Plant Physiol. (1992) 99, 372–377
0032-0889/92/99/0372/06/$01.00/0

Received for publication August 23, 1991
Accepted December 16, 1991

Gibberellin A_1 Biosynthesis in *Pisum sativum* L.[1]

II. Biological and Biochemical Consequences of the *le* Mutation

Valerie A. Smith

Department of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom

ABSTRACT

A comparative study of the metabolism of radiolabeled gibberellin (GA) 1, 19, and 20 in isolated vegetative tissues of isogenic *Le* and *le* pea (*Pisum sativum*) plants incubated *in vitro* with the appropriate GA substrate is described. The results of this study provide evidence that the enzymes involved in the latter stages of GA biosynthesis are spatially separated within the growing pea plant. Apical buds were not apparently involved in the production of bioactive GA_1 or its immediate precursors. The primary site of synthesis of GA_{20} from GA_{19} was immature leaflets and tendrils, and the synthesis of bioactive GA_1 and its inactive catabolite GA_8 occurred predominantly in stem tissue. GA_{29}, the inactive catabolite of GA_{20}, was produced to varying extents in all the tissues examined. Little or no difference was observed in the ability of corresponding *Le* and *le* tissues to metabolize radiolabeled GA_1, GA_{19}, or even GA_{20}. During a fixed period of 24 hours, stems of plants carrying the *le* mutation produced slightly more [^{3}H]GA_1 (and [^{3}H]GA_{29}) than those of *Le* plants. It has been concluded that the *le* mutation does not lie within the gene encoding the GA_{20} 3β-hydroxylase protein.

Pea (*Pisum sativum*) plants that carry the *le* mutation are dwarfed and deficient GA_1[2] (5). Although it has been shown that the GA_1 deficiency is characteristic of all immature vegetative tissues of *le* pea plants (15), it is only with respect to the stem that a clear correlation between growth (final internode length) and endogenous GA_1 concentration exists (4, 14). The role of GA_1 in tissues other than the stem is therefore uncertain. Indeed, the presence of GA_1 in the various tissues could simply be a fortuitous result of the mobility of this compound within the plant, depending on the site(s) of synthesis of GA_1. To date, there exists little information pertaining to this topic. The functional basis of the *le* mutation is also unknown at the present time, but, the most obvious and favored hypothesis is that of impaired catalytic performance of the enzyme, the GA_{20} 3β-hydroxylase, that produces GA_1 (10).

The present communication is the second in a series concerned with the role of GA_1 as a natural plant growth regulator. The steady-state distribution of the 13-hydroxy GAs in *P. sativum* was the subject of the first paper (15). The biosynthesis of GA_1 and the nature of the *le* mutation are the subjects addressed here.

MATERIALS AND METHODS

GA metabolites

[17-$^3H_2$]GA_1 (2.2×10^{11} Bq mmol^{-1}) was synthesized by Dr. M.H. Beale, University of Bristol. [17-^{13}C,$^3H_2$]GA_{20} (1.27×10^{12} Bq mmol^{-1}) was synthesized by the method described by Ingram *et al.* (5). [17-$^3H_2$]GA_{19} (1.83×10^{15} Bq mmol^{-1}) and [17-$^2H_2$]GA_{19} were gifts from Professor L. Mander, Australian National University, Canberra, Australia.

Plant Material

Seeds of the tall (205^+ *Le*) and dwarf (205^- *le*) isogenic lines of pea (*Pisum sativum*) were gifts of Professor J.B. Reid, University of Tasmania. The conditions adopted for seed germination and plant growth have been described previously (15). Tissues were harvested after the emergence of four internodes.

Metabolic Studies

The tissue used for each incubation was obtained from six to 12 plants. The apical buds, upper two internodes, and their accompanying leaflets: petioles, and tendrils were separated, weighed, and placed in vials containing sterile plant tissue culture medium (Murashige-Skoog; Flow Labs, Ayrshire, Scotland). In separate experiments, the component epidermal and cortical tissues of the internodes were also separated. After the tissue was washed, the samples were blotted dry in a laminar flow sterile cabinet and transferred to fresh sterile medium (2–3 mL). The various radioactive GA metabolites were then added at a concentration of 10 μM, unless otherwise stated. In the case of GA_{19}, the radioactive ^{3}H tracer was added to a known mass of the deuterated compound, and its specific activity was subsequently determined. All tissues were incubated at 25°C under fluorescent lighting and routinely extracted for analysis of their [^{3}H]GA content by HPLC after 24 h.

