Interaction of $[^3\text{H}]$Gibberellin A$_1$ with a Sub-Cellular Fraction from Lettuce (Lactuca sativa L.) Hypocotyls

Requirement for Protein Synthesis

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Abstract. The relationship between protein synthesis and the incorporation of $[^3\text{H}]$gibberellin A$_1$ ($[^3\text{H}]$GA$_1$) into a 2,000 × g pelletable (2KP) fraction from lettuce (Lactuca sativa L.) hypocotyl sections has been investigated. Concentrations of D-2-(4-methyl-2,6-dinitroanilino)-N-methylpropionamide (MDMP) between 10$^{-7}$ M and 10$^{-4}$ M caused increasing inhibition of growth, 2KP labelling and incorporation of $[^{14}\text{C}]$leucine into soluble protein. Growth and 2KP radioactivity were highly correlated ($r=0.996$). Transfer to MDMP early or late in the course of GA response caused reductions in both growth and incorporation into the 2KP fraction. Exposure to the inhibitor had more effect at 4 h than at 20 h. The proportions of alkali-soluble and insoluble radioactivity in the 2KP fraction were also altered by this treatment. The implications of these findings are discussed.

Key words: Gibberellin - Growth - Lactuca - Protein-synthesis.

Introduction

It has been demonstrated that $[^3\text{H}]$gibberellin A$_1$ ($[^3\text{H}]$GA$_1$) is incorporated into a 2,000 g pelletable fraction (designated 2KP) from lettuce (Lactuca sativa L.) hypocotyls and that the radioactivity in this fraction increases with both time and external concentration (Stoddart, 1979a). Hypocotyl section growth was also found to be correlated with the incorporation of GA into the low-speed pellet. The process was shown to be a property of the intact cell and there were suggestions that the growth regulator was 'processed' in some way prior to incorporation. The implication of protein synthesis in the transfer of $[^3\text{H}]$GA$_1$ from the soluble to the insoluble fraction has been investigated as part of a series of studies aimed at assessing whether growth and 2KP-GA incorporation are causally or consequentially related.

Materials and Methods

Lettuce (Lactuca sativa L. cv. 'Arctic') seeds, obtained from Dobies Seeds Ltd., Llangollen, U.K. were sown on moistened Whatman No. 1 filter paper in 9 cm petri dishes and maintained in darkness for 40 h at 20°C. Sections were then prepared as previously described (Stoddart, 1979a) and held in distilled water at 5°C until required.

Incubations were conducted at 28°C with a light intensity of 6,000 lx, in petri dishes containing 1.0 ml of a solution prepared by drying down 25 gl of 1,2-$[^3\text{H}]$GA$_1$ (1.5·10$^{12}$ Bq mmol$^{-1}$) in 1:1 methanol/ethyl acetate (= 2.5·10$^6$ c. rain$^{-1}$) in each dish prior to the addition of distilled water. For some experiments a bulk of labelled solution was subdivided amongst the individual treatments. Solutions for growth experiments also contained unlabelled GA$_1$ at a concentration of 10$^{-5}$ mol 1$^{-1}$.

Homogenisation and Washing Procedures

These were as previously described (Stoddart, 1979a) with a standard regime of four resuspensions followed by extraction of the final 2,000 × g pellet (2KP) with 1.0 M potassium hydroxide for 2 h at 37°C. Aliquots of the KOH extract were counted in a toluene/triton X-100 scintillation fluid (2:1) using an LKB Rackbeta 1215 liquid scintillation counter. The residual material after extraction was suspended for counting in a gel medium (LKB Lumagel). Quench corrections were applied using automatic external standardisation and efficiencies were typically in the 30–40% range. Uptake was estimated by counting aliquots of the 2,000 g supernatants.
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Table 1. Effects of concentration ranges of D-MDMP and L-MDMP on protein synthesis in excised lettuce hypocotyl sections (expressed as percentage inhibition)

<table>
<thead>
<tr>
<th>Concentration M</th>
<th>10\textsuperscript{-4}</th>
<th>10\textsuperscript{-5}</th>
<th>10\textsuperscript{-6}</th>
<th>10\textsuperscript{-7}</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-MDMP</td>
<td>82</td>
<td>78</td>
<td>52</td>
<td>8</td>
</tr>
<tr>
<td>L-MDMP</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Measured as incorporation of [\textsuperscript{14}C]leucine into trichloroacetic acid precipitable material. Control incorporation = 8.4 \times 10^5 dpm/mg soluble protein. MDMP = 2-(4-methyl-2,6-dinitroanilino)-N-methylpropionamide

![Graph showing the effect of MDMP concentration on growth and 2,000 g (2KP) pelletable radioactivity in lettuce hypocotyl sections. Inset: Relationship between MDMP concentration and incorporation of [\textsuperscript{14}C]leucine into trichloroacetic acid precipitable material. All incubations contained 10\textsuperscript{-5} M unlabelled GA\textsubscript{4} and 2 \times 10^6 ct min\textsuperscript{-1} [\textsuperscript{3}H]GA\textsubscript{1} in a total volume of 1 ml. 25 sections harvested at each point. Data collected after 18 h growth. 2KP values refer only to alkali-soluble radioactivity.]

Protein Biosynthesis Inhibitors

The active (D) and inactive (L) isomers of 2-(4-methyl-2,6-dinitroanilino)-N-methylpropionamide (MDMP) were obtained from Dr. R. Baxter, Shell Research Ltd., Sittingbourne, Kent, U.K. and cycloheximide from Sigma (U.K.) Ltd. All were dispensed as aqueous solutions at the required concentrations. Protein synthesis was measured by the incorporation of [\textsuperscript{14}C]leucine (1.3 \times 10^{10} Bq mmol\textsuperscript{-1}) into trichloroacetic acid (TCA) precipitable material using a glass-fibre disc (Whatman GF/A) technique (Bol lum, 1968; Jones and Stoddart, 1970). Soluble protein was measured with the Folin phenol reagent (Lowery et al., 1951).

![Graph showing the relationship between [\textsuperscript{3}H]gibberelin A\textsubscript{1} uptake and percentage incorporation in 2,000 g pelletable (2KP) material in lettuce hypocotyl sections at various concentrations of MDMP. All incubations contained 2 \times 10^6 ct min\textsuperscript{-1} [\textsuperscript{3}H]GA\textsubscript{1} in a total volume of 1 ml. 25 sections harvested at each point. Data collected after 18 h growth. 2KP values refer only to alkali-soluble radioactivity.]

Growth Measurements

Sections were measured by projection at 20 x magnification. Outlines were traced with a map reader and converted to basic units by comparison with a projected 0.5 cm grid. Growth was expressed as percentage change in length (%AL) (Silk and Jones, 1975).

Results

The protein biosynthesis inhibitor MDMP acts upon 80s ribosomes (Baxter et al., 1973) and has the advantage of being available as active (D) and inactive (L) stereoisomers, thus allowing proper controls to be applied for the elimination of non-specific effects on tissue metabolism. A check that this isomer differentiation applied in the lettuce hypocotyl section system was carried out and the results are shown in Table 1.

Optimal inhibition was obtained at 10\textsuperscript{-5} M with the active isomer whereas the L form was confirmed as being inactive in this tissue over the practical concentration range. The effect of MDMP on both extension growth and incorporation of [\textsuperscript{3}H]GA\textsubscript{1} into the 2KP fraction was examined over a comparable concentration range. As shown in Fig. 1, there was a high correla-