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DOPAMINE BIOSYNTHESIS AT DIFFERENT STAGES OF PLANT DEVELOPMENT IN *PAPAVER SOMNIFERUM*

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ABSTRACT.—Latex and cell-free extracts of various organs and stages of plant and capsule development in *Papaver somniferum*, the opium poppy, synthesized dopamine, an alkaloid precursor, from ^{14}C-dopa. The 1000 g × 30 min supernatant from latex of the pedicel-capsule junction converted more dopa than latex supernatant from the upper capsule or lower pedicel regions, although there was more protein in the latex from the capsule. Percent conversion of pedicel-capsule latex into dopamine was maximum in unopened flower buds and decreased within 14 days after flowering. Dopamine biosynthesis in latex and cell-free extracts also varied with the stage of organ development. Extracts from capsule tissue converted more labeled dopa into dopamine than did extracts from pedicels, leaves from vegetative plants at the rosette stage, leaves from flowering plants, or pedicels connected to capsules.

L-3-4 Dihydroxyphenylalanine (dopa) and dopamine have been established as alkaloid precursors in intact plants of *Papaver somniferum* L. (1-3). When 2-^{14}C-dopa or 1-^{14}C-dopamine was injected into intact poppy capsules, all of the radioactive label, upon extraction and degradation of the labeled morphine, was specifically at the ethanamine bridge position.

Laticifer cell protoplasm has been studied as a site of alkaloid biosynthesis, with ^{14}C-dopa as the alkaloid precursor. There was evidence for the presence of dopa decarboxylase in the 1000 g × 30 min latex supernatant fraction that converted dopa to dopamine (4,5). The 1000 g × 30 min latex pellet fraction synthesized five major alkaloids—morphine, codeine, thebaine, papaverine, and narcotine—when the pellet was incubated with labeled dopa (6,7).

In addition, *Papaver* alkaloid biosynthesis has been studied in cell-free systems (8-11). There are several advantages of a cell-free system for studying biosynthetic reactions. With such an extract, one avoids the problems of precursor penetration into the plant tissue, translocation within the plant, possible subcellular compartmentation of metabolic pools (12), and as in this study, cell-free extracts allow one to study laticifer protoplasm in conjunction with other tissues from regions of the plant, such as the leaves, where it is difficult to collect substantial amounts of latex for *in vitro* latex studies.

The alkaloid and dopamine concentration in poppy organs varies during stages of plant development and in different organs (7,13-21). Recently, it was reported that latex collected from plants in the floral bud stage contained higher levels of dopamine than did latex collected 3-12 days after the plants had flowered. These variations in alkaloid concentration may reflect translocation of an alkaloid or its precursor to a particular plant organ rather than differences in alkaloid biosynthetic activity for various organs at different stage of plant development.

The purpose of this study was to examine the capacities of isolated latex and cell-free extracts to synthesize dopamine, an alkaloid precursor, from ^{14}C-dopa. Various organs and tissues at different stages in development were examined to evaluate dopamine synthesize in both the latex and cell-free systems.

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EXPERIMENTAL

ANALYSIS OF LATEX FOR DOPAMINE BIOSYNTHESIS.—The pedicel-capsule was lanced when submerged in buffer (0.5 M mannitol, 0.1 M phosphate, pH 7.2) on ice so as to prevent latex oxidation. In all experiments, the 1000 g × 30 min latex supernatant was used because dopa decarboxylase occurs only in the supernatant (4,5).

The dopamine assay method for incubating latex with ^{14}C-dopa and the preparation of samples for the CG-50 column were similar to the procedure of Roberts and Antoun (4). Immediately after latex collection, the latex supernatant was added to a tube containing 0.18 μCi [3-^{14}C]dopa, specific activity 1.85 mCi/mmole (Amersham), previously dried down in the tube with N_2. The tubes were swirled, gassed for 3 min with N_2, and then incubated at 35° for 20 min. For controls, the latex was boiled for 30 min. Samples were stored at −20° until ready to run on the ion-exchange column.

