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Communication

Abscisic Acid Biosynthesis in Isolated Embryos of *Zea mays* L.[1]

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ABSTRACT

Previous labeling experiments with $^{18}O_2$ have supported the hypothesis that stress-induced abscisic acid (ABA) is synthesized through an indirect pathway involving an oxygenated carotenoid (xanthophyll) as a precursor. To investigate ABA formation under nonstress conditions, an $^{18}O_2$ labeling experiment was conducted with isolated embryos from *in vitro* grown maize (*Zea mays* L.) kernels. Of the ABA produced during the incubation in $^{18}O_2$, three-fourths contained a single ^{18}O atom located in the carboxyl group. Approximately one-fourth of the ABA synthesized during the experiment contained two ^{18}O atoms. These results suggest that ABA synthesized in maize embryos under nonstress conditions also proceeds via the indirect pathway, requiring a xanthophyll precursor. It was also found that the newly synthesized ABA was preferentially released into the surrounding medium.

Although $^{18}O_2$ labeling experiments have strongly implicated the indirect biosynthetic pathway to ABA via an oxygenated carotenoid precursor in water-stressed leaves and roots (3, 4), little is known of the biosynthesis of ABA in nonstressed tissues. Labeling experiments with turgid *Xanthium* leaves suggest a similar pathway may be operating under nonstress conditions, but the rate of synthesis was so low that only 6% incorporation of a single ^{18}O atom could be observed after 72 h (3). Immature maize embryos isolated from *in vitro*-grown kernels provide a convenient system to investigate the formation of ABA under nonstress conditions, since they produce large quantities of ABA 15 to 26 DAP[3] (11). An added advantage is that the kernels can be cultured on agar, thus eliminating any maternal source of ABA.

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[3]Abbreviations: DAP, days after pollination; Me-ABA, methyl ester of ABA; GC-NCl, gas chromatography-negative chemical ionization; GC-El-SIM, gas chromatography-electron impact-selected ion monitoring.

Several lines of evidence indicate that reductions in carotenoid biosynthesis are associated with reduced ABA accumulation in developing maize kernels. For example, viviparous mutants (*vp2*, *vp5*, *vp7*, *vp9*, *w3*) show reductions in both carotenoid and ABA content (6, 9, 11). Also, chemical inhibitors of carotenoid desaturation (6, 7), and cyclization reactions (5) similarly result in reduced carotenoids and ABA. Since carotenoids are required for the formation of photosynthetically active leaves, it is difficult to separate studies on carotenoids without secondarily affecting photosynthesis. The developing maize kernel is totally dependent on external sources of nutrients, and carotenoid accumulation is thus independent of any photosynthetic function. We have examined the labeling patterns of ABA produced by isolated embryos to determine if the same labeling patterns are found in photosynthetic and nonphotosynthetic tissues, and in stress and nonstress situations.

In this study, cultured maize embryos were incubated in an atmosphere containing $^{18}O_2$ during the period of maximum ABA synthesis. The pattern of ^{18}O incorporation into ABA produced by the embryo tissue was then determined by MS.

MATERIALS AND METHODS

Plant Material

Developing cobs of field grown inbred yellow dent maize (*Zea mays* L., cv Tx 5855) were harvested 5 DAP, then sectioned into pieces containing approximately 10 kernels and placed on defined medium with agar (11) at 29°C. Embryos were dissected from the whole kernels 17 DAP and transferred to fresh agar in the bottom of a 125 mL Erlenmeyer flask sealed with a new serum stopper. The flask was then filled with an atmosphere of 21% $^{18}O_2$ and 79% N_2 as described previously (4). The embryos were maintained in this atmosphere for 24 h, during which time the O_2 concentration in the flask was monitored and replenished when needed as described (4).

Extraction and Purification of ABA

The embryo tissue and the agar were extracted separately (2, 3). ABA was purified from the extracts as described previously (3).

Mass Specrometry

GC-NCl and GC-El-SIM mass spectrometry of Me-ABA were performed on a JEOL HX-110 HF double focusing mass spectrometer equipped with a Hewlett-Packard 5890 gas chromatograph (single stage jet separator) with He as the carrier gas (flow rate 12 mL/min). The column used was a DB-1 megabore (15 m × 0.53 mm; J&W Scientific, Inc. Rancho Cordova, CA). GLC conditions were: oven temperature programmed from 180 to 250°C at 5°C/min. For GC-NCl MS methane was used as the reagent gas.

Chemicals

$^{18}O_2$ (99%) was purchased from Cambridge Isotopes (Woburn, MA).

RESULTS

Oxygen Labeling of Abscisic Acid

Analysis of the ABA (as Me-ABA) isolated from the embryo tissue by GC-NCl MS demonstrated that most of the compound was unlabeled, while 13.4% contained a single ^{18}O atom and 4.3% contained two ^{18}O atoms; only a trace was triply labeled (Table I). This indicates that most of the ABA in the embryo tissue was synthesized prior to the incubation in the $^{18}O_2$ atmosphere. GC-El-SIM MS measurements of the ions at m/z 125 and 127 confirmed that the first oxygen atom was located in the carboxyl group (3, 8). The ratio of the intensity of the ion at m/z 127 relative to that of the ion at m/z 125 was increased in the embryo Me-ABA sample compared to the ratio found in an unlabeled Me-ABA standard (data not shown). This pair of ions represents fragments containing the side chain of Me-ABA, including the two carboxyl oxygen atoms, where m/z 125 is unlabeled and m/z 127 is labeled with a single ^{18}O atom (8).

