Rapid Metabolism of Propylene by Pea Seedlings

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ABSTRACT

Propylene uptake by intact pea seedlings (Pisum sativum L. cv. Alaska) was easily detected using standard gas chromatographic techniques suggesting rapid metabolism. Comparative studies with highly purified 14C2H6 and 14C3H6 under aseptic conditions verified that propylene was rapidly metabolized and indicated that some aspects of its metabolism were similar to that of ethylene since 14C2H6, like 14C3H6 (Beyer, Nature 1975, 255: 144-147), was oxidized to 14CO2 and incorporated into water-soluble tissue metabolites. However, 14C3H6 was metabolized at a substantially faster rate and unlike 14C2H6 the rate of 14C3H6 tissue incorporation exceeded its rate of oxidation to 14CO2. In addition the neutral 14C-metabolites derived from 14C3H6 were chromatographically distinct from those formed from 14C2H6.

In contrast to ethylene (2, 3, 5, 7), the metabolism of propylene has not been investigated even though it can clearly mimic ethylene action if applied at a concentration about 100 times that of ethylene (1). Because of its structural similarity, propylene undoubtedly attaches to the same receptor site as ethylene. Therefore, it was of interest to determine if propylene, like ethylene, is also actively metabolized by plant tissues. A preliminary report of this work has been published (6).

MATERIALS AND METHODS

Initial experiments were conducted with nonradioactive propylene. Ten healthy, or hot-water-killed (70 C, 2 min), aseptically grown 3-day-old etiolated pea seedlings were enclosed in each of several 240-ml Lucite acrylic resin chambers and gassed with either 1.3, 12.3, or 104 µL/1 of nonlabeled propylene for 96 hr. Each chamber contained a beaker with 4 ml of 3 N NaOH to trap evolved CO2. Chambers without seedlings were also included to evaluate sampling losses. O2 was added to each chamber after each 24-hr propylene sampling period to restore chambers to atmospheric pressure and prevent O2 deficiencies.

In subsequent work the nonlabeled propylene was replaced with [1-14C]propylene purified by preparative gas chromatographic techniques (4) using a Porapak N column rather than the standard Porapak T column. Following a 24-, 48-, 72-, or 96-hr incubation period at 1 and 9 µL/1 of 14C3H6 the seedlings and NaOH were removed and counted for radioactivity as previously described (2, 3). For comparative purposes purified 14C2H6 (4) was also run in parallel with 14C3H6.

RESULTS

Attempts to measure the uptake of nonlabeled ethylene by conventional gas chromatographic techniques have failed (1) primarily since this technique is not precise enough to detect the small amount of uptake that has been shown to occur by 14C-tracer studies (2, 3, 5). Such measurements are further complicated by the fact that they must be made against a background of continuous endogenous ethylene production. In contrast to ethylene no previous attempt has apparently been made to measure the uptake of propylene by plant tissues. This is unfortunate since, as seen in Figure 1, such measurements would have revealed a very rapid uptake of propylene that could have been readily detected by conventional gas chromatographic techniques and, unlike ethylene, would not have required the use of labeled material. Starting with an initial propylene concentration of 1.3 µL/1 (Fig. 1A) or 7.9 nmol of propylene for each g dry wt of pea seedling tissue, over half of the propylene in the chamber had disappeared (4.2 nmol/g dry wt) at the end of the first 24 hr. By the end of 72 hr, 97% (7.7 nmol/g dry wt) of the propylene was gone from the gas phase in the chamber. Increasing the propylene concentration to 12.3 µL/1 (Fig. 1B) or 73 nmol/g dry wt did not significantly change the percentage of total propylene taken up at various times, clearly indicating that the uptake rate increased with increasing concentration. The rate of propylene uptake at both 1.3 and 12.3 µL/1 (Fig. 1A and B) was fairly linear during the first 48 hr of incubation but fell off quickly thereafter because of the rapidly diminishing amount of propylene left in the chamber. When high levels of propylene were applied (104 µL/1 or 619 nmol/g dry wt), and propylene never became limiting, the rate of propylene uptake was constant over the entire 96-hr incubation period (Fig. 1C).

The uptake was clearly dependent on living tissue since no uptake was observed in those chambers containing the hot-water-killed seedlings (Fig. 1, A, B, and C). Microorganisms were not involved in the uptake of propylene since no uptake occurred even when nonaseptic conditions prevailed in the chambers containing the killed seedlings and massive contamination was apparent.