Sample Processing

All tissue samples were washed extensively in aqueous methanol (5% v/v) and blotted dry. They were frozen in

[1] This work was supported by Imperial Chemical Industries Plant Protection, Jealott's Hill, Bracknell, Berkshire, United Kingdom, and the Science and Engineering Research Council.

[2] Abbreviation: GA, gibberellin.

liquid nitrogen, pulverized, placed in vials containing aqueous methanol (60%), and stored at 4°C for a minimum period of 16 h. The samples were then filtered through glass fiber filters (Whatman GF/C, Whatman Ltd, Maidstone, Kent, United Kingdom) to remove tissue debris. The methanolic extracts obtained were partitioned against light petroleum ether (60–80°C boiling point), evaporated to near dryness by heating in a stream of N_2 gas, resuspended in methanol, and stored at −20°C. Finally, particulate and precipitated materials were removed by centrifugation, and aliquots of the resultant supernatants were counted for radioactivity to determine total GA uptake by each tissue.

Product Analysis and Quantification

GC-MS-selected ion monitoring analysis of the GAs present in the various vegetative tissues of 205^+ and 205^- isogenic pea plants failed to detect endogenous epi-GA_1, epi-GA_{29}, and GA_3 in any significant quantity (15). Additionally, from total ion current data obtained from full-scan GC-MS analysis of immunoaffinity chromatography-purified GAs from various vegetative tissues of a different variety of pea (Aldeman), it was shown that epi-GA_1 and epi-GA_{29}, where present, represented <10% of the amount of GA_1 recovered (data not shown). Thus, reverse phase HPLC on ODS Hypersil (5 μm) (Shandon Solution Products, Runcorn, Cheshire, United Kingdom), packed into a stainless steel column (250 mm long, 8 mm i.d.) and fitted to an LDC HPLC apparatus (Riviera Beach, Florida), was used to separate and quantify GA_1, GA_8, GA_{20}, and GA_{29}. These were expected to be the major detectable GA products formed from radiolabeled GA_{19}, GA_{20}, and GA_1 (6). All samples of similarly incubated *Le* and *le* tissues that were applied to the column contained equivalent quantities of total radioactivity. Corresponding amounts of the particular radiolabeled GA substrate used were also run as a baseline control. Prepared samples were loaded onto the column preequilibrated with 30% methanol in water containing 0.5% (v/v) phosphoric acid at a flow rate of 1 mL min^{-1}. To separate GA_1, GA_8, GA_{20}, and GA_{29}, the column was eluted with an exponential methanol gradient of 30 to 70% (v/v, MeOH:H_2O) for 30 min. Fractions (0.5 or 1.0 mL) were collected and counted for radioactivity. Radioactivity recovery from the column was routinely in excess of 90%. The retention volumes of authentic samples of GA_1, GA_8, GA_{20}, and GA_{29} were 12, 4.5, 22, and 6.5 mL, respectively. For separating GA_{19} and GA_{20}, the column was operated isocratically in 30% aqueous methanol containing 0.5% H_3PO_4 until GA_1 had eluted. The methanol concentration of the elution solvent was then stepped up to 45% (v/v). Under these conditions, the elution volumes of GA_{19} and GA_{20} were 31.5 and 25 mL, respectively.

RESULTS

GA Uptake

The total quantities of radiolabeled GA present in extracts of the *Le* and *le* apical bud, leaflet, petiole, tendril, and stem tissues incubated for 24 h with equimolar concentrations of GA_1, GA_{19}, and GA_{20} (10 μM) were determined before analysis by HPLC. It was observed that the amounts of radiolabeled GA in *Le* and *le* apical buds incubated with [^{3}H]GA_1 were somewhat higher than in other tissues and that the amounts of radiolabeled GA recovered in *Le* and *le* petioles were relatively low. However, on the whole, the data obtained were relatively uniform. The *le* mutation had no apparent effect on GA uptake into the tissues, and individual tissues failed to discriminate among GA_1, GA_{19}, and GA_{20}.

GA_{19} Metabolism

The calculated concentrations of the individual radiolabeled GA metabolites recovered after incubating the various vegetative tissues *in vitro* with [^{3}H]GA_{19} for 24 h are given in Table I. Whereas 90% of the recovered radioactivity in the apical buds, stems, and petioles remained as GA_{19}, in leaflet and tendril tissues only 50% remained as GA_{19}.

