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REGULATION OF STEROL BIOSYNTHESIS IN ETIOLATED MUNG BEAN HYPOCOTYL SECTIONS

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Key Word Index—*Phaseolus aureus*; Leguminosae; mung bean; transmethylation; wounding; NAA; sterol biosynthesis.

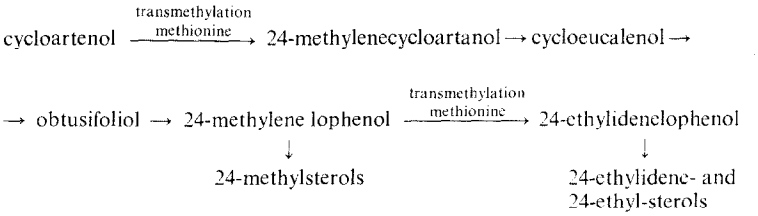
Abstract—The incorporation of radioactivity into sterols by transmethylation of methionine-[^{14}C-methyl] was studied in mung bean hypocotyl sections. Young hypocotyl sections (1 cm) synthesized 4 times more radioactive sterols than older sections (5 cm). The transmethylation reactions may be rate limiting in older tissues. Wounding has only a quantitative effect on sterol biosynthesis, as seen by incorporation experiments with MVA-[2-^{14}C]. Naphthalene acetic acid (NAA) stimulates sterol biosynthesis in both wounded surfaces and intact tissues of mung bean hypocotyl sections.

INTRODUCTION

Sterol biosynthesis occurs mainly in the 1 cm zone (region of elongation growth) of etiolated mung bean hypocotyls, and the sterol biosynthesis capacity diminishes from the 1 cm zone to the 3 cm and 5 cm zones of the hypocotyl [1, 2]. The rate limiting step for sterol synthesis in the older tissues (3 cm and 5 cm zones of the hypocotyls) might be found in the conversion of cycloartenol into sterols. NAA stimulates this conversion rate and so may stimulate the overall sterol biosynthesis. Baisted [3] studying sterol biosynthesis in peas, and Hartmann and Benveniste [4] in potato tuber discs, suggested that the enzyme *S*-adenosylmethionine-Δ^{24} sterol methyl transferase could regulate sterol biosynthesis. This too implicates a regulation between cycloartenol and the 4-demethylsterols. We now provide more direct evidence that this enzyme regulates sterol synthesis in the young and old mung bean hypocotyl sections, although other regulating enzyme systems cannot be excluded. In our previous experiments [1, 2] we studied mung bean hypocotyl sections, each having 2 wound surfaces, and the criticism could be made that the results were due to wound effects. We now show that wounding the tissues has only a quantitative effect on the incorporation of label from MVA-[2-^{14}C] into sterols and that the previously reported stimulation of sterol biosynthesis by NAA takes place in both wounded and intact tissue.

RESULTS AND DISCUSSION

Because of the suggestion that the activity of *S*-adenosyl methionine Δ^{24} sterol methyl transferase could regulate sterol biosynthesis [3, 4] and as we found that sterol biosynthesis may be regulated at a point between cycloartenol and the 4-demethylsterols [1, 2], we studied the incorporation of label from methionine-[^{14}C-methyl] into 4-demethylsterols, 4-monomethylsterols and 4,4-dimethylsterols in the 1 cm (young, growing) and the 5 cm (older, non-growing) zones of etiolated mung bean hypocotyls. Castle *et al.* [5, 6] showed that the alkyl groups at C-24 of sterols are introduced by a transmethylation from *S*-adenosyl methionine. The Δ^{24} sterol precursor, cycloartenol, is methylated to produce 24-methylene cycloartanol. A second transmethylation will lead to the conversion of 24-methylene lophenol into 24-ethylidene lophenol [7].



The results obtained with the 1 cm and 5 cm zones of the hypocotyls after an incubation period of 3, 6 and 20 hr in methionine-[^{14}C-methyl] with (for the 20 hr incubation period only) or without 2 ppm NAA are summarized in Table 1.

The incorporation of label by transmethylation from methionine-[^{14}C-methyl] in the sterols of the 1 cm zone is much higher (*ca* 5 ×) than in the corresponding 5 cm sections. The labelling of the sterol fraction increases with incubation time, in both the 1 cm and the 5 cm zone, as expected. Because there is no significant difference in uptake of methionine-[^{14}C-methyl] between the 1 and 5 cm zones of the hypocotyls, the lower incorporation of label into the sterols in the 5 cm zone cannot be explained by a lower uptake of precursor. As expected, the uptake from the medium also increases as a function of incubation time in both the 1 and 5 cm zones. The reduced sterol synthesis in the 5 cm zone cannot be explained by a reduced synthesis of cycloartenol (the starting material to be transmethylated), because in previous studies we showed that in both the 1 and 5 cm zones the 4,4-dimethylsterols became equally labelled from applied MVA-[2-^{14}C] [1, 2]. Thus the reduced sterol biosynthesis found in the 5 cm zone is further proof that the rate limiting step lies between cycloartenol and the 4-demethylsterols. It is also clear that not only the 4-monomethylsterols become labelled, but also the 4,4-dimethylsterols, thus implicating the transmethylation of cycloartenol. The labelling of these 2 groups of sterols was more pronounced in the 1 cm zone of the hypocotyls. This indicates that the transmethylation reactions become rate limiting in the 5 cm zone of the hypocotyl. However, other enzyme systems may be limiting for the conversion of the labelled 4,4-dimethylsterols and the 4-monomethylsterols into the 4-demethylsterols.

