

Auxin Synthesis in Crown Gall Tumor Tissue: A Comparison of Three Putative Precursors

By

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Abstract

Axenic crown gall tumor callus (from *Vinca rosea* L.) which is known to synthesize its own auxin is able to convert exogenous ¹⁴C-indole or tryptamine to indoleacetic acid [5.4 and 10×10^{-6} $\mu\text{mol} \times \text{h}^{-1} \times (\text{g fr wt})^{-1}$ respectively], but little or no ³H-tryptophan is converted [less than 6.4×10^{-8} $\mu\text{mol} \times \text{h}^{-1} \times (\text{g fr wt})^{-1}$].

Introduction

Although indoleacetic acid (IAA) plays an important role in regulating plant development, and in spite of a large and impressive literature, its biosynthesis in higher plants is still uncertain. The main reason for this uncertainty is the instability of the indoles which are suspected to be precursors of IAA. Studies on cell-free systems are difficult to interpret, because many enzymes such as peroxidase (4), which may not normally be involved in IAA synthesis, can convert tryptophan and other indoles to IAA. In addition, some phenolic compounds present in plants can cause similar conversions (4, 6). Such artifactual reactions are less likely to occur in whole organs or whole cells; however, studies on whole cells or organs suffer from the possible influence of epiphytic bacteria, which are very difficult to exclude completely. Even small numbers of epiphytic bacteria can produce relatively large amounts of IAA, and this is especially significant when radioactive IAA precursors are used (2). Thus studies of auxin biosynthesis, particularly those employing radioactive tracers, have been clouded by uncertainty over what is artifact and what is not. In an effort to avoid as many known artifacts as possible, we have studied the ability of a crown gall tumor callus,

known to be able to synthesize its own auxin and to be axenic (1), to convert 3 putative auxin precursors to IAA.

Materials and Methods

Culture and incubations: *Vinca rosea* L. crown gall tumor callus tissue obtained from Dr. A. C. Braun (Rockefeller University, N.Y.) was grown under axenic conditions on a modified White's medium (8). Two pieces of the inocula weighing approximately 2 g each were cultured in darkness at 25°C for a period of one month or until each piece reached a fresh weight of 15–20 g. Thirty-five g of this material were then transferred to 50 ml of sterile distilled water containing tryptophan-UL-H³ (750 mCi/mmol), indole-2-¹⁴C (8.9 mCi/mmol) or tryptamine-2-¹⁴C (8.9 mCi/mmol). Radiochemical purity was checked by paper chromatography as described below. The tumor callus was incubated in a water bath at 25°C under constant gentle agitation for a period of 6 h.

Extractions: After incubation, the material was washed 3 times with 100 ml of distilled water; each time the cell-tissue suspension was centrifuged at about 3000 g for 5 min. The pellet was removed and ground with mortar and pestle plus a small amount of washed sand at 4°C in 15 ml of 0.1 N sodium phosphate buffer, pH 7.4, and 0.1 ml of mercaptoethanol. The homogenate was then centrifuged at 0–4°C for 10 min at 10,000 g. After collection of the supernatant, the pellet was resuspended 2 additional times in 15 ml of buffer and centrifuged as above. The 3 supernatants were combined, yielding a total of 65 ml. The final supernatant was extracted 3 times with 2 volumes of peroxide-free diethyl ether. The combined ether fractions, designated the neutral ether extract, were evaporated to dryness and redissolved in 2.0 ml of 80% ethanol. The aqueous phase was divided into 2 equal parts. One portion was acidified to pH 3.5 with 1 N HCl, and the other was

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adjusted to pH 8.5 with 1 N NaOH. Each portion was extracted as above with diethyl ether, and the ether phases were combined, evaporated to dryness, and redissolved in 2.0 ml of 80% ethanol, representing the final acidic and basic ether extracts respectively.

Partitioning tests using ^{14}C -IAA were performed to determine the efficiency of our technique for extracting and purifying IAA. Ninety-two per cent of the total counts appeared in the acidic ether extract, 4% in the neutral and 4% in the basic fraction. The amount of IAA present in the neutral and basic ether extracts from tissue homogenates was also checked by paper chromatography and found to be negligible.

Chromatography and measurement of the radioactive compounds: 100 μl of each final extract was spotted on Whatman no. 1 paper and chromatographed in descending fashion with ^{14}C -IAA, ^{14}C -indole, ^{14}C -tryptamine and ^3H -tryptophan run in parallel as standards. The solvent systems employed were isopropanol-ammonia-water at 20:1:3 v/v/v (IPA), and benzene-acetic acid-water at 2:1:1 v/v/v (BAA). After drying, the developed chromatograms were divided into 2-cm sections, placed in 10 ml of scintillation medium [5.0 g of 2,5-diphenyloxazole (PPO) and 0.30 g of 1,4-bis-2-(5-phenyloxazolyl) benzene (POPOP) per liter of toluene] and counted on a Beckman LS 100 at an efficiency of about 50% for ^{14}C and 5% for ^3H .

