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The Effect of Ethylene on [1-^{14}C]Glycerol Incorporation into Phospholipids of Etiolated Pea Stems

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The effect of ethylene (10 p.p.m.) on the rate of incorporation of [1-^{14}C]glycerol into phospholipids of etiolated pea stems was studied. After 2–3h treatment with ethylene, incorporation was decreased by 50%. It remained at this value for as long as ethylene was supplied (8h). Handling the plants also caused a temporary decrease in incorporation, which we attribute to the production of endogenous 'wound' ethylene. The percentage decrease in incorporation was the same in four major phospholipid fractions, i.e. phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol.

The morphological effects of ethylene as a plant hormone are well documented (Abeles, 1972; Burg, 1973), but the biochemical changes in the cell caused by application of low concentrations of ethylene to whole plants have not been so fully studied. Most investigations on protein or RNA synthesis and enzyme activities have involved high concentrations of ethylene, the treatment of excised plant parts, or long treatment time of 24h or more.

Modifications in cell membrane systems frequently occur during differentiation in animal and plant tissues. Many changes are induced by hormones, and are reflected in alterations in phospholipid metabolism (Tata, 1971; Valdovinos *et al.*, 1971; Koehler *et al.*, 1972). A particularly rapid change in phospholipid turnover takes place in cell cultures released from contact inhibition by serum (Pasternak, 1972).

In etiolated pea stems, applications of ethylene alter cell-wall synthesis and deposition (Ridge & Osborne, 1971). These effects can occur within 3–6h (Ridge, 1973), and, since wall synthesis has been shown to be linked to the activity of vesiculate membrane systems (Northcote, 1969), an early change in phospholipid metabolism might be expected.

The large and rapid effect of ethylene on [1-^{14}C]-glycerol incorporation into pea stem phospholipids is briefly reported here.

Materials and methods

Etiolated peas. Seeds of *Pisum sativum* (var. Meteor) were sterilized by treatment for 5min in conc. H_2SO_4 before being planted in trays of sand. The seedlings were grown at 24°C in the dark and used when the third internode was about 1cm long.

Ethylene treatment. Trays of peas were placed in 20-litre chromatography tanks and ethylene (Cambrian Chemicals, Croydon, U.K.) was injected, as 4ml of a 1:20 (v/v) dilution in air, through rubber self-sealing caps. Pellets of KOH were present to absorb CO_2, and control tanks also contained solutions of 0.25M-mercuric perchlorate in 0.25M-$HClO_4$ to absorb stray ethylene. The air and ethylene in the tanks were renewed every hour.

[1-^{14}C]*Glycerol.* This (sp. radioactivity 26mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

[1-^{14}C]*Glycerol infiltration.* A 1cm segment was cut from just below the sub-apical hook of ten uniform plants. The segments were immediately placed in 2ml of an aqueous solution (4.8–58μM) of the [1-^{14}C]glycerol (0.25–3.0μCi/ml) in a 10ml Erlenmeyer flask. Each flask was evacuated in a desiccator to 3–4mmHg (400–530Pa) within 1.5–2min in order to infiltrate the segments. This treatment causes no ultrastructurally detectable damage to the cells. On returning to atmospheric pressure, the segments were shaken in a minimal volume of [1-^{14}C]glycerol solution at 25°C. At 45min from infiltration the segments were washed once in unlabelled 20mM-glycerol. Pieces (2mm) were removed from either end [to eliminate 'cut end' effects (Palmer & Loughman, 1964)] and the segments immediately homogenized at 0°C in 10% (w/v) trichloroacetic acid containing unlabelled 20mM-glycerol. After standing at 0°C, the precipitate was centrifuged, washed once with 5% trichloroacetic acid containing unlabelled 10mM-glycerol and resuspended in propan-2-ol for the first stage of lipid extraction.

