Effects of N⁶-Benzyladenine on the Rate of Turnover of [³H]GA₂₀ by Shoots of Dwarf Pisum sativum

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Summary. Application of the cytokinin, N⁶-benzyladenine, to light-grown shoots of dwarf “Meteor” pea seedlings (Pisum sativum L.) markedly increased the rate of turnover of GA₂₀, a native pea gibberellin. It is suggested that endogenous cytokinins may affect gibberellin metabolism in plants by controlling rates of gibberellin turnover.

Introduction

It has been known for some time that applied cytokinins affect the levels of endogenous gibberellin-like (GA-like) substances in seedlings of Pisum sativum (Sebanek, 1966; Karanov and Vassilev, 1969) and other higher plants (Michniewicz and Kamienska, 1967; Chin and Beevers, 1970; Loveys and Wareing, 1971; Reid and Railton, 1974a, b). Recent evidence (Railton and Reid, 1973) suggests that root-produced cytokinins play a major role in the control of shoot growth in tomato plants and that this control is exerted to some extent via an effect on GA metabolism (Reid and Railton, 1974a, b).

Recent studies on GA biosynthesis in seedlings of dwarf Pisum now provide an opportunity to study more specifically, the effect of cytokinins on GA metabolism. Two possible GA biosynthetic pathways have been demonstrated in dwarf “Meteor” pea seedlings. One pathway utilizes the C-20 gibberellin, GA₁₄ as a precursor and proceeds via GA₁₈, GA₂₈, and GA₃₂ to the C-19 gibberellins, GA₁ and GA₅ (Durley et al., 1974a, b). The other pathway proceeds via GA₅, which is converted via GA₂₀ into GA₄₀ (Railton et al., 1974a, b, c). The availability of suitable methods for labelling GA₅ and GA₂₀ allowed, for the first time, a demonstration of the rate of turnover of a higher-plant GA, GA₂₀, in dwarf pea seedlings (Railton, 1974). The strong circumstantial evidence implicating cytokinins as controlling factors in GA metabolism in plants, prompted a study of the effects of a cytokinin, N⁶-benzyladenine (BA) on the turnover rate of GA₂₀ in dwarf pea seedlings.

Materials and Methods

Seedlings of dwarf “Meteor” pea (Pisum sativum L.) were grown in moist vermiculite and maintained under continuous illumination at 23°C for 6 days.

BA was applied as a spray to the aerial parts of the plants at a concentration of 50 mg/l containing 0.01% Tween 20. The plants were sprayed until runoff; about 1 ml of solution (ca. 50 μg BA) was required to achieve this. Seedlings to be treated with BA were sprayed on 3 consecutive days prior to the application of radioactive GAs. Control plants were sprayed with distilled water containing 0.01% Tween 20.

[³H]-2,3-GA₂₀ was prepared from GA₅ by the method of Murofushi et al. (1974). Gibberellin A₅ methyl ester was converted to GA₅ methyl ester-16, 17-epoxide by oxidation with meta-
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Fig. 1A and B. Effect of BA on the metabolism of [3H]GA9 in dwarf pea seedlings. Extracts were chromatographed on TLC using the solvent system, ethylacetate, chloroform, formic acid (50:50:1 v/v). (A) without BA. (B) with BA.

chloroperbenzoic acid. The epoxide so formed was reduced with a mixture of hydrogen and tritium to afford GA20 methyl ester-16, 17-epoxide. Removal of the epoxide oxygen was achieved using iodine and zinc dust and the resulting GA20 methyl ester hydrolyzed with 0.2 N NaOH in methanol to give [3H]-2,3-GA20, sp.act. 2 Ci/mM.

[3H]-16,17-GA9 was prepared via a Wittig reaction in a similar manner to that described by Cross et al. (1968) to yield a product, sp.act. 18 mCi/mM.

Radioactive GAs were applied in 10 μl droplets of 50% ethanol (1 μCi of each GA) to shoots of dwarf pea seedlings treated with or without BA. A total of 10 plants were used per treatment and these were harvested 22 h after application of [3H]GA9 and [3H]GA20.

Gibberellins were extracted from the shoots by homogenizing in ice-cold 80% methanol. After removing all methanol in vacuo at 35°C, the aqueous phase was adjusted to pH 9.0 with an equal volume of 0.5 M phosphate buffer and partitioned against diethyl ether. The aqueous phase was then adjusted to pH 3.0 with 1 N hydrochloric acid and partitioned against ethyl acetate. The ethyl acetate fraction was reduced to dryness and a portion of the residue chromatographed on thin layer sheets of silica gel (Eastman-Kodak) using the solvent system, ethyl acetate, chloroform, formic acid (50:50:1, v/v).

Thin layer sheets were divided into strips, the gel eluted with absolute methanol and the levels of radioactivity associated with each strip determined by scintillation spectrometry using a toluene-based scintillant containing 2,5-diphenyloxazole (PPO) at a concentration of 5 g/1 of toluene.

Results and Discussion

It was previously shown by Sebanek (1966) and Karanov and Vassilev (1969) that both kinetin and BA increased the levels of endogenous GA-like substances in seedlings of dwarf Pisum. Karanov and Vassilev (1969) suggested that this effect was indirect and resulted from a cytokinin-induced inhibition of growth, resulting in less GA utilization and a subsequent increase in GA levels.

When [3H]GA9 was applied to BA treated dwarf pea seedlings it was metabolized into its products more rapidly than in non-BA treated control plants, as indicated by the larger amount of [3H]GA9 remaining after 22 h (Fig. 1). The low levels of radioactivity associated with the products, GA20 and 2,3 dihydro GA31 (Fig. 1B) in BA treated seedlings suggested that these products were themselves...