In contrast to the embryo tissue, the agar extract contained a much higher proportion of labeled ABA. Although unlabeled Me-ABA was still the predominant component, 40.0% of the total contained a single ^{18}O atom and 12.9% was doubly labeled. Again, only a small amount contained three ^{18}O atoms (Table I). The higher percentage of labeled ABA isolated from the agar extract suggests that 'newly synthesized ABA' (*i.e.* the ABA formed during the incubation in the $^{18}O_2$ atmosphere) was preferentially excreted from the embryo. Alternatively, ABA released into the agar was no longer available for conversion to phaseic acid. In both the embryo tissue and the agar extract the ratio of singly labeled Me-ABA to doubly labeled Me-ABA was the same, namely 3:1 (Table I). The single ^{18}O label was again found to be located in the carboxyl group by GC-El-SIM. As expected, the intensity of the ion at m/z 127 relative to the ion at m/z 125 was increased in the agar Me-ABA sample over that of the embryo Me-ABA sample. This is in accord with the larger fraction of labeled Me-ABA in the agar sample. The position of the second ^{18}O atom in the doubly labeled Me-ABA could not be confirmed by an MS-MS experiment (3) due to the small sample sizes available. However, the absence of increased intensity of the ion at m/z 129 in the GC-El-SIM MS analysis of either sample indicates the second oxygen label is located on the ring.

Table I. *Incorporation of ^{18}O into Abscisic Acid in Maize Embryos*

Excised maize embryos were incubated on agar in the presence of $^{18}O_2$ for 24 h. ABA was extracted from both the embryos and agar, and analyzed as Me-ABA. The data shown were obtained by GC-NCl. A value of 100% indicates that it was the most prominent ion (base peak). All other ions are relative to the base peak. Values in parentheses are percentages of total.

ABA Isolated from	Relative Abundance at m/z:			
	278	280	282	284
Embryos	100	16.4	5.3	0.5
	(81.8)	(13.4)	(4.3)	(0.4)
Agar	100	85.5	27.5	0.9
	(46.8)	(40.0)	(12.9)	(0.4)

DISCUSSION

Incorporation of ^{18}O into ABA from $^{18}O_2$

The results of the mass spectrometric analyses of the Me-ABA isolated in this experiment suggest a similar biosynthetic pathway is operating in stressed tissue (3) and in cultured excised maize embryos. Although a large proportion of the ABA isolated from the embryo tissue and agar media was synthesized prior to the incubation in $^{18}O_2$, the labeling pattern of the ABA synthesized during the experiment was similar to that observed in stressed tissues. Moreover, the location of the labeled oxygen in the carboxyl group in the singly labeled ABA is identical to that previously reported for water stressed leaves and roots (3, 4). The position of the second oxygen label in the ring (position C-1′ or C-4′ of ABA) is also consistent with previous observations of the labeling pattern in stressed roots (3). All of these results further support the hypothesis that ABA is formed by an initial oxidative cleavage of a xanthophyll precursor. The primary xanthophyll precursor would contain both ring oxygen atoms, yielding the singly labeled ABA. This compound could be one of the 'polyoxy' xanthophylls reported in maize kernels (10). A second xanthophyll precursor (*e.g.* zeaxanthin, lutein, or cryptoxanthin) containing only one ring oxygen atom would lead to the doubly labeled ABA. Time course experiments are required to determine whether the primary xanthophyll precursor is depleted over time, allowing the second deoxy-carotenoid to feed into the precursor pool. Alternatively, both xanthophyll precursors may contribute to ABA biosynthesis, no matter what the concentration of the primary precursor (see Ref. 3 for discussion). Although the proportion of doubly labeled ABA produced by the maize embryos is higher than that observed in stressed *Xanthium* leaves (3), other tissues, such as barley leaves and avocado fruit (JAD Zeevaart, DA Gage, unpublished results), have produced a similar pattern in $^{18}O_2$ labeling experiments.

Release of ABA from Embryo Tissue

One unexpected result of this experiment was the large proportion of ^{18}O labeled ABA detected in the agar medium surrounding the embryo tissue. The higher ratio of labeled to unlabeled ABA in the agar relative to that found in the embryo

tissue (Table I) suggests that newly synthesized ABA may diffuse from the outer layers of the embryo tissue into the surrounding media. There is no apparent vascular system in the developing embryo which could account for movement of ABA from more interior cells (6). Thus, it seems likely that the site of ABA synthesis in maize embryo tissue is localized in the outer cell layers. During normal development of maize kernels the ABA formed in the embryo would likely accumulate in the endosperm (11).

The present labeling studies with isolated maize embryos further support evidence from vivipary mutants (6, 9, 11), chemical inhibitors of carotenoid biosynthesis (5–7), and lipoxygenase activity changes during embryo development (1) that carotenoids are the precursor to ABA and that this biosynthetic pathway is present in a variety of plant tissues.

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