Uptake and Metabolism of Radioactive Gibberellins by Barley Aleurone Layers*

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Received August 20, 1971

Summary. When aleurone layers were treated with labeled gibberellin A₁ (3H-GA₁), gibberellin A₅ (3H-GA₅) and the methyl ester of 3H-GA₅ (3H-GA₅-ME), radioactivity was accumulated by the tissue for a period of 20–30 h. After this time, radioactivity was released into the medium. Concomitantly, ribonuclease was also liberated by the tissue. The radioactivity accumulated by aleurone layers was associated with polar metabolites of the respective GAs, and the extent of accumulation was a function of the degree of GA metabolism (GA₅-ME > GA₅ > GA₁). Accumulation of radioactivity was inhibited in the cold and by the metabolic poisons NaF and dinitrophenol. This was thought to be due to inhibition of GA metabolism. The accumulation of 3H-GA₁ in aleurone tissue did not reach saturation when unlabeled GA₃ up to 10⁻⁸ M was added to the incubation medium.

Introduction

The accumulation of animal hormones in target tissues is a well-documented phenomenon (see e.g. Jensen and Jacobson, 1962). Such in vivo binding studies have led to the isolation and characterization of hormonal receptor proteins (see e.g. Gorski et al., 1968). The accumulation of plant hormones in their respective target tissues or target cells has been demonstrated in only two cases: Radioactive gibberellins (GAs) accumulate in the apical, GA-sensitive part of the dwarf pea stem (Musgrave et al., 1969) and a labeled cytokinin, benzyladenine, in those cells of moss protonemata which form buds in response to the hormone, and in the developing buds themselves (Brades and Kende, 1968).

The experiments described below were performed in order to study the uptake, accumulation and metabolism of GAs by barley aleurone cells which synthesize a number of hydrolytic enzymes in response to GA.

* Abbreviations: GA, gibberellin; GA₅-ME, gibberellin A₅ methyl ester; RNase, ribonuclease.
Materials and Methods

Incubation. Aleurone layers of barley (*Hordeum vulgare*, L., cv. Himalaya, 1964 harvest) were prepared according to Chrispeels and Varner (1967), and incubated at 25°C on a reciprocating shaker in filter-sterilized Na-acetate buffer (0.001 M, pH 4.8) containing 0.01 M CaCl₂ and 10 µg/ml chloramphenicol. Where indicated, the following compounds were included in the incubation medium: ³H-GA₁ (spec. activity 48 mc/mmole; Kende, 1967), ³H-GA₅ (spec. activity 110 mc/mmole; Musgrave and Kende, 1970), ³H-GA₅-ME (spec. activity 110 mc/mmole; Musgrave et al., 1969) all at a concentration of 2 × 10⁻⁷ M, and NaF and dinitrophenol at 10⁻³ M.

Enzyme Assays. At appropriate intervals, samples of the incubation media were removed aseptically, frozen and kept at −20°C until further analysis. α-Amylase and RNase activities were measured as described by Chrispeels and Varner (1967).

Assay of Radioactivity. The aleurone layers were blotted, weighed and shaken overnight in vials containing Bray's (1960) scintillation fluid. The radioactivity was determined with a Packard Liquid Scintillation Spectrometer (Model 3375). Zones of thin-layer chromatograms were scraped off and counted directly in Bray's solution. When necessary, the results were corrected for quenching.

Extraction of GAs and GA-Metabolites. The layers were removed from the incubation medium and washed for 1 h in two fresh batches of GA-free medium. The medium and the combined wash solutions were partitioned against ethyl acetate, first at pH 8.5, then at 2.5. The resulting three phases—non-acidic ethyl-acetate, acidic ethyl-acetate and the remaining aqueous phase—were tested separately for radioactivity. The aleurone layers were ground in methanol using a glass mortar and pestle. The mixture was centrifuged, the methanol decanted, and the residue extracted twice more with methanol. The methanol extracts were combined, and the solvent was evaporated under vacuum. The residue was taken up in ethyl acetate and phosphate buffer at pH 8.4 and partitioned. The pH of the aqueous phase was adjusted to 2.5 with HCl and partitioning against ethyl acetate was repeated. The radioactivity of the non-acidic ethyl-acetate, acidic ethyl-acetate and aqueous phases was determined. The methanol-insoluble residue was dried, combusted in a Packard Sample Oxidizer and the radioactivity of the resulting tritiated water was counted.

Chromatography. The relative distribution of GAs and GA-metabolites in the 2 ethyl-acetate phases was determined by thin-layer chromatography using Silica gel H and the following solvent systems: A. benzene—n-butanol—acetic acid (70:20:2, v/v), B. chloroform—ethyl acetate—acetic acid (60:40:5, v/v).

Results

GA₁ and, to a lesser extent, GA₅ enhanced the production of α-amylase in isolated aleurone layers (Fig. 1). The earliest effect of GA₁, like that of GA₅, was measurable after 7–8 h, while the earliest response to GA₅ could be detected only after 12 h. GA₅-ME did not enhance the formation of α-amylase. The level of the enzyme in control and GA₅-ME-treated aleurone layers increased slowly beginning about 15 h after the start of incubation.

Aleurone layers incubated in GA₁ solution at 4°C maintained a water content of 50% by weight, whereas the water content of layers kept at 25°C rose to 80% over a period of 50 h. If the water in the tissue had