The total concentration of the GA_{19} metabolites in leaflet and tendril tissues were, at minimum, an order of magnitude

Table I. *GA_{19} Metabolism in Isolated Tissues Incubated in Vitro*

Plant Tissue and Phenotype	Total GA in Tissue	Metabolite Recovery				
		GA_{19}	GA_{20}	GA_1	GA_{29}	GA_8
	nmol g^{-1}			%		
Apical bud						
Le	6.7	88.0	10.0	1.0	<1.0	<1.0
le	5.3	83.0	15.0	1.0	<1.0	<1.0
Leaflet						
Le	6.4	42.0	46.0	4.0	8.0	<1.0
le	7.2	44.0	46.0	5.0	5.0	<1.0
Tendril						
Le	12.7	52.0	40.0	4.0	3.0	<1.0
le	12.5	56.0	39.0	3.0	2.0	
Petiole						
Le	2.3	83.0	14.0	1.5	1.0	<1.0
le	5.4	82.0	15.0	2.0	1.0	<1.0
Stem						
Le	2.4	89.0	8.0	<1.0	1.0	<1.0
le	7.9	80.0	17.0	<1.0	1.0	<1.0

Table II. *Metabolism of GA_{20} (10 μM) in Isolated Tissues Incubated in Vitro*

Plant Tissue and Phenotype	Total GA in Tissue	Metabolite Recovery			
		GA_{20}	GA_1	GA_{29}	GA_8
	nmol g^{-1}	%			
Apical bud					
Le	10.0	88.2	0.7	10.7	0.4
le	10.2	86.8	0.8	12.2	0.2
Leaflet					
Le	15.5	62.3	0.5	36.4	0.8
le	13.3	64.4	0.6	33.8	1.0
Tendril					
Le	10.8	65.1	1.8	31.7	1.4
le	12.2	83.1	1.3	15.2	0.4
Petiole					
Le	2.8	54.4	1.8	43.5	0.3
le	3.7	58.6	1.5	39.7	0.2
Stem					
Le	12.3	39.0	3.0	57.4	0.6
le	9.7	16.7	4.7	77.6	1.0

greater than the sum total of GA_{20}, GA_{29}, GA_1, and GA_8 in apical bud, petiole, and stem tissues. The leaf and tendril tissues of both *Le* and *le* plants accumulated GA_{20} as the major GA_{19} metabolite. Although further metabolism of GA_{20} appeared to be restricted in these tissues, they produced similar quantities of GA_1 (bioactive 3β-hydroxy GA_{20}) and GA_{29} (inactive 2β-hydroxy GA_{20}). GA_8, the inactive 2β-hydroxylated derivative of GA_1, did not feature as a major metabolite in any of the tissues under consideration.

From these data, it was concluded that the GA_{19} oxidase that catalyzes the synthesis of GA_{20} from GA_{19} was located predominantly in the leaflet and tendril tissues, that the GA_{20} 2β- and 3β-hydroxylase activities and the GA_1 2β-hydroxylase activity were low in the leaflet and tendril tissues, and, finally, that the *le* mutation was not expressed under the experimental conditions.

GA_{20} Metabolism

The calculated concentrations of labeled GA metabolites in apical bud, leaflet, stem, and petiole tissues after a 24-h incubation period with [17-$^3H_2$]GA_{20} (10 μM) are given in Table II. These data show that the relative contributions of unmetabolized GA_{20} to the total radiolabeled GA pools varied considerably, even between corresponding *Le* and *le* tissues. Whereas in apical buds, leaflets, and tendrils of either *Le* or *le* phenotype the GA_{20} accounted for approximately 90, 65, and 55% of the total GA, respectively, in *Le* stem tissue, GA_{20} represented about 40% of the total GA and in *le* stem tissue GA_{20} represented <20% of the total GA. In tendril tissues of the *Le* and *le* phenotype, respectively, GA_{20} accounted for 65 and >80% of the total GA.

In all the tissues investigated, the major metabolic product of GA_{20} was its inactive 2β-hydroxylated derivative, GA_{29}. Apparently stem tissue of the *le* phenotype contained the most GA_{29} synthetic activity and the apical bud tissues least.

