In Vitro Protection of Indoleacetic Acid during Thin Layer Chromatography

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Abstract. The antioxidant sodium diethyldithiocarbamate (DDC) was evaluated for use as a protectant of indole-3-acetic acid (IAA) during application onto cellulose thin layer strips as well as during subsequent ascending chromatographic development. An IAA standard of 5 μg, applied directly over a 100 μg pretreatment spot of DDC at the origin, revealed eightfold more auxin activity than did 5 μg of IAA alone as determined by the Avena coleoptile bioassay. However, DDC alone at this dosage produced an inhibitory biological response at the same chromatographic zone where auxin banded in an isopropanol, ammonia, water (10:1:1 by volume) solvent system. Reduction of the DDC dosage to 1 μg spot–1 gave threefold greater net auxin activity than did 100 μg DDC, and 20% better protection than did 0.5 μg DDC, while eliminating its own inhibitory action at this lower dosage level.

Even in this age of sophisticated and refined techniques for detection, quantification, and identification of auxin in plant tissues, there remains a role for time-honored, traditional methods of extraction and bioassay, if for nothing more than a low-cost first step to justify commitment to more precise methods, including isotope dilution (1), gas-liquid chromatography of fluorinated (8) or silylated derivatives (1), high-performance liquid chromatography (9), and mass spectrometry. Recovery of extracted IAA previously has been poor due to its labile nature, with losses of at least 30% occurring routinely during the extraction process (4). Additional losses of 30% can occur each time chromatography is performed (5), and up to 50% if chromatograms are not eluted immediately (3). The time between drying and recovery of auxin is critical, but more acute if silica gel rather than cellulose thin layer sheets are used (7). Such losses are amplified to an even greater extent if low levels of IAA are involved, leading to ultimate losses of 72–100% of the initial amount (2).

Various techniques have been used to avert losses of IAA during extraction, such as rapidly grinding tissues under a N2 atmosphere, use of a reducing agent in the initial extraction solvent, and reducing ether extracts in volume without vacuum to prevent sublimation (4). Oxidative losses occurring during application of the extract to a chromatogram have been reduced by applying a 100 μg spot of Santoquin sulfate to the origin before spotting the sample (4). This substance, however, is not widely commercially available. Sodium diethyldithiocarbamate (DDC), which has been used as an antioxidant addition to the solvent during initial extraction (4), was additionally evaluated in our study as a potential auxin protectant during thin layer chromatography.

Initially, DDC was evaluated as a pretreatment spot at the origin of the chromatogram at the dosage recommended for Santoquin sulfate (100 μg spot–1) (4) since a preliminary experiment suggested some potential value at this level. Four experimental treatments were examined: 5 μg of standard IAA applied at the origin either alone, or on top of (but not exceeding the boundaries of) a 100 μg pretreatment spot of DDC alone, or a blank consisting of solvent alone. Samples were applied quickly in a dimly lighted room under a directed stream of N2 to the origin of 2.5 cm wide × 20 cm long cellulose thin layer strips. Development in an ascending direction followed immediately in a Gelman model 51325-1 sandwich chamber under a N2 atmosphere using a solvent system consisting of freshly redistilled isopropanol, ammonia, and water (10:1:1 by volume). Strips were allowed to develop 15 cm and were then dried in darkness under N2. Nitrogen atmospheres were obtained by conducting all chromatographic procedures in an inflatable glove bag continuously purged

Table 1. Effect of DDC applied at the origin on activity of IAA in the Avena coleoptile assay following thin layer chromatography.

<table>
<thead>
<tr>
<th>DDC (μg)</th>
<th>IAA (μg)</th>
<th>Magnitude of significant response (relative units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>5</td>
<td>1.0†</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>1.0</td>
<td>5</td>
<td>24.5</td>
</tr>
<tr>
<td>100.0</td>
<td>5</td>
<td>8.1</td>
</tr>
</tbody>
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†Represents the summation only of histogram areas which exceed 5% confidence limits.

Fig. 1. Protection of 5 μg IAA using 0.5 or 1.0 μg spot–1 DDC at the origin of the chromatogram. Dark areas represent significant (5% confidence limits) growth promotion above oat coleoptile response to multiple solvent blanks.
with N₂ under slight positive pressure. Dried chromatograms were cut into 10 equal Rf zones and analyzed immediately for auxin activity by the Avena coleoptile bioassay (6). Section length was measured after incubation using a binocular microscope with an ocular micrometer. Baseline bioassay values were determined by coleoptile growth response of various chromatographic (RF) zones after developing blank chromatographic strips in solvent.

Two additional doses of DDC, 0.5 or 1.0 µg spot⁻¹, were subsequently evaluated to determine whether lower DDC levels would have protective capacity similar to that of higher levels, but without bioassay interference.

Five µg of IAA applied alone to the origin of cellulose thin layer strips gave barely significant biological activity at Rf zone 0.3 to 0.4 (Table 1). Preliminary experiments using 100 µg DDC alone revealed significant inhibition of bioassay activity below the background level of solvent blanks. However, even this high dosage of DDC gave an eightfold net increase in the biological activity of 5 µg IAA, which masked the inhibitory activity of 100 µg DDC. Both smaller dosages of DDC (0.5 or 1.0 µg spot⁻¹) greatly enhanced IAA activity relative to that of IAA alone (Table 1), while neither dosage exhibited significant inhibition due to DDC per se (Fig. 1). A 1.0 µg dose was slightly more effective than was a 0.5 µg dose.

This preliminary study demonstrated that 1.0 µg DDC, applied as a pretreatment spot at the origin of TLC plates would significantly reduce oxidative destruction of a 5 µg IAA sample spotted directly over it. This protective action was offset by a tendency for hundredfold higher DDC dosages to inhibit coleoptile growth in the 0.5 to 0.6 Rf region of developed chromatograms. Investigators who choose to include DDC in their preparative procedures for auxin should establish a dose-response curve over a range of DDC concentrations in the presence of their particular plant extract.

Inclusion of DDC to minimize losses of IAA during extraction and purification appears worthwhile considering that it is readily available, easily incorporated into the procedure, and, effectively decreases losses during thin layer chromatography.

Literature Cited


In Vitro Propagation of Dicentra spectabilis

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Abstract. Tissue culture propagation of Dicentra spectabilis (L.) Lem. was achieved by overcrowding of 2.0- to 2.5-cm-long shoot tips placed on solidified Murashige and Skoog (MS) salts and vitamins medium containing 2.0 mg/liter kethin. Each shoot tip yielded a 50-fold increase within 6 weeks. Proliferated shoots rooted 100% in 25 days on MS medium containing selected levels of either indolebutyric acid (IBA) or naphthaleneacetic acid (NAA). Optimum transfer of rooted shoots to the greenhouse occurred when an initial high humidity was followed by a gradual reduction over 5-7 days in the ambient environment.

Dicentra spectabilis, bleeding-heart, is an ornamental herbaceous perennial grown for its pink, heart-shaped flowers that appear in early spring. The white-flowered cultivar, D. spectabilis alba, is less common, probably due to its lower inherent vigor and concomitant higher costs of propagation and production. Dicentra spectabilis is asexually propagated either by root cuttings during a limited favorable period in late summer or by terminal stem cuttings rooted under mist in early spring. Both methods are successful, but timing is critical; thus, propagation is limited to two short, annual periods. Tissue culture propagation (1) may provide an alternative method to alleviate the constraint of seasonality for commercial perennial propaga-