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SERINE IN STEROL SYNTHESIS IN *EUPHORBIA LATHYRIS* SEEDLINGS

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Key Word Index—*Euphorbia lathyris*; Euphorbiaceae; phytosterol synthesis; serine; seedling; endosperm.

Abstract—During germination serine was shown to be taken up from the endosperm by the cotyledons and partly translocated to the hypocotyl. Up to 1.7% of the [^{14}C]serine taken up by the seedling was involved in phytosterol synthesis in which the hypocotyl was the most effective plant part. Compared with [^{14}C]sucrose, serine was found to be 12 times more effective in this synthesis. From the data obtained it could be calculated that this amino acid may yield about 7% of all the free sterols in an etiolated seedling of *Euphorbia lathyris*, making this substrate a suitable marker in sterol synthesis in endospermous seedlings.

INTRODUCTION

For a number of plant species the composition and synthesis of sterols have been investigated [1–3]. In most cases labelled mevalonic acid was used as a tracer but methionine, leucine and valine were also found to be involved in triterpenoid synthesis in plants [4–6]. In a previous paper [7] we reported the involvement of various amino acids in triterpene synthesis in the laticifers of *Euphorbia lathyris* seedlings. The contribution of these substrates (normally taken up from the endosperm) towards the total triterpene production was estimated to be a few percent. The 4-desmethylsterols were also found to contain significant amounts of ^{14}C after [^{14}C]-amino acid uptake. In the latex 4-desmethylsterols are detectable as traces only and the obtained [^{14}C]phytosterols originated outside the laticiferous system. Of all the amino acids used, serine tended to be more specifically involved in phytosterol synthesis. The total quantity of amino acids produced by the endosperm during germination can be estimated and the metabolic fate of these substrates can be studied quantitatively after active uptake by the seedling. We used this system to study the involvement of serine in phytosterol synthesis in an intact plant and the results obtained with *Euphorbia lathyris* seedlings are presented in this paper.

RESULTS AND DISCUSSION

About 7 days after the onset of germination a massive breakdown of proteins occurs in the endosperm and the resulting mixture of amino acids was shown to contain about 4.5% of serine [7]. As not all the amino acids produced are taken up immediately by the cotyledons a significant modification of the amino acid composition may occur.

If for example ^{14}C-labelled alanine, glycine or serine were fed to the endosperm of germinating castor bean, they were found to be converted to sucrose *in situ*. This was accompanied by the production of glutamine which appeared to act as a sink for amide nitrogen [8]. To bypass a possible serine metabolism in *Euphorbia lathyris* endosperm we analysed the amino acid composition in the free space of the cotyledons. Results in Table 1 demonstrate that serine still forms a 5% of the amino acid pool ready for uptake by the seedling.

In a previous paper it was shown that excised cotyledons with a part of the hypocotyl attached were very useful in substrate uptake and metabolic studies [7]. A time-course experiment on [U-^{14}C]serine uptake by these tissues revealed that about 100% of the 20 nmoles of substrate supplied to one pair of cotyledons was taken up within 10 hours (Fig. 1). In the same period labelled 4-desmethylsterols, 4α-methylsterols and 4,4-dimethylsterols (triterpenols) were synthesized. Phytosterol synthesis leveled off after about 40 hr as was found for the triterpenols. The 4α-methylsterols reached a maximum in ^{14}C-content after 20–30 hr of incorporation. The decrease thereafter is in support of their role as intermediates in phytosterol synthesis. Several triterpenols are supposed to play a similar role in sterol synthesis but a constant ^{14}C level in this fraction indicates that not all the triterpenols synthesized are to be considered as intermediates. The ester fraction, containing triterpene esters and sterol esters and squalene continued to accumulate ^{14}C in the 60 hr incorporation period. As less than 4% of the phytosterols was found to occur in the esterified form no detailed analysis of the ^{14}C-distribution in this complex fraction was carried out in the course of this study.

If serine was taken up by the cotyledons of intact growing seedlings similar results of ^{14}C distribution over the apolar lipids were obtained (Table 2).