By comparison with GA_{29}, the relative quantities of GA_1 and its inactive catabolite GA_8 that were formed under these experimental conditions were low. Nevertheless, whereas GA_1 was barely detectable as a radiolabeled metabolite in the apical buds and leaflets, its presence was most apparent in the stem. Furthermore, the expectation that GA_1 synthesis would be suppressed in the *le* tissue was not fulfilled. Indeed, as with GA_{29}, the concentration of GA_1 was greater in stem tissue of the *le* phenotype. This discrepancy was not affected by GA_8 synthesis; the observed concentrations of GA_8 were low but proportional to the GA_1 concentrations in the respective *Le* and *le* tissues.

The conclusions that were drawn from data presented in Table II were substantiated by the results of similar experiments in which [^{3}H]GA_{20}, at a concentration of 50 μM, was added to the culture medium of apical bud, tendril, and separated stem cortex and epidermis. These data (Table III) again indicated that the stem was the principal site of GA_1 biosynthesis within the plant. Moreover, the GA_{20} 3β-hydroxylase that catalyzes GA_1 production was located in cortical rather than in epidermal cells, and its activity was not impaired as a consequence of the *le* genetic mutation. Comparison of the data presented in Tables II and III also showed that the fivefold increase in GA_{20} substrate concentration resulted in approximately a fivefold increase in GA_1 yield in apical bud, tendril, and stem cortical tissue after the 24-h incubation *in vitro*. Corresponding increases in GA_{29} production were not observed. Indeed, in the stem cortical tissue, GA_{29} production was apparently suppressed. In apical buds and the tendrils, GA_{29} production proceeded at similar rates with 10 and 50 μM GA_{20}. Thus, unlike the GA_{20} 3β-hydroxylase, the GA_{20} 2β-hydroxylase would appear to be substrate saturated and operating at maximal rate when these tissues were incubated with 10 μM GA_{20}.

GA_1 Metabolism

The results presented in Table IV show that apical bud tissue abstracted GA_1 from the culture medium more efficiently than leaflet, tendril, and stem tissues. Despite the fivefold excess of GA_1 in this tissue, very little GA_8 was recovered. The estimated yield was similar to that produced by leaflet and tendril tissues.

Table III. *Metabolism of GA_{20} (50 μM) in Isolated Tissues Incubated in Vitro*

Plant Tissue and Phenotype	Total GA in Tissue	Metabolite Recovery			
		GA_{20}	GA_1	GA_{29}	GA_8
	nmol g^{-1}	%			
Apical bud					
Le	62.4	97.5	0.5	2.0	<0.1
le	51.9	96.2	0.7	3.1	<0.1
Tendril					
Le	137.4	93.3	0.6	6.0	<0.1
le	95.9	94.1	0.6	5.2	<0.1
Stem cortex					
Le	14.9	73.5	16.9	8.2	1.4
le	11.3	63.2	22.1	12.1	1.8
Stem epidermis					
Le	8.4	95.2	1.0	3.6	0.2
le	8.1	94.9	1.3	3.6	0.2

Table IV. *GA_1 Metabolism in Isolated Tissues Incubated in Vitro*

Plant Tissue and Phenotype	Total GA in Tissue	Metabolite Recovery	
		GA_1	GA_8
	nmol g^{-1}	%	
Apical bud			
Le	36.3	98.0	2.0
le	27.3	98.0	2.0
Leaflet			
Le	12.7	94.0	6.0
le	6.9	92.0	8.0
Tendril			
Le	6.3	93.0	7.0
le	5.6	96.0	4.0
Stem			
Le	6.7	58.0	42.0
le	6.4	49.0	51.0

By contrast, the observed concentration of GA_8 was equivalent to that of GA_1 in the stem tissues of both *Le* and *le* plants. Therefore, it was concluded that the enzyme responsible for inactivating GA_1, the GA_1 2β-hydroxylase, was located predominantly in stem tissue. Furthermore, the *le* genetic mutation had no effect on GA_1 catabolism in this tissue.