Table 1. Radioactivity recovered in the different sterol bands after TLC

	1st cm				5th cm			
	C 3 (hr)	C 6 (hr)	C 20 (hr)	NAA 20 (hr)	C 3 (hr)	C 6 (hr)	C 20 (hr)	NAA 20 (hr)
Sterols								
Exp. 1	1800	3830	15·550	18·300	430	590	3·900	4·620
Exp. 2	1600	2890	21·570	27·320	340	570	5·520	6·270
Mean	1700	3360	18·560	22·810	385	580	4·710	5·445
	(100%)	(100%)	(100%)	(122%) [100%]	(22%)	(17%)	(25%)	(29%) [23%]
4. Monomethylsterols								
Exp. 1	660	1040	1370	550	120	580	350	240
Exp. 2	610	890	600	920	280	460	220	430
Mean	635	960	985	735	200	520	285	335
	(100%)	(100%)	(100%)	(74%) [100%]	(31%)	(54%)	(28%)	(34%) [45%]
4,4 dimethylsterols								
Exp. 1	500	390	390	540	290	300	170	270
Exp. 2	390	310	710	850	190	270	280	260
Mean	445	350	550	695	240	285	225	265
	(100%)	(100%)	(100%)	(126%) [100%]	(53%)	(81%)	(40%)	(48%) [38%]
Uptake (%)								
Exp. 1	22	36	59	59	15	37	66	58
Exp. 2	21	40	62	65	15	51	63	67

The medium before each incorporation expt contained 0·04 μM/3 ml Methionine-[^{14}C-Methyl] (2·7 × 10^6 cpm). Values are given in cpm, counting efficiency being 70%.

The applied NAA has no clear effect on sterol biosynthesis, but it is known that auxins stimulate protein synthesis in mung beans [8], and this may interfere with the stimulating effect of NAA on sterol biosynthesis.

The wound surfaces (outer 2 mm segments) of the 1 cm zone synthesized more sterols than the middle segments of the sections (Table 2). In the 5 cm zone the sterol biosynthesis capacity is much lower, as seen also from Table 1 and from our previous work [1, 2]. In both cases NAA has a stimulatory effect on incorporation of label from MVA-[2-^{14}C] into the sterol fraction as reported earlier [2]. If we take cpm sterols/cpm 4,4-dimethylsterols as a measure of the conversion of cycloartenol into sterols, it is clear that NAA stimulates sterol biosynthesis by stimulating the conversion rate of cycloartenol into sterols in both the wound surfaces and the central segments of the sections. Thus

Table 2. Radioactivity recovered in the sterol and 4,4-dimethylsterol bands after TLC

		Central segments		Cut surfaces*	
		Control	NAA	Control	NAA
1 cm zone	Sterols	1380 ± 159	3270 ± 405	2710 ± 248	4910 ± 167
	4,4-dimethylsterols	940 ± 41	1060 ± 126	1070 ± 79	1180 ± 105
	cpm sterols / cpm 4,4-dim.ster.	1·46	3·08	2·53	4·16
5-cm zone	sterols	480 ± 47	930 ± 102	600 ± 35	1070 ± 61
	4,4-dimethylsterols	930 ± 46	1180 ± 25	1030 ± 81	1240 ± 63
	cpm sterols / cpm 4,4-dim.ster.	0·51	0·78	0·58	0·86

The medium before each incorporation expt contained 4 μM MVA-[2-^{14}C], 0·06 μCi/3 ml. After the 20 hr incubation period the uptake from each medium by the 25 hypocotyl sections was about 60%. Values are given in cpm, counting efficiency being 70%. For the 1 cm zone results are the mean of 5 expts, for the 5 cm zone the mean of 3 experiments. * See Experimental.

wounding the tissues of mung bean hypocotyl sections has only a quantitative effect on sterol biosynthesis. The higher sterol biosynthesis rate of the wound surfaces may be due to a better uptake of precursor or to the enhanced metabolic activity of the wound surfaces, as seen from the higher conversion rate of cycloartenol into sterols (Table 2).

