid nitrogen, and the high speed supernatant fraction was prepared as described (9), except that homogenization was by a Waring Blender at full speed for 2.5 min. Purified RNA polymerase was prepared as the diethylaminoethyl cellulose fraction of Stout and Mans (9).

RNA polymerase activity was measured as the incorporation of 3H-AMP from 3H-ATP into acid-insoluble product by the filter paper disk method (9). The standard reaction mixture contained in 0.2 ml: 40 μmoles of tris-HCl, pH 8.4, 0.5 μmole each of CTP, GTP, UTP, and 3H-ATP (3.15 cpm/μmole), 2.5 μmoles of magnesium acetate, 5 μmoles of dithiothreitol, 20 μmoles of (NH₄)₂SO₄, 50 μg of calf thymus DNA and 235 μg of high speed supernatant protein or 50 μg of purified enzyme protein. A unit of RNA polymerase activity is the amount of enzyme catalyzing the incorporation of 1 pmole of 3H-AMP per 5 min of incubation at 30°C. Specific activity is units per mg of protein. Protein concentrations were determined by the method of Lowry et al. (5) with bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Treatment of maize seedlings with 2,4-D, 48 hr prior to harvest, enhanced the activity of the RNA polymerase in the high speed supernatant preparations. However, it was necessary to dialyze the protein solutions for the enhancement to be observed. In Figure 1A, the undialyzed RNA polymerase for control seedlings and those treated with 2,4-D had the same specific activity (833 ± 28). Dialysis of these solutions for 2 hr against 50 mM tris-HCl, pH 8.4, containing 0.1 mM EDTA, 1 mM MgCl₂, and 10 mM 2-mercaptoethanol increased the specific activity of the RNA polymerase. While the control increased only from 833 to 926 (11%), that from 2,4-D-treated seedlings increased to 1,478 (177%) at 1 mM (Fig. 1A).

In Figure 1B, seedlings were treated with 0.1 M 2,4-D for

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1 This research was supported in part by a grant from the Research Division, Virginia Polytechnic Institute and State University.
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0, 12, 24, and 48 hr. The undialyzed RNA polymerase from control and treated seedlings had the same specific activity. After dialysis, the specific activity of all had increased. Again the control was 111% of the undialyzed enzyme, whereas the 48 hr treatment was 154% (Fig. 1B).

The protein concentration of all the fractions in Figure 1 was 4.7 ± 0.2 mg/ml of high speed supernatant or 5.9 ± 0.4 mg/g fresh weight of tissue. There was no difference in the amount of protein extracted from control and treated seedlings. Thus, the total RNA polymerase activity increased in response to 2,4-D treatment as did the specific activity.

2,4-D added to purified maize RNA polymerase (9) did not enhance the activity of the enzyme (Table I). The only discernible effect was an inhibition at high concentrations (10 mm) of 2,4-D, IAA, GA, and kinetin also failed to produce a significant effect on the activity of the purified maize RNA polymerase (Table I). This enzyme has been characterized as being of the "nucleoplasmic type" based on differential inhibitor sensitivity (1, 11). It is inhibited more than 95% by 0.2 µg/ml of α-amanitin, whereas the same concentration of rifamycin SV had no effect on the activity of the enzyme (Arens and Stout, unpublished data). The addition of 2,4-D to the high speed supernatant enzyme or at any point in the isolation procedure also failed to elicit any measurable effect on the activity of the enzyme. It seems improbable, therefore, that the enhancement of RNA polymerase activity in Figure 1 is the consequence of a direct interaction of 2,4-D with the enzyme.

The enhancement of RNA polymerase activity by 2,4-D in maize seedlings is in agreement with reports for soybean and cucumber seedlings (3, 6). However, the maize polymerase is a soluble enzyme, whereas those from soybean and cucumber are chromatin-bound. Since the maize polymerase requires an exogenous template, the interpretation of the results is not complicated by possible changes in template sites on the DNA. Also during the isolation of chromatin, the soluble portion of the homogenate is discarded. If those plants had contained a soluble inhibitor of RNA polymerase analogous to that from maize, it would have been lost prior to the RNA polymerase assay. The purification and characterization of the maize RNA polymerase inhibitor will be reported elsewhere.

Table I. Effects of 2,4-D, IAA, GA and Kinetin on the Activity of Purified RNA Polymerase

Maize RNA polymerase was purified and assayed as described in the text. The concentrations given are the final concentrations in the reaction mixture.

<table>
<thead>
<tr>
<th>Conc</th>
<th>RNA Polymerase Activity</th>
<th>% of control</th>
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<tr>
<td></td>
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<td>IAA</td>
</tr>
<tr>
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LITERATURE CITED