Effect of Abscisic Acid on Uptake and Metabolism of
[\textsuperscript{3}H]Gibberellin A\textsubscript{1} and [\textsuperscript{3}H]Pseudogibberellin A\textsubscript{1}
by Barley Half-seeds\textsuperscript{1}

CHARLES F. STOLP, RONN NADEAU, AND LAWRENCE RAPPAPORT
Department of Vegetable Crops, University of California, Davis, California 95616

Received for publication May 11, 1973

ABSTRACT

Uptake and metabolism of 1,2-[\textsuperscript{3}H]gibberellin A\textsubscript{1} (\[\textsuperscript{3}H\]GA\textsubscript{1}, I) and its 3-hydroxy epimer (\[\textsuperscript{3}H\]pseudoGA\textsubscript{1}, II) by barley (Hordeum vulgare L.) half-seeds were measured after 24 hours of incubation, in the presence or absence of abscisic acid in the media. Uptake of both compounds was enhanced by abscisic acid, and abscisic acid enhanced the extent of metabolism of \[\textsuperscript{3}H\]GA\textsubscript{1}. However, \[\textsuperscript{3}H\]pseudoGA\textsubscript{1} was not metabolized, even in the presence of abscisic acid. The significance of the stereochemistry of the 3-hydroxyl position is discussed.

Barley aleurone layers readily accumulate and metabolize [\textsuperscript{3}H]GA\textsubscript{1}, and these processes are markedly enhanced by the presence of ABA in the incubation medium (4). However, it is not known from those studies whether ABA specifically affects [\textsuperscript{3}H]GA\textsubscript{1} uptake, metabolism, or both. Nadeau et al. (4) speculated that ABA enhanced conversion of \[\textsuperscript{3}H\]GA\textsubscript{1} to more polar, sequestered metabolites, resulting in the establishment of a gradient which favored greater net uptake of the labeled GA\textsubscript{1}. This speculation was supported by the observation that ABA had no apparent effect on uptake of \[\textsuperscript{3}H\]GA\textsubscript{1} from the medium until after the 4th hr of incubation, about the same time that metabolites were first detected in extracts of the aleurones. This left open the possibility that ABA simply enhanced uptake directly so that more \[\textsuperscript{3}H\]GA\textsubscript{1} was made available at the site of conversion. One approach to resolving the question of whether ABA influences uptake or metabolism is to study an analogue of \[\textsuperscript{3}H\]GA\textsubscript{1} which is not metabolized. PseudoGA\textsubscript{1} (3\textalpha-OH-GA\textsubscript{1}) differs chemically from GA\textsubscript{1} (3\beta-OH-GA\textsubscript{1}) only in the orientation of the 3-hydroxyl (Fig. 1). In GA\textsubscript{1}, the 3-hydroxyl group is axial, whereas in pseudoGA\textsubscript{1}, it is equatorial. As a consequence pseudoGA\textsubscript{1} is virtually devoid of biological activity when applied at hormonal concentration. It was decided, therefore, to compare \[\textsuperscript{3}H\]pseudoGA\textsubscript{1} and \[\textsuperscript{3}H\]GA\textsubscript{1} as to uptake, metabolism, and capacity to promote \alpha-amylase synthesis, with and without ABA, in barley half-seeds.

MATERIALS AND METHODS

The \[\textsuperscript{3}H\]GA\textsubscript{1} was synthesized (New England Nuclear Corp.) by selective reduction of the 1,2-double bond in GA\textsubscript{1}, using the method described by Pitel and Vining (5). However, the preparation produced a low yield of \[\textsuperscript{3}H\]GA\textsubscript{1}, and extensive purification was necessary to obtain pure product (Nadeau and Rappaport, in preparation). Carrier-free tritium gas was used in the reduction, resulting in \[\textsuperscript{3}H\]GA\textsubscript{1} of high specific radioactivity (43 c/mmole, 2.9 \times 10\textsuperscript{6} dpm/\mu g, 1 \times 10\textsuperscript{8} cpm/\mu g). \[\textsuperscript{3}H\]PseudoGA\textsubscript{1} was prepared from \[\textsuperscript{3}H\]GA\textsubscript{1} by a variation of the method described by Cross et al. (1), in a reaction which occurred without the loss of \textsuperscript{3}H from C-2 (Nadeau, in preparation).

All procedures for incubation of barley (Hordeum vulgare L.) half-seeds and measurement of \alpha-amylase have been reported in detail (4). Preimbibed half-seeds of Himalaya barley (1972 harvest) were incubated in flasks containing 2.0 ml of medium. The \[\textsuperscript{3}H\]GA\textsubscript{1} and \[\textsuperscript{3}H\]pseudoGA\textsubscript{1} concentrations were 500,000 cpm, 5 \times 10\textsuperscript{6} \mu g, per 2.0 ml of medium (7.2 nm). When ABA was present, it was in concentration of 10 \mu g/2.0 ml (19 \mu M). Each treatment was run in duplicate. After the 24-hr incubation period the media were decanted, and the half-seeds were washed twice with distilled water (10 sec each time) to remove surface radioactivity. The media and washings were combined and brought up to a volume of 6 ml. Aliquots were taken for \alpha-amylase determination and for liquid scintillation counting to measure the amount of label remaining in each flask. The replicated half-seeds were then combined and homogenized in 6 ml of 80% ethanol. The homogenates were centrifuged, and the resulting pellets were resuspended in fresh 80% ethanol and recentrifuged. The combined supernatants were evaporated almost to dryness under vacuum. The concentrated extracts were brought up to a volume of 2 ml with 10% (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, which had been adjusted to pH 2.0. The (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} was added to facilitate subsequent partitioning of