Lipid extraction. This was by the methods of Dawson (1960) with the modification of the first propan-2-ol extraction (Hitchcock & Nichols, 1971). The extract was finally dried by passing through 200mg of anhydrous Na_2SO_4 held between two glass-wool

plugs and evaporated to dryness under vacuum at 40°C. The lipids were resuspended in approx. 2ml of chloroform, and two 1ml samples were removed; one was dried and counted for radioactivity, and the other was divided equally for duplicate phospholipid determinations.

Phospholipid determination. The method of Rouser *et al.* (1966) was used on the whole extract. Separation of the phospholipids by t.l.c. showed that more than 95% of the phosphate in the extract was associated with phospholipids.

Thin-layer chromatography. Two-dimensional separation of phospholipids was carried out by the method of Hitchcock & Nichols (1971) on silica gel G plates (E. Merck A.G., Darmstadt, Germany). One-dimensional neutral-lipid separation was by the method of Clayton *et al.* (1970). Lipids were identified as described by Hitchcock & Nichols (1971) and by the use of standards.

Scintillation counting. For lipids 0.7% (w/v) 5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole in toluene was used as scintillant (Scales, 1967). For counting of radioactivity of whole segments, a 1:1 (v/v) mixture of methanol and toluene was substituted for toluene.

Results and discussion

The rate of incorporation of [1-^{14}C]glycerol into pea segments was linear for about 2h, and under these conditions 30–50% of the radioactivity taken up was found in the lipid extract. Of this 95% was associated with identifiable phospholipids and the rest with neutral lipids (mono-, di- and triglycerides). By addition of a large excess of [1-^{14}C]-glycerol during the grinding, the background counts due to non-specific binding of radioactive glycerol, or to its carry-over during partitioning, were found to be less than 0.1% of the total counts in the lipid extract.

In order to eliminate bacteria on their surface, some pea shoots were painted with ethanol before infiltration of the segments. This did not lower the incorporation values obtained, indicating minimal bacterial contamination. Application of 10p.p.m. of ethylene to plants for 5½h, taking segments at zero time and every half hour, gave c.p.m./μg of phosphate in the lipid extracts of 117, 98, 113, 118, 71, 73, 55, 49, 53, 60, 55 and 46. Control plants incorporated a mean of 110c.p.m./μg of phosphate during this period. Two further experiments were carried out. The composite results are presented in Fig. 1 in terms of c.p.m. incorporated as a percentage of the control and clearly indicate the decrease in incorporation to about 50% after 2–3h.

Of the total radioactivity incorporated after the ethylene treatments, the proportion present in each

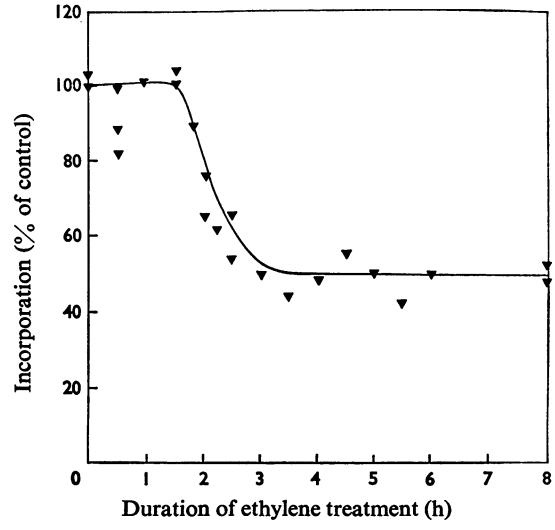

Fig. 1. *Effect of applied ethylene on* [1-^{14}C]*glycerol incorporation into phospholipids*

Intact plants were treated with ethylene (10p.p.m.) for the times shown. Segments were then cut and given [1-^{14}C]glycerol for 45min as described under 'Materials and methods'. The radioactivity incorporated in the lipid extracts (c.p.m./μg of phosphate) is expressed as a percentage of controls.

of the four major phospholipid fractions (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol) was the same as in the controls. The radioactivity in neutral lipid fractions was also lowered by 50%. This implies a lowering of activity of all lipid-biosynthesis pathways which incorporate [1-^{14}C]glycerol.