Comparative Kinetics of GA_1 and GA_{29} Formation in *Le* and *le* Stem Tissue

The net production of GA_1 and GA_{29} in *Le* and *le* stem tissue was determined within a GA_{20} substrate concentration range of 0.25 to 5.0 μM. Over this range, the relationship between the tissue concentration of total radiolabeled GA after a 24-h incubation period and the initial concentration of [^{3}H]GA_{20} in the incubation medium was shown to be linear

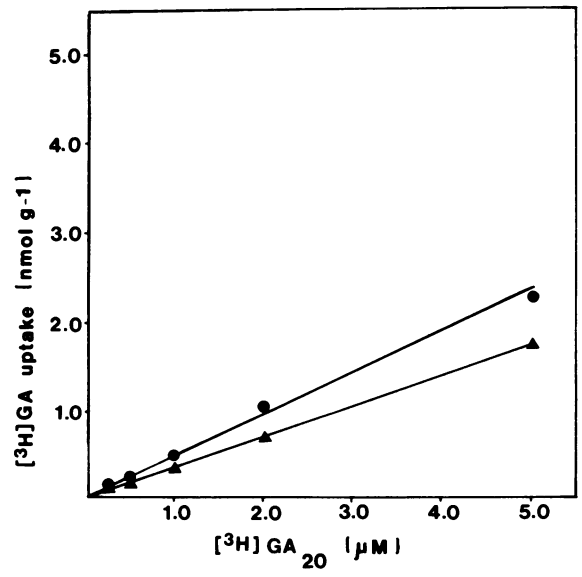

Figure 1. Comparison of radiolabeled GA uptake into Le (▲) and *le* (●) stem tissues incubated *in vitro* at increasing concentration of [^{3}H]GA_{20}.

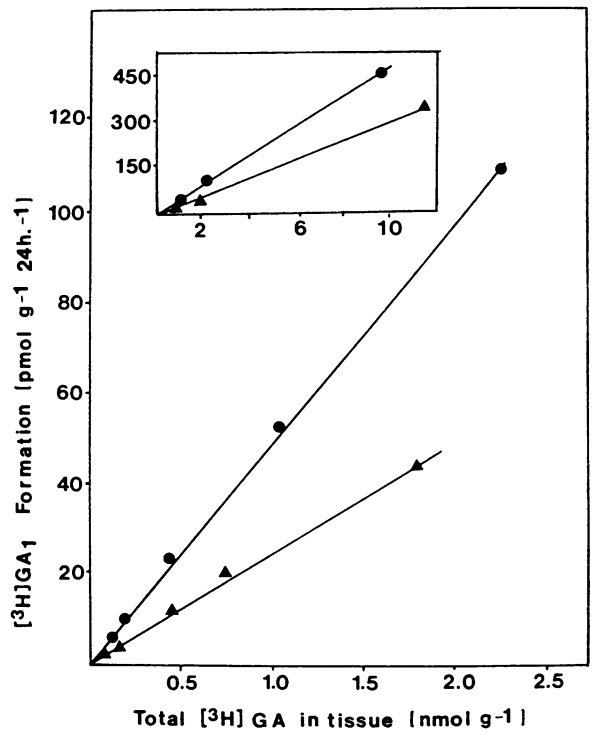

Figure 2. Relationship between [^{3}H]GA_1 formation and the total radiolabeled GA content of *Le* (▲) and *le* (●) stem tissues after 24-h incubation *in vitro* with [^{3}H]GA_{20}. Inset, Linear plot extrapolated to include data presented in Table III.

(Fig. 1). The data presented in Figure 2 pertaining to GA_1 formation in stem tissue of *Le* and *le* plants showed that the apparent rates of [^{3}H]GA_1 synthesis were linear with respect to the total radiolabeled GA recovered in these tissues and also higher in *le* stem tissue than in *Le* stem tissue. These observations were also true for [^{3}H]GA_{29} synthesis in stem tissues of *Le* and *le* plants (Fig. 3). However, within the experimental GA_{20} concentration range, the apparent rate of GA_{29} production was an order of magnitude greater than that of GA_1 production. Additionally, in the *le* stem tissue, GA_{29} production was beginning to plateau at the highest substrate concentration.

