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Occurrence of L-DOPA and dopamine in plants and cell cultures of *Mucuna pruriens* and effects of 2,4-D and NaCl on these compounds

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Abstract

The development of the L-DOPA-content of roots, stems and leaves of *Mucuna pruriens* during growth of the plants is described. Besides L-DOPA, the leaves, but not the stems and the roots, also contain the related catechol dopamine. The time course of dopamine accumulation is compared to that of L-DOPA.

In cell suspension cultures of *M. pruriens* dopamine can be detected as well. Its level is strongly increased by addition of the growth regulator 2,4-D to the medium, a condition that suppresses cell growth and L-DOPA-accumulation. Dopamine induction appears to be a specific metabolic effect of 2,4-D. Salt stress, as caused by the addition of NaCl, gives no induction of dopamine formation, whereas L-DOPA is released into the medium.

Abbreviations: 2,4-D – 2,4-dichlorophenoxyacetic acid, DW – dry weight, HPLC – high performance liquid chromatography, L-DOPA – (3,4-dihydroxyphenyl)-L-alanine

Introduction

(3,4-Dihydroxyphenyl)-L-alanine (L-DOPA) is a catecholic amino acid whose presence in plant taxa was established several years ago (Daxenbichler et al. 1972). Besides L-DOPA, other catecholic compounds, particularly isoquinolines, can be found in *Mucuna* species. 3-Carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline was isolated from the seeds of *M. mutisiana* (Bell et al. 1971), and 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline was extracted from the seeds of *M. deeringiana* (Daxenbichler et al. 1971).

More recently, the presence of L-DOPA in callus cultures of *Stizolobium hassjoo* and in callus and cell suspension cultures of *M. pruriens* has been reported (Obata-Sasamoto & Komamine 1983; Brain 1976; Huizing et al. 1985). In callus cultures of *S. hassjoo* the presence of the isoquinolines mentioned above was demonstrated as well. In addition, stizolobic acid was formed (Saito et al. 1982). This latter compound might also be formed from L-DOPA in cell cultures of *M. deeringiana* (Remmen & Ellis 1980). At a high concentration of the synthetic auxin 2,4-D, a condition suppressing L-DOPA accumulation, the metabolism of L-tyrosine is claimed to be directed towards incorporation into protein (Obata-Sasamoto et al. 1981). Remmen & Ellis (1980) reported the active catabolism of L-DOPA in cultures of *M. deeringiana*.

Although L-DOPA accumulation in cell cul-

tures is relatively well documented, only few reports on the occurrence of this compound on the plant level other than in seeds have appeared.

In earlier experiments on the regulation of L-DOPA biosynthesis in cell suspension cultures of *M. pruriens*, we used a high performance liquid chromatograph equipped with electrochemical detection for the quantification of L-DOPA (Huizing et al. 1985). When this detection system was applied to extracts of leaves of *M. pruriens*, L-DOPA and another readily oxidizable compound could be detected. In this paper we give evidence that this compound is dopamine. The time course of dopamine formation is compared to that of L-DOPA in roots, stems and leaves of growing plants of *M. pruriens*. The presence of dopamine and the inducibility of its formation in cell suspension cultures of *M. pruriens* is also reported. Finally, the possible role of dopamine in tyrosine metabolism is discussed.

Materials and methods

Cultivation of plants

Seeds from *Mucuna pruriens* (L.) DC f. utilis (Wall. ex Wight) Back cv White were obtained from Quimatilal Sevenseas Linkimpex, Amritsar, India. Plants were grown in pots in a greenhouse under a photoperiod of 16 h (Philips 40W/57 lamps) followed by 8 h in the dark, at a temperature of 18–23°C.

Seedlings or plants were harvested at intervals after germination as indicated in the text.

Cell cultures

Callus and cell suspension cultures were initiated from leaf explants of plants of *M. pruriens* and maintained on Murashige and Skoog medium supplemented with 1 mg l^{-1} indole-3-acetic acid, 1 mg l^{-1} benzyladenine and 4% (w/v) sucrose as described earlier (Huizing et al. 1985).

Sample preparation and determination of L-DOPA and dopamine

Samples of ca. 2 g of fresh material from leaves, stems and roots of seedlings or plants were homogenized in 50 ml of 5% (v/v) formic acid with a Sorvall Omnimixer type 17106 at maximum speed under cooling in ice. Samples from cell suspension cultures were acidified with formic acid to a final concentration of 5% (v/v) and homogenized as described above. The homogenized samples were filtered through a 0.45 μm membrane filter prior to analysis.