Hartmann and Benveniste [3] studying sterol biosynthesis in potato tuber discs found that sterol biosynthesis capacity increased as a function of time after excision of the discs. Discs incubated for 2 hr in NaOAc-[1-^{14}C] immediately after excision synthesized only cycloartenol, but when the 2 hr incubation period in acetate-[1-^{14}C] was given after longer time intervals from excision, 24-methylenecycloartanol, 4-monomethylsterols and of the sterols mainly 28-isofucosterol also became labelled. From these results the intact potato sterol biosynthesis appears to be blocked after the formation of cycloartenol, and that cells near the wound surfaces became dedifferentiated progressively after excision. Lange [9] and Lange *et al* [10] studied the influence of derepression on the respiration, suberin synthesis and cell proliferation in potato tuber tissues. Under some experimental conditions, even callus proliferations could be seen as early as 32 hr after excision. Therefore, it might be better to interpret the results of Hartmann and Benveniste [4] not as a result of ageing, but rather as a result of dedifferentiation of the cells near the wound surfaces, thus giving physiologically younger cells with a higher sterol biosynthesis capacity. From a physiological point of view these considerations are important because they permit us to come to a more unifying principle about the regulation of sterol biosynthesis. In actively growing and differentiating tissues there is a high sterol synthesis rate (1 cm zone of mung bean hypocotyls [1, 2]; during earlier stages of developing pea seeds and the development of young pea seedlings [3]). As a result of the higher degree of differentiation, sterol biosynthesis is diminished by a blockage after the formation of cycloartenol [1–4]. This is perhaps also the reason why cycloartenol accumulates in seeds of *Strychnos nux vomica* [11] and in fruits of *Artocarpus incisa* (unpublished results). In the case of dedifferentiation (giving physiologically younger cells), for example, by wounding, sterol biosynthesis is again activated by the unblocking of the pathway after cycloartenol (potato tuber discs [4], wound surfaces of mung bean hypocotyl sections (Table 2)). In mung bean hypocotyl sections the termination of growth is accompanied by a diminished sterol biosynthesis capacity together with a considerable increase in the stigmasterol:sitosterol ratio [1, 2].

This could be a reflection of different membrane properties during and after the growth period of the hypocotyl sections. It is known that in plant systems sitosterol and stigmasterol have different effects on membrane permeability [12, 13]. The MeOH-initiated leakage of β-cyanin from red beet discs can be reduced by certain sterols. Most effective is cholesterol, followed by sitosterol and then

by stigmasterol. Ergosterol increased the leakage of betacyanin. The ethanol-induced leakage of electrolytes from barley roots can be reduced by cholesterol and campesterol. Sitosterol and stigmasterol had no clear effect in influencing leakage. In animal systems, the ease of incorporation of different sterols in the membranes of erythrocytes and in liposomes is in the order: cholesterol > campesterol > sitosterol > stigmasterol [14–16]. Thus the possibility exists that a changed permeability of the cell membranes due to a changed stigmasterol:sitosterol ratio is concerned with the termination of elongation growth in the older mung bean hypocotyl sections. Besides the supposed role of sterols in membranes, they may function as precursors of steroid hormones such as corticosteroids, that were found to double the growth of mung bean roots, to double the lateral root number and to stimulate the hypocotyl growth by about 60% [17].

EXPERIMENTAL

For each treatment, 25 5-day-old etiolated mung bean seedlings (*Phaseolus aureus* Roxb.) with a hypocotyl length of 6 cm were selected. The 1 and 5 cm zones from the top of the hypocotyls were removed in dim green light and soaked in H_2O for 1 hr. Sections were dried on a filter paper and incubated in 3 ml medium for 20 hr with continuous shaking.

Incorporation experiments. Various hypocotyl sections were incubated in 3 ml methionine-[^{14}C-methyl] soln, sp. act. 50 mCi/mmol (0·04 μmol = 1·7 μCi; dark, 23°) for 3, 6 or 20 hr or in 3 ml MVA-[2-^{14}C] soln, sp. act. 0·015 mCi/mmol (4 μmol = 0·06 μCi; dark, 23°) for 20 hr with continuous shaking with/without 2 ppm NAA. After incubation, the sections were rinsed with H_2O and the vol. made up to 50 ml. Aliquots were counted in a liquid scintillator.

Distribution of radioactivity in the sections. Because NAA stimulated the elongation growth of the sections of the 1 cm by about 45% compared to the control sections, each section was divided into 5 equal parts. The various parts were collected in scintillation vials, squashed, and the counting medium (1 ml H_2O and 3·4 ml of a soln containing 4 g PPO in 500 ml Triton X 100 and 1 l. toluene) was added. Because wound surfaces incorporated more label from added MVA-[2-^{14}C], the outer 2 mm (called "wound surfaces") and the central sections were extracted separately.

Sterol extraction and analysis. The non-saponifiable lipid fractions were submitted to TLC. The bands corresponding to the sterols, 4-monomethyl- and 4,4-dimethylsterols were counted in a liquid scintillator [1, 2].

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