The column (Pasteur pipet) was packed with 6 cm of prepared (22,23) Amberlite CG-50 resin. Samples (3 ml each) were loaded onto the column and chromatographed with 15 ml of 0.05 M ammonium acetate, pH 6.2 followed by 20 ml of 1 M ammonium acetate, pH 6.2. The dopamine fraction (the first 12 ml) of the 1 M ammonium acetate pH 6.2 wash were collected for analysis. The percent conversion of ^{14}C-dopa to dopamine was calculated using liquid scintillation. Dpm was calculated by internal standardization using D-[U-^{14}C]glucose. specific activity 4.5 mCi/mmole. The dopamine concentration was determined from an aliquot of the 1 M ammonium acetate dopamine-containing fraction. Total dpm was calculated from all fractions obtained from the ion-exchange column.

The dopamine product collected from the ion-exchange column was verified by two-dimensional pc on Whatman No. 1 chromatography paper. The solvent system was *n*-PrOH-H_2O-*n*-BuOH (5:7:10) (4) for the first dimension and H_2O-*n*-BuOH-pyridine (1:1:1) (24) for the second. Dopamine was identified by its Rf values in the two different solvent systems and its color when sprayed with a mixture of equal volumes of 15% (w/v) $FeCl_3$ and 1% (w/v) $K_3Fe(CN)_6$ (25).

Autoradiograms were prepared from the paper chromatograms to determine the presence of labeled dopamine and the possible presence of contaminants. Paper chromatograms were sprayed with Enhance Surface Spray (New England Nuclear) and then exposed to Kodak X Omat AR, XAR-5 film at −70°.

PROTEIN DETERMINATION.—The protein in the latex sample was determined according to the method of Lowry (26) using latex supernatant diluted with buffer.

ANALYSIS OF CELL-FREE SYSTEM FOR DOPAMINE BIOSYNTHESIS.—This assay was based upon the methods of Roberts and Antoun (4) and Hodges and Rapoport (11). Plant organs were homogenized in a phosphate buffer (0.1 M phosphate, 10 mM DIECA, 20 mM ascorbic acid, pH 7.2) to prepare the cell-free extract. Specific organs (capsules, pedicels, and leaves from plants at the rosette or flowering stages) were obtained from plants up to 5 days after flowering, except for leaves from vegetative plants in the rosette stage of growth. Flowering was defined as the first day the corolla opened.

The cell-free filtrate was prepared from homogenized tissue filtered through #80 mesh nylon cloth (Tetko Company). The 3-ml incubation mixture included 2.5 ml of the filtrate and 0.51 μCi L-[3-^{14}C]dopa, specific activity 1.7 mCi/mmole.

The reaction was initiated by adding ^{14}C-dopa (1×10^{-4} M) diluted with 0.1 M phosphate buffer, pH 7.2 buffer. The tubes were incubated at 35° for 60 min, and the tubes were inverted every 20 min during the incubation period. Tissue boiled for 30 min served as the control.

The reaction was terminated by adding 3% (w/v) sulfosalicylic acid (0.2 ml), 5% (w/v) EDTA (0.2 ml), and 5% (w/v) sodium metabisulfite (0.2 ml). Cold dopamine carrier (2 mg) was added to the incubation mixture. Protein was precipitated by 10% TCA followed by centrifugation at 4500 g × 15 min. The pH of the supernatant was titrated to pH 6.2 with NH_4OH. Each sample volume was brought to 6 ml with the 0.1 M phosphate buffer. Samples were kept at −20° until they were run on the ion-exchange column as for latex samples.

The calculation of ^{14}C-dopa percent conversion to dopamine was determined by liquid scintillation using the method as for latex samples. In addition, the method for analyzing dopamine by two-dimensional chromatography and autoradiography was identical to that used for examining the dopamine samples from latex.