\begin{align}
I, & \ [\textsuperscript{3}H\]GA\textsubscript{1} ; \ R\textsubscript{1} = \textsuperscript{3}H, \ R\textsubscript{2} = \textsuperscript{3}H, \ R\textsubscript{3} = \textsuperscript{3}H \\
\textbullet I, & \ [\textsuperscript{3}H\]pseudoGA\textsubscript{1} ; \ R\textsubscript{1} = \textsuperscript{3}H, \ R\textsubscript{2} = \textsuperscript{3}H, \ R\textsubscript{3} = \textsuperscript{3}H \\
\textbullet I, & \ [\textsuperscript{3}H\]GA\textsubscript{1} glycoside; \ R\textsubscript{1} = \textsuperscript{3}H, \ R\textsubscript{2} = 0-glycosyl, \ R\textsubscript{3} = \textsuperscript{3}H \\
\textbullet I, & \ [\textsuperscript{3}H\]GA\textsubscript{1} glycoside; \ R\textsubscript{1} = 0-glycosyl, \ R\textsubscript{2} = \textsuperscript{3}H, \ R\textsubscript{3} = \textsuperscript{3}H
\end{align}

Fig. 1.

\textsuperscript{1}This research was supported by United States Public Health Service Grant GM12885 and National Science Foundation Grant GB21241.
labeled metabolites. The acidified extracts were extracted first with three 1-ml portions of ethyl acetate, then with three 1-ml portions of butanol. The ethyl acetate fractions were dried over NaSO₄, and the butanol fractions were concentrated to small volume under nitrogen. The remaining water fractions were concentrated to dryness under nitrogen, redissolved in the minimum amount of water, and treated with 5 ml of methanol to precipitate the (NH₄)₂SO₄. Virtually all of the radioactivity remained in solution. This procedure was repeated once, and the reconcentrated fractions were streaked on ChromAR. The ethyl acetate fractions were run in ether-benzene-acetic acid (10:10:1) and the butanol and water fractions in isopropanol-3 N ammonia (5:1). The peaks, located with a radiochromatogram scanner, were eluted, and aliquots of each were taken for liquid scintillation counting.

RESULTS

Accumulation of [³H]pseudoGA, in half-seeds after 24 hr was greater than that of [³H]GA, in the presence and absence of ABA (Table I). The ethyl acetate fractions from the [³H]GA, incubation treatments (with or without ABA) were found to contain only one radioactive peak with an RF corresponding to that of unchanged [³H]GA,. The butanol fractions also contained a small amount of [³H]GA, which did not completely fractionate into ethyl acetate. In addition, the butanol phase yielded a second radioactive zone, detected by radio-scanning, at RF values corresponding to glycosides III and IV. The percentage of each compound is shown completely fractionate into ethyl acetate. In addition, the butanol phase yielded a second radioactive zone, detected by radio-scanning, at RF values corresponding to glycosides III and IV. The percentage of each compound is shown (4).

The ethyl acetate fractions from the [³H]pseudoGA, treatments, with and without ABA, each contained only one radioactive substance with an RF corresponding to that of unchanged [³H]pseudoGA,. A small amount of [³H]pseudoGA, was also found in the butanol extract. No other peaks occurred in any of the extracts of the [³H]pseudoGA, incubations. Thus, virtually no metabolism of the [³H]pseudoGA, occurred, whether or not ABA was present in the incubation medium. Moreover, [³H]pseudoGA, was completely ineffective in stimulating α-amylase synthesis, whereas [³H]GA, in identical concentration typically stimulated enzyme synthesis (Table III).

DISCUSSION

The results indicate the remarkable significance of the stereochemistry of the 3-OH position in relation to uptake and metabolism of [³H]GA, and its capacity to induce α-amylase synthesis in barley half-seeds. They also indicate that ABA enhances accumulation of both GA isomers but only affects metabolism of the bioactive hormone. It appears that the enzymes which hydroxylate and/or glycosylate GA, are so specific as to be unable to react with pseudoGA,. Such specificity has been found in a cell-free enzyme preparation from bean seeds that converts [³H]GA, to [³H]GA, but leaves [³H]pseudoGA, totally unaffected (Patterson and Rappaport, in preparation). However, alternative explanations are possible, e.g., that the necessary enzymes must be induced by an active GA like GA, (but not pseudoGA,). Finally, there is the more remote possibility that pseudoGA, for unknown reasons, may never reach the vicinity of the enzymes.

Since [³H]pseudoGA, is taken up readily but is not metabolized by barley half-seeds, it is of special interest to consider the effect of ABA which enhances total accumulation of [³H]GA,. The data of Table I show that over a 24-hr period the increase in [³H]pseudoGA, uptake is considerably higher in the presence of ABA than in its absence. It is clear, therefore, that ABA may enhance uptake of GAs with very similar structure, but that susceptibility of a GA to metabolism may be restricted by configurational characteristics. Although the relation between metabolism of GAs and biological activity is not yet understood, it is conceivable that differences in biological activity may be linked to susceptibility to metabolism.

It should be noted that in some respects barley half-seeds differ from barley aleurones with regard to metabolism of...
For example, in half-seeds \(^{3}\text{H}\)GA-X is a detectable product only when ABA is present in the medium, whereas with isolated barley aleurones \(^{3}\text{H}\)GA-X is formed both in the presence and absence of ABA, but to a greater extent in the presence of ABA.

Acknowledgments—ABA was generously supplied by Hoffman-La Roche and GAs, GAs, and GAs-glucoside by G. Sembdner, J. MacMillan, N. Takahashi, and D. Mertz.

LITERATURE CITED