After three days of incubation with [U-^{14}C]serine, the latex expelled after inscision contained a trace of [^{14}C]triterpenols. The hypocotyl proved to be the most effective plant part in phytosterol synthesis. In the cotyledons the triterpenols and the ester fraction accumulated a considerable amount of ^{14}C. If [U-^{14}C]sucrose was fed to the seedling substantial amounts of [^{14}C]triterpenes were measured in the latex after three days of incorporation. In the cotyledons only 5.3% of the labeled apolar lipids were found to be sterols. The hypocotyl was again more productive in the elaboration of these compounds. Two different ^{14}C distributions over the three classes of

Table 1. Amino acid composition in the free space of the cotyledons of *Euphorbia lathyris*

	μmol
Aspartate	2.7
Threonine	3.4
Serine	5.0
Asparagine	7.0
Glutamate	3.7
Glutamine	21.7
Proline	13.9
Glycine	1.5
Alanine	3.5
Valine	8.2
Methionine	0.7
Isoleucine	8.1
Leucine	6.1
Tyrosine	2.2
Phenylalanine	3.9
Ornithine	0.3
Lysine	2.7
Histidine	2.4
Arginine	3.0

lipids in the hypocotyl after [^{14}C]serine and [^{14}C]sucrose supply indicate that both these substrates have their own translocation profile and metabolic fate. A substantial conversion of serine into sucrose is not to be anticipated. Similar incorporation patterns in hypocotyl and cotyledons were also observed when excised cotyledons with a variable hypocotyl length were used. Results presented in Table 3 show that the longer the hypocotyl the more [^{14}C]sterols were produced and in tissues of 2 cm and longer only 4–5% of the label in the apolar lipids was measured in the 4α-methylsterols. Comparatively, too much radioactivity was accumulated in the triterpenols to consider this fraction as solely an intermediate in phytosterol synthesis.

Gas chromatography revealed that 80% of the free sterols co-chromatographed with sitosterol, two other peaks co-chromatographed with campesterol (13%) and stigmasterol (7%). At the end of the germination period (when the endosperm was completely absorbed) the total sterol content of the etiolated seedling was found to range from 18 to 22 μg. If we assume that in phytosterol synthesis serine is converted to pyruvate via 3-phosphoserine, 3-phospho-hydroxypyruvate, 3-phosphoglycerate, 2-phospho-glycerate and phosphoenolpyruvate, its contribution to these lipids can be estimated quantitatively. Therefore 18 molecules of serine are required to produce one molecule of squalene and 27 carbon atoms of this linear triterpene end up in the phytosterol carbon skeleton. Using [U-^{14}C]serine 50% of the ^{14}C is incorporated into the sterols, the other 50% is lost as CO_2. Therefore the radioactivity measured in the 4-desmethylsterols reflects only 50% of the [U-^{14}C]serine used in this synthesis. From the data presented in Table 2 it can be calculated that 1.7% of the serine supplied was metabolized to sterols. A similar calculation for sucrose reveals that only 0.14% of this substrate ends up as a phytosterol. Therefore serine is about 12 times more effective in this synthesis than sucrose.

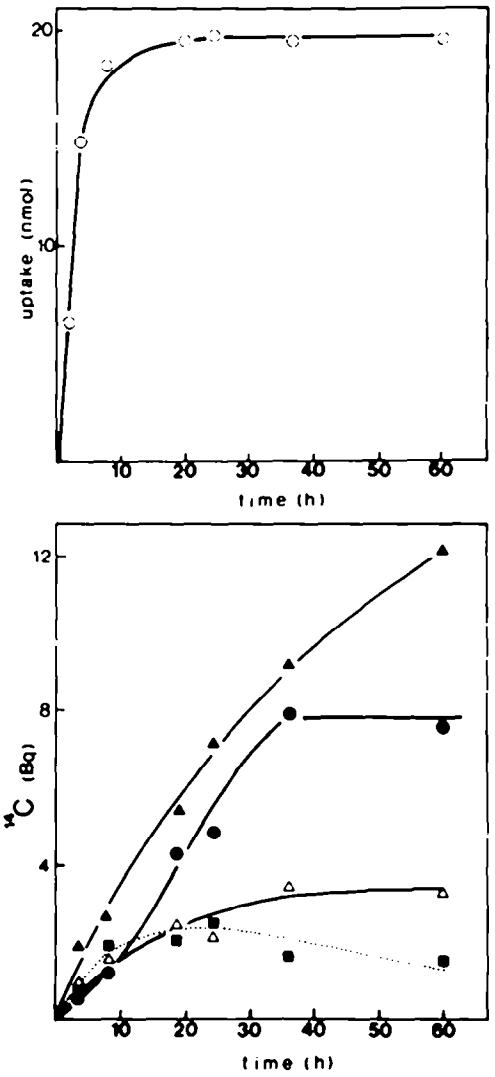

Fig. 1. Uptake of [U-^{14}C]serine (2822 Bq) and ^{14}C incorporation into esters (▲), triterpenols (△), 4α-methylsterols (■) and 4-desmethylsterols (●) *versus* time by a single pair of cotyledons with 0.5 cm hypocotyl.