PLANT CULTIVATION.—*P. somniferum* plants were grown from seeds (UNL 376 from Rennes, France) in a greenhouse or outside when weather permitted. Plants were grown 2 months under short-day conditions (8 h light and 16 h dark) and subsequently induced to flower under long-day conditions (15 h light and 9 h dark) using incandescent light.

RESULTS AND DISCUSSION

DOPAMINE BIOSYNTHESIS IN LATEX COLLECTED FROM DIFFERENT PLANT REGIONS.—In this comparative study of dopamine biosynthesis, latex was collected from

three regions of the floral axis: the capsule, the pedicel-capsule junction, and the pedicel base region. Equal amounts of latex were collected from plants within 5 days after flowering. DIECA was added to the supernatant to inhibit dopa oxidation by polyphenol oxidase. The supernatant may have been contaminated by this enzyme, which is localized in the 1000 g $\times$ 30 min pellet (5). Our results agreed with Roberts (5) that polyphenol oxidase was present in the 1000 g $\times$ 30 min pellet, while dopamine biosynthetic activity was in the 1000 g $\times$ 30 min latex supernatant fraction.

Incorporation experiments showed more conversion of ^{14}C-dopa into dopamine in latex of pedicel-capsule origin than in latex from other regions (Table 1). Latex from the pedicel-capsule region converted nearly twice as much dopa as did the latex from the lower pedicel position. These results were unexpected because there was considerably more protein in latex from the capsule than in latex from the pedicel-capsule position (Table 1). Latex from the three different regions differed in physical appearance, protein concentration, and dopamine biosynthetic activity.

TABLE 1. Latex from Different Regions of the Plant Analyzed for ^{14}C-dopa Conversion into Dopamine[a]

Plant part	Dpm	Conversion (%)	mg protein/ml diluted latex
Capsule	4,428	1.26	130
Pedicel-capsule junction	6,432	1.79	42
Lower pedicel	3,036	0.93	40

[a]The incubation mixture contained 1000 g $\times$ 30 min latex supernatant, 0.18 μCi ^{14}C-dopa (1.85 mCi/mmole), 0.1 M phosphate, 0.5 M mannitol, 20 mM ascorbic acid, 10 mM DIECA buffer, pH 7.2 to a total volume of 0.5 ml. Incubation was for 20 min at 35°. The values listed represent the average of two samples, and each sample was collected from 35-80 plants depending on the plant region.

The presence of labeled dopamine in the dopamine fraction collected from the CG-50 ion-exchange column was verified by two-dimensional pc. The Rf values (0.39 in the first dimension and 0.69 in the second dimension) and the purple color in response to $FeCl_3$-$K_3Fe(CN)_6$ spray were similar for both the sample and standard dopamine. The autoradiogram of the sample contained only one radioactive spot with the same Rf as standard dopamine in the two solvent systems. A 3-week exposure period was optimal for the chromatograms. Exposures for 2 and 6 weeks showed no other radioactive compounds present on the autoradiograms. The same results were obtained for the control autoradiograms, which showed no radioactive spot for dopamine. Counts in the dopamine control fraction were negligible (50-60 dpm).

DOPAMINE BIOSYNTHESIS IN LATEX COLLECTED FROM CAPSULES OF DIFFERENT AGES.—Latex was collected from the pedicel-capsule junction of floral buds several days prior to flowering, and subsequently 1-2, 5-6, and 13-14 days after flowering. From each age the latex of 40 plants set was pooled. Protein determinations showed the latex from these developmental stages contained approximately the same amount of protein, 25-30 mg protein/ml diluted latex (Table 2).