The decrease in incorporation of [1-^{14}C]glycerol into phospholipids caused by ethylene cannot be attributed to a decrease in the total uptake of the radioactive precursor by the segments. For example, 45min after infiltration, control segments contained a total of 3580c.p.m., of which 1220 were recovered in the lipid extract [i.e. were incorporated into phospholipids (105c.p.m./μg of phosphate)]. In contrast, segments from ethylene-treated plants contained 3000c.p.m., of which only 630 were recovered in the lipid extract (55c.p.m./μg of phosphate).

Provided that plants are placed in the experimental tanks (with the lids off) at least 12h before treatment, phospholipid metabolism of control plants, as judged by [1-^{14}C]glycerol incorporation, is constant for the 8h of the experiments. However, if trays of plants were placed in the tanks immediately before the start of the experiment and no precautions were taken to prevent handling of

the plants or exposure to a brief period of light, a decrease in [1-^{14}C]glycerol incorporation was obtained after 3–4h (Fig. 2).

We attribute this transient lowering of [1-^{14}C]-glycerol incorporation to the burst of 'wound' ethylene (Osborne, 1972) that is induced within 1h of handling and occurs 2–3h before the lowered incorporation (see inset in Fig. 2). 'Wound' ethylene production was of the order of 1nl/h per plant, as measured by monitoring the concentrations of ethylene when the plants were placed in small sealed tubes (for method see Jackson & Osborne, 1970). The concentration of ethylene reached in the large experimental tanks, however, was never detectable by gas chromatography (i.e. <0.001p.p.m.) and was therefore well below that at which applied ethylene would inhibit [1-^{14}C]glycerol incorporation into phospholipids. The incorporation response caused by endogenous 'wound' ethylene approximated to that caused by 2p.p.m. of applied ethylene.

It should be emphasized that the 3–4h lag before the response to handling is much longer than the period of glycerol incorporation (45min), and it must be concluded that any 'wound' ethylene induced by handling at infiltration will not affect the incorporation values obtained.

The results described here are the most rapid biochemical effects of ethylene in plants reported so far. The 50% decrease in [1-^{14}C]glycerol incorporation into phospholipids after 2–3h of application of ethylene, and a similar response induced by endogenous 'wound' ethylene, implicates membranes and their metabolism as central to the mediation of the ethylene response.

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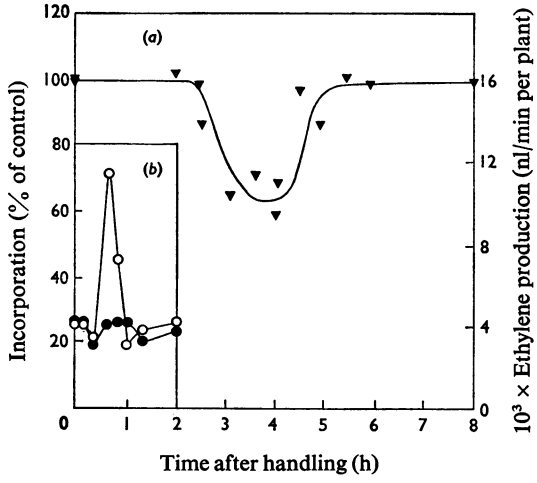

Fig. 2. *Effect of handling plants on* [1-^{14}C]*glycerol incorporation into phospholipids and ethylene production*

(*a*) Assays were done as described in Fig. 1 (▼). Comparison is made between plants set up in tanks immediately before the experiment (i.e. handled at 0h) and controls set up 12h previously (i.e. no handling at 0h). Inset (*b*): ethylene production by plants handled at 0h (○) and by control plants (●).

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