Studies with radioactive propylene confirmed the uptake results obtained with nonlabeled propylene (Fig. 2A). Like ethylene (2, 3, 7; Fig. 2B), [1-14C]propylene applied at 1 µL/1 was oxidized to 14CO2 and incorporated into tissue metabolites. The rate for total propylene metabolism during the first 48 hr, when uptake was linear, was approximately 25 times faster than with 14C3H4 (Fig. 2, upper curve A versus B). Unlike 14C3H4, the rate of 14C3H6 tissue incorporation exceeded the rate of oxidation to 14CO2. (Compare lower two curves in A and B.) Similar results were obtained when 9 µL/1 of 14C3H6 was applied (data not shown).

By comparing the total metabolism curve in Figure 2A with the uptake curve in Figure 1A, a similar pattern of concentration was applied, it can be seen that tissue incorporation plus oxidation completely account for the nonlabeled propylene lost in Figure 1A.

Because 14C3H6 and 14C2H6 were oxidized and incorporated into pea tissues at substantially different rates at 1 µL/1 (Fig. 2) a direct comparison was made between 14C3H6 and 14C2H6 over a wide concentration range using gases of equal specific radioactivity. As
previously reported (3) \(^1\)C\(_2\)H\(_4\) tissue incorporation and oxidation were found to increase steadily with increasing \(^1\)C\(_2\)H\(_4\) concentration during a 24-hr incubation period. The rate of \(^1\)C\(_2\)H\(_4\) conversion to \(^1\)CO\(_2\) was found to exceed the rate of tissue incorporation over the concentration range shown in Figure 3. In contrast, the rates of \(^1\)C\(_2\)H\(_4\) oxidation to CO\(_2\) and tissue incorporation were many times higher with both rates falling off rapidly above 100 \(\mu\)l/l. At 1 \(\mu\)l/l the rate of propylene oxidation was 10 times greater and incorporation 45 times greater than that of ethylene. A comparative study of the distribution pattern of \(^1\)C metabolites derived from \(^{14}\)C\(_2\)H\(_4\) and \(^1\)C\(_2\)H\(_4\) using previously described ethanol extraction, ion exchange fractionation, and paper chromatography techniques (7) revealed clear similarities as well as some noticeable differences. As with \(^{12}\)C\(_2\)H\(_4\), the majority of the \(^1\)C\(_2\)H\(_4\)-derived metabolites were water-soluble (70%) with about half separating by ion exchange chromatography into the cationic fraction and the other half into the neutral fraction. Very few of the water-soluble counts were recovered in the anionic fraction. The distribution pattern of the neutral fraction from \(^{14}\)C\(_2\)H\(_4\)- and \(^1\)C\(_2\)H\(_4\)-treated pea seedlings (Fig. 4) clearly shows that two major labeled metabolites are formed following treatment with either olefin. However, it is also clear by their \(R_p\) values that they are not the same two metabolites. Presumably, this difference is derived from the additional methyl group in propylene.

DISCUSSION

These results demonstrate that propylene, like ethylene, is metabolized by plants. The rate of propylene uptake is so rapid that...
it can be detected easily by standard gas chromatographic methods; therefore, it is surprising this uptake has gone undetected for so long. The metabolic system responsible for the oxidation and incorporation of propylene appears to be the same one responsible for ethylene metabolism since nonlabeled propylene competitively inhibits $^{14}\text{C}_2\text{H}_4$ metabolism and vice versa (data not shown).

In view of the suggestion that ethylene metabolism is directly related to action (2, 3), it can be argued that propylene, being more actively metabolized, should therefore be more active biologically. In this regard it must be remembered that while ethylene and propylene metabolisms are similar they are by no means identical. From these initial studies two major differences are already apparent. First, in the case of propylene the ratio of oxidation to tissue incorporation is completely reversed from that observed with ethylene (Fig. 3); and second, the neutral tissue metabolites derived from propylene are different from those from ethylene. While the over-all metabolisms of $^{14}\text{C}_2\text{H}_6$ and $^{14}\text{C}_2\text{H}_4$ seem to be similar (i.e. both are oxidized and incorporated into pea tissues), there are sufficient differences possibly to account for the weaker biological activity of propylene even though it is metabolized more rapidly. For example, if the initial product(s) of ethylene metabolism were an active intermediate in the reaction sequence leading to an ethylene response, then both its rate of formation and chemical nature would be important. Since the neutral end products of propylene metabolism are different from those derived from ethylene (Fig. 4), the possibility exists that the initial propylene product(s) is also different and is simply a much less active intermediate. This could then account for the over-all lower biological activity of propylene even though it is metabolized more rapidly. Additionally, if the relative rate of oxidation to tissue incorporation were important, this may also contribute to differences in biological activity since with propylene tissue incorporation exceeded oxidation whereas the opposite was true with ethylene (Fig. 3).

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**LITERATURE CITED**