Quantification was performed with HPLC, using a Nucleosil 5C18 column (150 × 4.6 mm), and eluted with McIlvaine buffer pH 4.7 at a flow rate of 1.0 ml min^{-1}. Catechols were detected with a rotating electrochemical detector as described previously (Huizing et al. 1985; Oosterhuis et al. 1980) using authentic samples as reference compounds.

For identification of dopamine by means of mass spectrometry a leaf extract was concentrated to one tenth of the original volume under *vacuo* at 50°C and applied to a Nucleosil RP-18 HPLC-column (150 × 4.6 mm, i.d.). The relevant compound was collected from the effluent of the column. In this case, the mobile phase consisted of 0.1 M ammonium acetate/acetic acid buffer, pH 4.7. The fractions from several runs were pooled and lyophilized.

Mass spectrometry

Mass spectrometry (thermal desorption combined with chemical ionization) was performed as described previously (Huizing et al. 1985) with NH_3 as the reactant gas.

Chemical preparation of tetrahydroisoquinolines

1 - Methyl - 3 - carboxy - 6,7 - dihydroxy - 1,2,3,4 - tetrahydroisoquinoline and 3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline were prepared from L-DOPA using acetaldehyde or formaldehyde according to the procedure as given by Saito et al. (1982). 1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline were pre-

pared analogously from dopamine. The identity of the compounds was verified with thermal desorption mass spectrometry.

Results and discussion

Influence of ontogeny of M. pruriens plants on the occurrence of L-DOPA

Although the presence of L-DOPA in the seeds of *Mucuna* species has been known for a considerable time (Damodaran & Ramaswami 1937; Daxenbichler et al. 1971), to our knowledge no information is available on the occurrence of L-DOPA in plant parts other than the seeds.

Figure 1 shows the time course of the L-DOPA-content of roots, stems and leaves of growing plants of *M. pruriens*. The conclusion

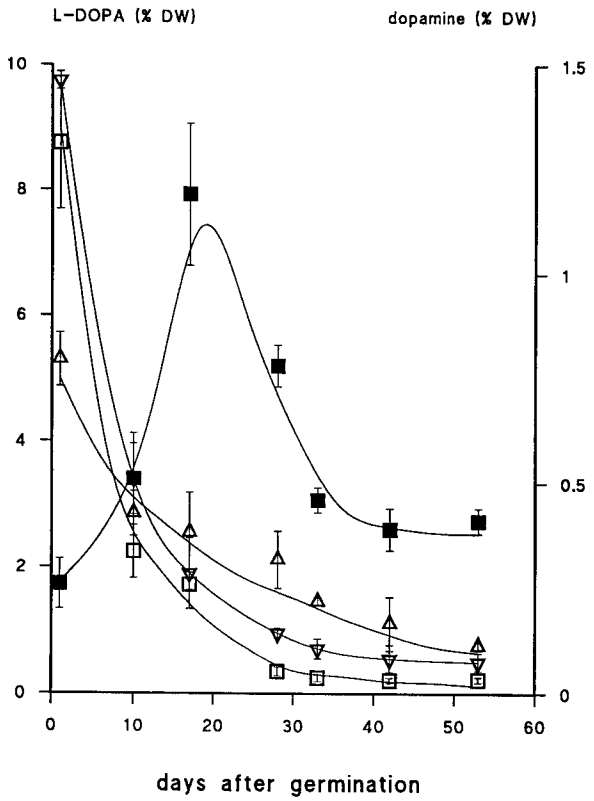

Fig. 1. L-DOPA-content (expressed as %DW) of roots (▽–▽), stems (△–△) and leaves (□–□) and dopamine-content of the leaves (■ – ■) of growing plants of *M. pruriens*. Values are the average of two replicate experiments.

can be drawn, that after germination the L-DOPA-content of the leaves and roots rapidly drops by one to two orders of magnitude within 4 weeks. Also in the stems there is a rapid decline in L-DOPA-content, albeit at a somewhat slower rate than in leaves and roots. An L-DOPA-content in the seeds of several percent of the dry weight had been measured before (Bell et al. 1971; Bell & Janzen 1971), which indeed suggests that the seeds are the only possible economical source of L-DOPA.