DISCUSSION

The results presented here provide for the first time information pertaining to the location of enzymes involved in the latter stages of the GA metabolic pathway in vegetatively growing pea plants. Significantly, the GA_{19} oxidase that catalyzes the synthesis of GA_{20} from GA_{19} was located predominantly in young expanding leaves (leaflets and tendrils), whereas the GA_{20} 3β-hydroxylase that catalyzes the formation of bioactive GA_1 and the GA_1 2β-hydroxylase that catalyzes the formation of biologically inactive GA_8 were both located predominantly in stem tissue. The GA_{20} 2β-hydroxylase that produces biologically inactive GA_{29} from GA_{20} was present in all the tissues examined, although its activity was relatively

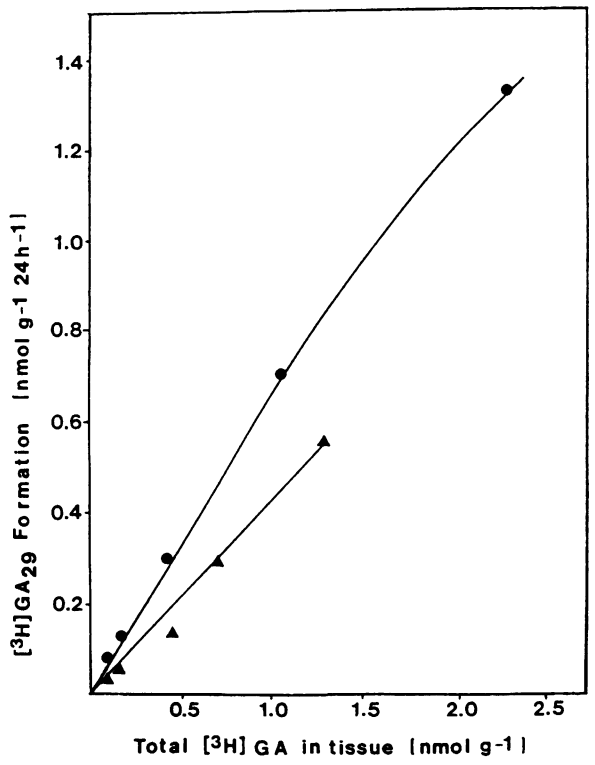

Figure 3. Relationship between [^{3}H]GA_{29} formation and the total radiolabeled GA content of *Le* (▲) and *le* (●) stem tissues after 24-h incubation *in vitro* with [^{3}H]GA_{20}.

low in apical bud tissue and highest in the stem. Growing plant vegetative tissues do not, therefore, individually contain all the GA biosynthetic enzymes. In a more general form, this conclusion was also deduced in a previous communication concerned with the steady-state distribution of GAs in vegetative tissues of *P. sativum* (15). The implications of this information are quite considerable with regard to the biology of GA action. Immature leaflets are the only vegetative tissues of *P. sativum* in which GA_{19} has been detected previously, albeit as a minor metabolite (15). Because the precursors of GA_{19} are relatively insoluble in aqueous solution and, from bioassay data (18), do not appear to move freely through plant vascular tissues, it was surmised that the GA_{19} biosynthetic enzymes, in addition to the GA_{19} oxidase, are probably all located in the young leaflets.

The fact that the GA_{19} oxidase is located in these leaflets may account for the finding that, in some plant species, this enzyme is under photoperiodic regulation (2). Moreover, without invoking any particular mechanism (*i.e.* phytochrome mediated), this location would also make the GA_{19} oxidase activity, and hence the production of GA_{20}, particularly susceptible to the influence of light, the intensity and quality of which determine the general metabolic status of the leaf. Furthermore, because the GA_{20} that is metabolized to GA_1 in the stem is necessarily transported from the leaflets, the amount of GA_{20} that is produced in the leaflets ultimately dictates the extent of stem elongation.

In detail, however, the steady-state concentration of GA_1 in stem tissue is dependent on the rate of its synthesis from GA_{20}, the rate of its catabolism to GA_8, and also the rate of production of GA_{29} from GA_{20} as common substrate. Comparison of data presented in Tables II and III indicates that at the lower concentration of the GA_{20} substrate (10 μM in the culture medium), the GA_{20} 3β-hydroxylase competes poorly with the GA_{20} 2β-hydroxylase for the available GA_{20} substrate. At 50 μM GA_{20}, the estimated intracellular concentration of GA_1 in stem (cortical) tissue had increased fivefold, whereas the GA_{29} concentration was in fact reduced. The explanation for this latter phenomenon is uncertain, but GA_1 is known to inhibit the GA_{20} 2β-hydroxylase activity (16). However, given that at subsaturating substrate concentrations the reaction rates are proportional to the substrate concentration, it would appear that the GA_{20} 2β-hydroxylase was operating at V_{max} when the stem tissues were supplied with 10 μM GA_{20}, but at 50 μM GA_{20}, the 3β-hydroxylase was still operating at or below K_m.

