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DOPA SYNTHESIS IN NON-PRODUCER CULTURES OF *MUCUNA DEERINGIANA*

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Key Word Index—*Mucuna deeringiana*; Leguminosae; velvet bean; non-protein amino acids; DOPA; stizolobic acid; cell cultures; catabolism.

Abstract—Cell cultures of *Mucuna deeringiana* on various media fail to accumulate DOPA or stizolobic acid. Radiotracer studies prove that both compounds are being synthesized in the cells; however, the apparent lack of production is due to metabolic emphasis on catabolism instead of storage.

INTRODUCTION

Numerous non-protein amino acids have been found in higher plants [1] and their high concentrations, particularly in seeds of legume species, often make the tissue toxic to consuming organisms, including Man. While the identification and distribution of these compounds have been extensively studied, much less is known about their metabolism in plants or about the mechanisms controlling their accumulation in specific tissues at specific times. Plant-cell cultures offer several advantages as material for metabolic studies [2, 3], and they are known to be capable of synthesizing and accumulating various amino acid-derived secondary products [4–8]. In the course of studies on the biosynthesis of the non-protein amino acid stizolobic acid from DOPA in *Mucuna deeringiana* [9], we had therefore established callus and suspension cultures of this species. DOPA is accumulated in seeds of *Mucuna* spp. [10] but, in contrast to recent reports of the formation of remarkable quantities of DOPA in cultures of *M. pruriens* [11] and *M. sloanei* [12], we found that *M. deeringiana* cells in culture did not accumulate chromatographically detectable quantities of DOPA or stizolobic acid. The observation that tyrosine accumulation could be induced in these cultures by exogenously supplied DOPA, however, led us to investigate the metabolic fate of tyrosine and DOPA in these cultures in more detail.

RESULTS

While the seeds of *M. deeringiana* contain the highest tissue concentrations of L-DOPA, the compound is found in all plant parts and has also been shown to be synthesized from tyrosine in these tissues [9]. Explants of root or hypocotyl segments readily formed callus on a modified B-5 medium and also grew as rather lumpy suspension cultures. At no time was any darkening of the growth medium observed, and necrotic cultured tissue did not turn black but rather a slightly deeper brown. This behaviour is typical of cell cultures which are not accumulating *ortho*-dihydroxy phenols and the absence of both L-DOPA and its ring-cleavage product, stizolobic acid, was confirmed by TLC, TLE and amino-acid ion exchange CC. The growth medium was modified in the following ways and the effect on non-protein amino acid accumulation was noted: 2,4-D concentration = 0.5–5 mg/l.; naphthalene acetic acid-deleted; IAA-deleted; kinetin-deleted; casein hydrolysate-deleted, or replaced with selected groups of L-amino acids at the same concentrations as in the hydrolysate. While some of these treatments depressed the growth of *M. deeringiana* suspension cultures, no non-protein amino acids appeared in the cells or medium. Incubation of suspension cultures with 0.3 and 3.0 mM L-tyrosine for 10 days also did not result in L-DOPA accumulation but parallel incubations with L-DOPA led to both an appearance of detectable amounts of DOPA in the cells and to a substantial and specific increase in the intracellular pool of L-tyrosine (Table 1).

To determine whether L-tyrosine was still being metabolized to L-DOPA and sitizolobic acid in these cultures, the tissue was incubated for shorter periods (6 and 24 hr) with L-tyrosine-[*ring*-^{14}C] in the presence and absence of unlabelled L-DOPA (0.2 mM). Label from the tyrosine could be found in both the L-DOPA and stizolobic acid fractions isolated from the tissue and the presence of unlabelled L-DOPA enhanced the labelling of these non-protein amino acids (Table 2). In all cases, some of the label also appeared in the respiratory carbon dioxide, indicating that at least part of the tyrosine pool has been completely catabolized. Label from L-DOPA-[*ring*-^{14}C] incubated with *M. deeringiana* suspension cultures for 24 hr also appeared in both stizolobic acid and carbon dioxide. For comparison, the extent of conversion of L-tyrosine to non-protein amino acids in young leaves of *M. deeringiana* during a 24 hr incubation was also determined.

Table 1. Effect of a 10-day incubation with amino-acid supplements on aromatic amino-acid contents of *M. deeringiana* suspension culture

Amino-acid supplement		Culture content of amino acid (μmol/g dry wt) Phenylalanine	Tyrosine	DOPA
None		1.2	1.1	—
L-Tyrosine	3.0 mM	2.2	2.0	—
L-DOPA	0.3 mM	1.8	5.4	trace
	3.0 mM	1.8	9.8	15.7

Values shown are the average of duplicate experiments.