Identification of dopamine and occurrence in plants of M. pruriens

When the elution profiles, obtained by HPLC with electrochemical detection as described in the Materials and methods section, of leaf extracts from 3 week old plants were compared with the elution profiles of leaf extracts from plants shortly after germination, a compound in addition to L-DOPA could be detected. This compound co-chromatographed with authentic dopamine (Fig. 2A–C). After collecting this compound from the effluent of the HPLC-column, the thermal desorption chemical ionization mass spectrum showed a base peak (RI = 100%) at m/z 154, indicating a molecular weight of 153 D for this compound, and a fragment at m/z 102 (11.3%). This spectrum was identical to the spectrum of an authentic sample of dopamine.

Based on this, we conclude that the new compound in the leaf extracts is dopamine.

Whereas the L-DOPA-content of all plant parts strongly diminished with time, the dopamine content of the leaves increased until a maximum was reached in 2–3 week old leaves (Fig. 1). At this stage, the dopamine content of the leaves even exceeded the L-DOPA-content. In the roots, stems and seeds of *M. pruriens* no dopamine could be detected at any stage of development.

Occurrence and inducibility of dopamine formation in cell suspension cultures

Dopamine was detected in two cell lines of leaf-derived suspension cultures of *M. pruriens* as

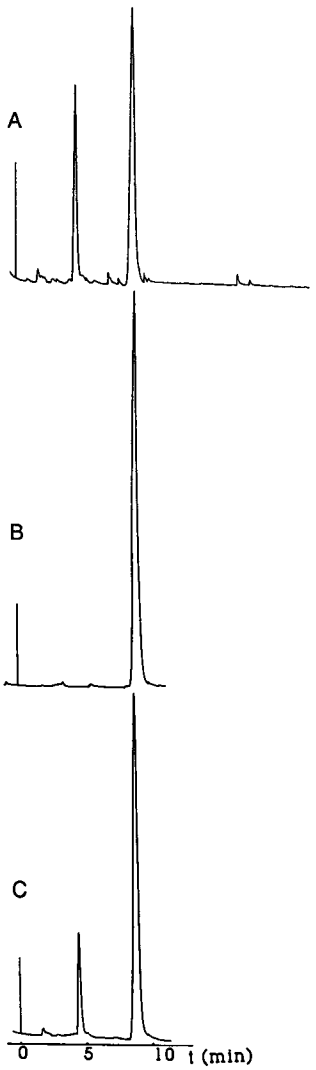

Fig. 2. HPLC elution profile of an extract of the leaves of a 3-week old plant of *M. pruriens*. (*A*) leaf extract; (*B*) reference dopamine; (*C*) leaf extract spiked with an equal amount of dopamine.

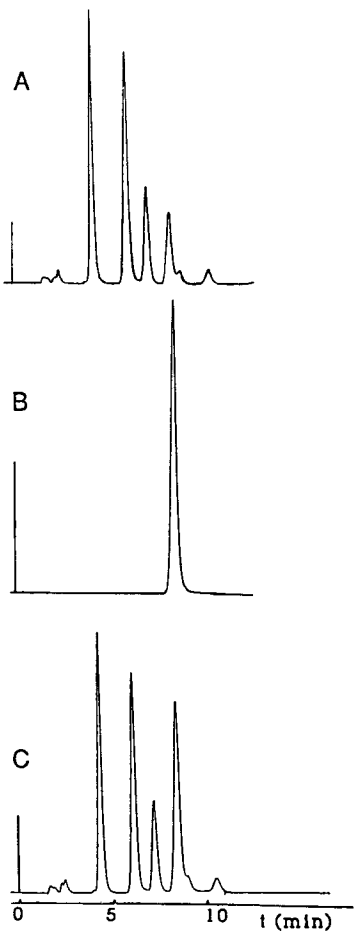

Fig. 3. HPLC elution profile of an extract of a cell suspension culture of *M. pruriens* (cell line MPL1/7), grown for 7 days in the presence of 10 mg l^{-1} 2,4-D. (*A*) cell extract; (*B*) reference dopamine; (*C*) cell extract spiked with an equal amount of dopamine.

well (Fig. 3, Table 1). Whereas in leaf extracts the dopamine content may exceed the L-DOPA-content (Fig. 1), in routinely grown cell suspension cultures the L-DOPA-content exceeded the dopamine content by a factor of 16–80 (Table 1).

As previously reported, the accumulation of L-DOPA in cell suspension cultures is suppressed by the addition of 2,4-D to the growth medium, while other compounds become dominant in the elution profiles from HPLC (Wichers et al. 1985). Also cell growth is inhibited by the addition of 2,4-D (Table 1). One of the compounds whose accumulation is induced by the addition of 2,4-D is dopamine, on the basis of retention time and cochromatography (Fig. 3). The dopamine content in cell line MPL 1/7 increased 5-fold (Table 1).