With regard to the GA_1 2β-hydroxylase, it was found that stem tissue incubated with GA_{20} at concentrations of 10 and 50 μM contained very little GA_8, either in full or expressed as a percentage of the synthesized GA_1. Significantly enhanced yields of GA_8, representing approximately 50% of the labeled intracellular GA, were obtained after incubating the stem tissue with GA_1 at a concentration of 10 μM. These data indicate that the K_m of the GA_1 2β-hydroxylase for the GA_1 substrate is greater than that of the GA_{20} 3β-hydroxylase for the GA_{20} substrate. The results presented in Tables II to IV show that both GA_1 and GA_8 are primarily synthesized in stem tissue and that this tissue is also the richest source of the GA_{20} 2β-hydroxylase. *In vivo,* however, GA_1, GA_8, and GA_{29} are found in all growing tissues and are particularly concentrated in apical bud, unexpanded leaflet, and tendril tissues (15). None of these tissues synthesize GA_1 and GA_8 effectively, and the apical bud appears to be particularly inert with respect to GA metabolism. Thus, whereas the stem does not accumulate GA_{20}, GA_1, or the 2β-hydroxylated GAs to any great extent *in vivo* (15), it clearly is the source of a large proportion of the GA_1 and GA_8 and possibly GA_{29} that *in vivo* is found in other growing tissues, particularly the apical buds, tendrils, and unexpanded leaflets. To meet these metabolic demands, the stem tissue GA_{20} 3β-hydroxylase, GA_{20} 2β-hydroxylase, and GA_1 2β-hydroxylase probably operate at or near V_{max}, thus requiring the leaflets to supply GA_{20} at an appropriately high concentration. That the enzymes operate under these conditions is possibly supported by the observation that the inability to produce GA_1 in *le* stem tissue *in vivo* is not counterbalanced by the formation of GA_{29}. The common substrate GA_{20} accumulates in *le* stem tissue relative to that of *Le* stem tissue (15).

The results presented in this paper clearly indicate that key intermediates in GA biosynthesis are synthesized in different tissues and that GA_1 is synthesized within the tissue that exhibits maximal biological response to its presence. However, they do not support the generally accepted hypothesis that the locus of the *le* mutation lies within the gene encoding the GA_{20} 3β-hydroxylase protein, causing a reduction in its catalytic performance (10). Because the uptake of GA_{20} into *Le* and *le* stem tissue incubated *in vitro* was shown to be a linear function of concentration (Fig. 1), it was possible to

measure, albeit indirectly, apparent rates of GA_1 and GA_{29} formation as functions of initial GA_{20} substrate concentrations. The data obtained (Figs. 2 and 3) showed that, on a fresh weight basis, the GA_{20} 3β-hydroxylase and GA_{20} 2β-hydroxylase activities of *le* stem tissue were, in fact, greater than those of *Le* stem tissue. This situation was not reversed when GA_{20} was added to the culture media of *Le* and *le* stem cortical tissues at a concentration of 50 μM (Table III). Indeed, under these experimental conditions in which GA_{20} was not apparently rate limiting and the GA_{20} 2β-hydroxylase activity was reduced, the rate of GA_1 formation continued to increase in both *Le* and *le* stem cortical tissues. Thus, it is considered extremely unlikely that the product of the *le* gene is an altered GA_{20} 3β-hydroxylase protein. The data do not indicate that the quantity of active enzyme is less in *le* stem tissue than in *Le* stem tissue; neither do they provide evidence for a change in either k_{cat} or k_{cat}/K_m.

The data also do not support the possibility that the *in vivo* depletion of GA_1 in *le* plant tissues might result from increased GA_{20} 2β-hydroxylase activity. Although the GA_{20} 2β-hydroxylase activity was apparently greater in *le* stem tissue than in *Le* stem tissue (Fig. 3), the balance between the GA_{20} 2β-hydroxylase and GA_{20} 3β-hydroxylase activities in these tissues was very similar. Thus, it is concluded that the functional basis of the *le* gene remains unknown. However, if the lesion does not lie within the GA_{20} 3β-hydroxylase protein itself or lead to an increase in the cellular concentration of the GA_{20} 2β-hydroxylase, then it is likely that another component is involved, one that can inhibit GA_{20} 3β-hydroxylation *in vivo*.