As was calculated before [7], the endosperm of a single seed of *Euphorbia lathyris* may produce 130 μmoles of sucrose and about 29 μmoles of amino acids. After depletion of this storage tissue at the end of the germination period 0.17 μmoles of sucrose have been used to produce 38 nmoles of sterols (15.7 μg). If 5% of the amino acids enter the cotyledon as serine the available 1.45 μmoles of serine will yield about 2.8 nmoles of sterols (= 1.15 μg). The calculated value for total sterol synthesis in an etiolated seedling is thus in the same order of magnitude as the sterol level measured at the end of the germination period. Although serine yields only 7% of the phytosterols in etiolated *Euphorbia lathyris* seedlings it may be used as a marker in 4-desmethylsterol synthesis in plant tissues, bypassing all the possible disadvantages of mevalonic acid or mevalonate as discussed in detail by Banthorpe *et al.* [9].

Table 2. *In vivo* incorporation of [U-^{14}C]serine and [U-^{14}C]sucrose into free sterols, triterpenols and esters by an *Euphorbia lathyris* seedling (20 nmol serine, 2 μmol sucrose, 3 days of incorporation)

		Latex (Bq)	Cotyledons (Bq)	Hypocotyl (Bq)	Total (Bq)
Serine	Esters	0.04	14.15	4.87	19.06
(1850 Bq)	Triterpenols	0.51	9.85	1.71	12.07
	Sterols	—	4.91	11.10	16.01
Sucrose	Esters	6.35	45.23	41.32	92.90
(33630 Bq)	Triterpenols	11.16	36.10	26.62	73.88
	Sterols	—	4.57	17.64	22.21

Table 3. Incorporation of ^{14}C into triterpenols, 4α-methylsterols and 4-desmethylsterols by cotyledons (cots) and hypocotyl (hyp) of an *Euphorbia lathyris* seedlings after [U-^{14}C]serine uptake (2937 Bq) by the excised cotyledons with 0, 1, 2 or 7 cm hypocotyl attached

	cots (Bq)	cots + 1 cm hyp (Bq)	cots + 2 cm hyp (Bq)	cots + 7 cm hyp (Bq)
Triterpenols	3.20	3.46 + 0.82	4.10 + 3.20	2.66 + 1.19
4α-Methylsterols	2.83	2.05 + 0.27	2.14 + 0.44	1.24 + 0.66
Sterols	6.33	5.91 + 2.10	5.51 + 7.61	2.47 + 11.45

EXPERIMENTAL

Plants. Seedlings of *Euphorbia lathyris* L. were grown on moist sand at 22° in the dark. Twelve-day-old seedlings with a 9–10 cm hypocotyl length were used in all the incorporation experiments.

Incorporation experiments. After removal of the endosperm the cotyledons of intact seedlings were provided with a snugly fitting 25 μl capillary filled with a labelled substrate soln. Groups of seven pairs of excised cotyledons were incubated in 1.5 ml cups containing 100 μl substrate soln. Incorporation occurred in the dark at 22°.

Radioisotopes. L-[U-^{14}C]Serine (10 mCi/mmol) and [U-^{14}C]sucrose, (3 μCi/mmol) were purchased from Amersham International. Serine was diluted with non-labelled serine to supply each pair of cotyledons with 20 nmoles of this substrate. Both serine and [^{14}C]sucrose were diluted with unlabelled 300 mM sucrose soln.

Lipid extraction. Tissues were frozen, refluxed in Me_2CO and extracted with petrol (40–60°) as described in ref. [10]. The petrol extracts were separated on silica gel G TLC plates developed in cyclohexane–EtOAc (5:1). References were visualized after chromatography by spraying with chlorosulphonic acid–HOAc (1:2) and heated to 90% for 2 min. Scrapings of the corresponding fractions were suspended in 3 ml aliquots of Me_2CO–petrol (40–60°) (1:1), diluted with 2 ml of H_2O after vigorous shaking and centrifuged in a tabletop centrifuge. The petrol fraction was used for ^{14}C assay. Sterols were analysed by GLC using 3% SE-30 at 255°. Quantitative data were obtained with 5α-cholestane as internal standard [11].

Amino acids. After removal of the endosperm, 60 pairs of cotyledons were rinsed for 2 sec in H_2O (0°) and subsequently extracted with H_2O (0°) for 2 min. Amino acids were adsorbed on a Dowex (H^+) column (5 × 0.3 cm) and eluted with 2 N NH_4OH. The eluate was slightly acidified and analysed with an LKB amino acid analyser. Experiments were carried out in duplicate. Groups of seven seedlings or pairs of cotyledons were used in the incorporation experiments but the data presented in this paper refer to one seedling or pair of cotyledons.

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