Inhibition of biomass production and L-DOPA-accumulation may be the result of stress as a result of the high level of 2,4-D or a specific effect of 2,4-D on growth and metabolism of L-DOPA. Therefore we investigated the effect of salt stress, resulting from the addition of NaCl to the growth medium, on cell growth and on the

Table 1. Effect of the addition of 2,4-D (10 mg l^{-1}) to the medium on growth and on the accumulation of L-DOPA and dopamine by a cell suspension culture of *M. pruriens*.

Cell line	Dry weight g l^{-1}	L-DOPA in homogenate		Dopamine in homogenate	
		mg l^{-1}	% of DW	mg l^{-1}	% of DW
MPL 1/7	26.7 ± 2.0	450 ± 16	1.7 ± 0.1	5.1 ± 0.3	0.02 ± 0.001
MPL 1/8	26.5 ± 2.5	85 ± 3	0.32 ± 0.02	5.0 ± 0.3	0.02 ± 0.001
MPL 1/7 +2,4-D	13.4 ± 1.5	44 ± 3	0.33 ± 0.01	13 ± 1.9	0.10 ± 0.004

Values given are averages of two replicate experiments. Cell lines: MPL 1/7 and MPL 1/8. Culture period: 7 days.

accumulation of L-DOPA and dopamine. Both cell growth and the accumulation of L-DOPA were suppressed by the addition of NaCl (Table 2). At higher NaCl-concentrations increasing amounts of L-DOPA, up to 74%, are detected in the medium. Microscopic examination revealed that the cells were plasmolysed. Nevertheless, no dopamine could be measured in the cell cultures. Therefore, the effect of 2,4-D on the metabolism of L-DOPA does not appear to be comparable to salt stress as caused by the addition of NaCl.

In cell suspension cultures of *M. pruriens* a phenoloxidase is present that is able to *ortho*-hydroxylate L-tyrosine into L-DOPA (Wichers et al. 1984). One of the monophenolic substrates of this enzyme that can also be converted *in vitro* is tyramine. Therefore, two possible ways of biosynthesis of dopamine may be assumed. Firstly, tyrosine may be *ortho*-hydroxylated into L-DOPA, after which L-DOPA may be decarboxylated into dopamine. In this case, the occurrence of a DOPA-decarboxylating enzyme in leaves and cell cultures should be postulated. Secondly, tyrosine may be decarboxylated into tyramine which is subsequently *ortho*-hydroxylated into dopamine by the phenoloxidase. In this situation, a tyrosine-decarboxylating enzyme must occur in leaves and cell cultures.

In the HPLC system used in this study, tyrosine and tyramine may be detected when UV-detection is applied instead of electrochemical detection. Whereas the presence of tyrosine is unequivocally demonstrated in extracts from leaves and cell cultures, tyramine has not been detected. Therefore, also taking the sequence of accumulation of L-DOPA and dopamine in the plant leaves into account, the *ortho*-hydroxylation of tyrosine followed by DOPA-decarboxylation appears to be the most likely sequence of conversions for the synthesis of dopamine.

Besides polymerization into melanin, catecholic compounds like L-DOPA and dopamine may be incorporated into tetrahydroisoquinolines (Bell et al. 1971; Saito et al. 1982) by *Mucuna* and related species. None of these tetrahydro-isoquinolines could be detected in extracts from leaves or cell cultures by cochromatography on HPLC. Therefore, the meta-

Table 2. Effect of addition of NaCl to the medium on growth and L-DOPA-production of a cell suspension culture of *M. pruriens*.

(NaCl) M	Dry weight g l^{-1}	L-DOPA in homogenate*		L-DOPA in cell free medium	
		mg l^{-1}	% of DW	mg l^{-1}	% of total
0	19.4 ± 1.9	405 ± 35	2.1 ± 0.04	0	0
0.10	14.8 ± 1.5	82 ± 5	0.6 ± 0.02	0	0
0.25	9.4 ± 1.0	55 ± 9	0.6 ± 0.03	21 ± 5	38 ± 3
0.50	6.2 ± 1.2	62 ± 0.9	1.0 ± 0.2	46 ± 5	74 ± 7

*A homogeneous sample of the culture broth was taken. Therefore, these figures represent the total L-DOPA-concentration in the culture broth. Values given are averages of two replicate experiments. Cell line: MPL 1/7. Cultivation period: 7 days.

bolic fate of the catecholic compounds, other than incorporation into melanin, in plants and cell cultures of *M. pruriens* remains unknown.

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