Evidence for the existence of a general light-induced GA_{20} hydroxylase inhibitor in *Le* pea seedlings has been obtained by the author (data not shown). It is thus hypothesized that such a component may be the product of the *Le* gene that is produced by the apex or immature leaflets of *Le* plants, dependent on the incident light intensity and/or quality, and transported to the stem. It is further hypothesized that the *le* mutation is "deregulatory," causing constitutive production of the component in both dark- and light-grown *le* pea plants. These hypotheses require further investigation. They are consistent with observations that light-mediated inhibition of stem growth can be reversed by application of GA_3 or GA_1 to pea plants (3, 7–9). They also reconcile apparently conflicting data (1, 11–13, 17) pertaining to the mechanism of control of GA_1 3β-hydroxylase activity and GA_1 production in light- and dark-grown pea plants.

LITERATURE CITED

1. **Campbell BR, Bonner BA** (1986) Evidence for phytochrome regulation of gibberellin A_{20} 3β-hydroxylation in shoots of dwarf (*le le*) *Pisum sativum* L. Plant Physiol **82:** 909–915
2. **Gilmour SJ, Zeevaart JAD, Schwenen L, Graebe JE** (1986) Gibberellin metabolism in cell free extracts from spinach leaves in relation to photoperiod. Plant Physiol **82:** 190–195
3. **Gorter CJ** (1961) Dwarfism of peas and the action of gibberellic acid. Physiol Plant **14:** 332–343
4. **Ingram TJ, Reid JB, MacMillan J** (1986) The quantitative relationship between gibberellin A_1 and internode elongation in *Pisum sativum* L. Planta **168:** 414–420
5. **Ingram TJ, Reid JB, Murfet IC, Gaskin P, Willis CL, MacMillan J** (1984) Internode length in *Pisum:* the Le gene controls the 3β-hydroxylation of gibberellin A_{20} to gibberellin A_1. Planta **160:** 455–463
6. **Kamiya K, Graebe JE** (1983) The biosynthesis of all major pea gibberellins in a cell free system from *Pisum sativum.* Phytochemistry **22:** 681–689
7. **Kende H, Lang A** (1964) Gibberellins and light inhibition of stem growth in peas. Plant Physiol **39:** 435–440
8. **Lockhart JA** (1956) Reversal of light inhibition of pea stem growth by gibberellins. Proc Natl Acad Sci USA **42:** 841–848
9. **Lockhart JA, Gottschall V** (1959) Growth responses of Alaska pea seedlings to visible radiation and gibberellin acid. Plant Physiol **34:** 460–465
10. **MacMillan J** (1988) Metabolism of gibberellins A_{20} and A_9 in plants: pathways and enzymology. *In* RP Pharis, SB Rood, eds, Plant Growth Substances. Springer-Verlag, New York, pp 307–313
11. **Reid JB** (1983) Internode length in *Pisum.* Do the internode length genes effect growth in dark-grown plants? Plant Physiol **72:** 759–763
12. **Reid JB** (1988) Internode length in *Pisum.* Comparison of genotypes in the light and dark. Physiol Plant **74:** 83–88
13. **Ross JJ, Reid JB** (1989) Internode length in *Pisum.* Biochemical expression of the le gene in darkness. Physiol Plant **76:** 164–172
14. **Ross JJ, Reid JB, Gaskin P, MacMillan J** (1989) Internode length in *Pisum.* Estimation of GA_1 levels in genotypes *Le,le* and *le*^{d}. Physiol Plant **76:** 173–176
15. **Smith VA, Knatt CJ, Gaskin P, Reid JB** (1991) The steady-state distribution of gibberellins in vegetative tissues of *Pisum sativum* L. Biological and biochemical consequences of the *le* mutation I. Plant Physiol **99:** 368–371
16. **Smith VA, MacMillan J** (1986) The partial purification and characterisation of gibberellin 2β-hydroxylases from seeds of *Pisum sativum.* Planta **167:** 9–18
17. **Sponsel VM** (1986) Gibberellins in dark- and red- light-grown shoots of dwarf and tall cultivars of *Pisum sativum:* the quantification metabolism and biological activity of gibberellins in Progress no. 9 and Alaska. Planta **168:** 119–129
18. **Waycott W, Taiz L** (1991) Phenotypic characterization of lettuce dwarf mutants and their response to applied gibberellins. Plant Physiol **95:** 1162–1168