The conversion of ^{14}C-dopa ranged from 0.8 to 2.1% (Table 2). More incorporation of labeled dopa occurred in latex from capsules of the preflowering stage than in latex collected 1-14 days after flowering. Thus, dopamine biosynthetic activity in latex declined as the capsule matured. This result is in agreement with Roberts (5) who reported that latex before flowering contained higher levels of dopamine than latex after flowering. Because our results indicated that preflowering latex had higher dopamine biosynthetic activity, the dopamine levels reported by Roberts may be the result of

TABLE 2. Latex Analyzed at Different Stages of Capsule Development for ^{14}C-dopa Conversion into Dopamine[a]

Developmental stage	Dpm	Conversion (%)	mg protein/ml diluted latex
Before flowering	6,576	2.1	27
1-2 days after flowering	4,296	1.3	26
5-6 days after flowering	5,508	1.7	30
13-14 days after flowering	3,348	0.8	25

[a]The incubation mixture contained 1000 g × 30 min latex supernatant, 0.18 μCi ^{14}C-dopa (1.85 mCi/mmole), 0.1 M phosphate, 0.5 M mannitol, 20 mM ascorbic acid, 10 mM DIECA buffer, pH 7.2 to a total volume of 0.5 ml. Incubation was for 20 min at 35°. The values listed represent the average of two samples, and each sample was collected from the pedicel-capsule of 40 plants.

dopamine biosynthesis rather than increased dopamine translocation to the capsule before flowering or of a dilution of dopamine in latex after flowering when the capsules and its laticifers rapidly expand.

DOPAMINE BIOSYNTHESIS IN CELL-FREE EXTRACTS DERIVED FROM VARIOUS PLANT ORGANS.—Cell-free extracts were prepared from: leaves at the rosette stage of vegetative plants, leaves from flowering plants, capsules, pedicels, and capsules connected to pedicels. These extracts were incubated with ^{14}C-dopa followed by ion-exchange and paper chromatography. An autoradiogram of the two-dimensional paper chromatogram from the cell-free sample showed one radioactive spot with the same Rf values as standard dopamine. This radioactive spot turned purple when sprayed with the solution of $FeCl_3$ and $K_3Fe(CN)_6$, indicating a positive reaction for dopamine. The control, also analyzed by two-dimensional pc, contained no labeled dopamine and had negligible counts (around 60 dpm).

Dopamine biosynthesis varied in cell-free extracts derived from various organs and different stages of plant growth. Extracts from leaves of the rosette stage converted more L-dopa (0.95%) than did leaves of flowering plants (0.38%) (Table 3). Maximal conversion (1.90%) occurred in cell-free extracts from capsules.

This study has demonstrated that the plant organs examined (leaves, capsules, and pedicels) possessed dopamine biosynthetic activity. Leaves of young plants in the rosette stage converted dopa to dopamine. After the plants matured and flowered, the leaves progressively lost this ability, but the capsules began to synthesize dopamine.

TABLE 3. ^{14}C-Dopa Conversion into Dopamine for Cell-Free Extracts of Different Organs[a]

Origin of Cell-free Extract	Dopamine Fraction	
	Dpm	Conversion (%)
Capsules	20,412	1.90
Pedicels	5,244	0.58
Capsules connected to pedicels	17,136	1.48
Leaves rosette stage	8,748	0.95
Leaves flowering stage	4,442	0.38

[a]The cell-free system contained cell-free extract (2.5 ml), 0.5 μCi L-dopa (1.67 mCi/mmole), 10 mM DIECA, 20 mM ascorbic acid and 100 mM phosphate buffer, pH 7.2 to a total volume of 3.0 ml. Incubation was for 60 min at 35°. Each value listed in the table represents the average of two samples, and each sample consisted of 10-35 plant organs depending on the plant part.

Examination of isolated latex showed that the greatest dopamine synthesis occurred prior to flowering. The dopamine synthetic capacity of latex declined as the capsule matured to 14 days after flowering. The differences in dopamine biosynthesis for various tissues and for tissues of different ages emphasize the importance of relating biosynthetic reactions to plant development.

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