DISCUSSION

The biosynthesis of DOPA requires a single step, the ring-3-hydroxylation of tyrosine, while one of the metabolic fates of DOPA in *Mucuna deeringiana* tissue is an oxidative ring-fission. The dicarboxylic acid ring-fission product can be enzymatically cyclized to form the α-pyrone amino acid stizolobic acid [9, 13, 14] but, alternatively, if it is also a substrate for further oxidation it could be regarded as a catabolic intermediate in the turnover of the DOPA pool. When the cell cultures of *M. deeringiana* were found to lack DOPA and stizolobic acid, two explanations seemed possible: either there was no synthesis of DOPA taking place, or there was no accumulation of the non-protein amino acids, i.e. synthesis was balanced by catabolism. Support for the second possibility was provided by the results of tyrosine and DOPA additions to the culture medium where the former had no effect but the latter resulted in tyrosine build-up in the cells. This is consistent with a system in which tyrosine is being converted to DOPA by a reaction that is subject to product inhibition. Short-term labelling of the cultures with L-tyrosine-[*ring*-^{14}C] confirmed that synthesis of DOPA is actively proceeding in the cells, as is further metabolism of the DOPA to produce both stizolobic acid and $^{14}CO_2$. The inclusion of unlabelled DOPA in the incubation system as a trap depressed the uptake of labelled tyrosine but was successful in producing a relatively more heavily labelled DOPA pool in the tissue. Surprisingly, the trapping procedure also resulted in a substantial increase in the relative amount of label recovered in the stizolobic acid pool and in the 24 hr CO_2 sample which suggests that the higher intracellular L-DOPA level may have stimulated a more rapid ring-fission/catabolism in the treated cells. Since the organized plant tissues contain far higher amounts of L-DOPA than those attained in the cultured cells, it must be assumed that a specialized storage mechanism exists in such tissue which normally segregates the bulk of the amino acid and prevents its catabolism. Cultured *M. deeringiana* cells appear to lack this storage ability and as a result are unable to accumulate any substantial amounts of L-DOPA. Similar results have been obtained in other laboratories with this species (J. Berlin, pers. commun.) and with cultures of *Stizolobium hassjoo* (= *Mucuna*) (A. Komamine, pers. commun.). We have also observed a complete disappearance of non-protein amino acids from cultured cells of species of *Lathyrus*, *Phaseolus* and *Canavalia* during rapid callus growth (unpublished results), which suggests that this may be a general phenomenon. Whether the disappearance in these cases represents a failure of synthesis or a rapid catabolism remains to be determined.

EXPERIMENTAL

Materials. Mucuna deeringiana (cv Early Jumbo) plants were grown as described previously [9]. Callus cultures were established from aseptically germinated seedling sections using B-5 medium [15] supplemented with 2 mg/l. 2,4-D, 0.5 mg/l. naphthalene–HOAc, 0.5 mg/l. IAA, 0.2 mg/l. kinetin and 2 g/l. casein hydrolysate. Suspension cultures grew in the same medium as small light-brown nodules of tissue which were subcultured every 3 weeks. L-Tyrosine-[*ring*-^{14}C] and L-DOPA-[*ring*-^{14}C] were synthesized from phenol-[U-^{14}C]

Table 2. Distribution of label from L-[*ring*-U-^{14}C]-tyrosine and L-[*ring*-U-^{14}C]-DOPA administered to young leaves and cell suspension cultures of *M. deeringiana*. Cultures were incubated with or without unlabelled L-DOPA (0.2 mM)

Tissue	Substrate	Incubation period (hr)	Labelled product	Total ^{14}C (dpm)	% of soluble label
Culture	Tyrosine (5 μCu; 0.1 μmol)	6	tyrosine	$2.7\times10^6(0.5\times10^6)$*	86(93)*
			DOPA	$3.1\times10^3(4.1\times10^3)$	0.1(0.8)
			stizolobic acid	$11.9\times10^3(14\times10^3)$	0.3(2.8)
			CO_2		0.7(0.4)
Culture	Tyrosine	24	tyrosine	$8.0\times10^5(0.8\times10^5)$	73(49)
			DOPA	$3.2\times10^3(7.4\times10^3)$	0.3(4.8)
			stizolobic acid	$16\times10^3(20\times10^3)$	1.5(12.9)
			CO_2		3.9(10.3)
Culture	DOPA (1 μCu; 0.1 μ mol)	24	DOPA	1.1×10^4	7.0
			stizolobic acid	7.4×10^3	4.9
			CO_2		5.0
Leaf	Tyrosine (20 μCu; 0.1 μmol)	24	tyrosine	1.3×10^6	20.2
			DOPA	0.9×10^6	13.7
			stizolobic acid	1.8×10^4	0.3

*Values in parentheses are from incubations which include unlabelled DOPA.