Although the figure and the table contain data from one typical determination, all experiments were done at least twice, and the results agreed to within 15% of the average.

Results and Discussion

Figure 1 shows the distribution of various radioactive compounds in the acidic ether extracts along descending chromatograms using IPA and BAA solvent systems. The total cpm in the IAA regions around R_f 0.29 in IPA and 0.68 in BAA were divided by the total counts in the centrifuged homogenate, yielding the percentage conversion to IAA (thereby correcting for uptake) given in Table 1. With ^3H -tryptophan, these were 0.17 and 0.16% or less for IPA and BAA respectively. Since there is no clear IAA peak in either chromatogram, there is a question whether or not any ^3H -labelled IAA is formed. Most of the ^3H -tryptophan in the supernatant is in the unbound form [soluble in cold 5% (w/w) trichloroacetic acid], thus it is not getting tied up in protein as quickly as it enters these cells. The chromatographic patterns of ^{14}C -labelled compounds in the acidic ether extract from tissue incubated with ^{14}C -indole were different from those observed for tryptophan. The conversion to IAA, calculated in the same manner as above, was considerably higher for indole, 0.67 and 0.65% with IPA and BAA solvents respectively. ^{14}C -tryptamine is metabolized by the crown gall tissue, producing chromatographic profiles similar to those from ^{14}C -indole (Figure 1).

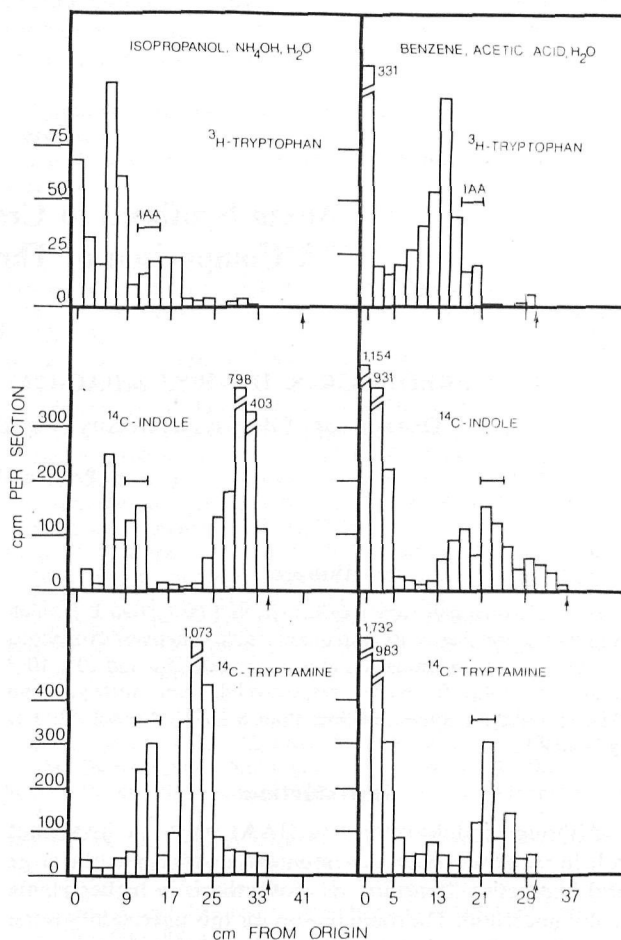


Figure 1. Chromatography of the acidic ether extracts obtained from *Vinca rosea* crown gall tumor tissue incubated with the radioactive precursor designated on each graph for 6 h. The horizontal bar on each chromatogram indicates the expected location of IAA. The chromatograms on the left were run in isopropanol, NH_4OH , H_2O (IPA) and on the right in benzene, acetic acid, H_2O (BAA). The arrows under each graph designate the solvent front.

^{14}C -IAA is formed; however, the conversion percentage (averaging 0.80%) is greater than observed for either tryptophan or indole. Thus both indole and tryptamine are clearly converted to IAA, but the conversion of tryptophan is uncertain. It is noteworthy that chromatograms developed in 2 different solvent systems show similar amounts of conversion.

The difference in the ability of crown gall tissue to convert the 3 exogenous radioactive precursors to IAA is most pronounced when the conversion rates are compared on a micromolar basis. The rates are 1.0×10^{-5} , 5.4×10^{-6} and less than $6.4 \times 10^{-8} \mu\text{mol} \times \text{h}^{-1} \times (\text{g fresh weight})^{-1}$ fresh weight for tryptamine, indole and tryptophan respectively (Table 1). Since these rates do not take into

Table 1. Quantitative comparison of the conversion of labelled precursors to indoleacetic acid. IPA and BAA chromatography solvents.

Precursor	Conversion to IAA, % of the radioactivity taken up		Rate of conversion to IAA, $\mu\text{mol} \times \text{h}^{-1} \times (\text{g fr. wt})^{-1}$
	IPA	BAA	
^3H -tryptophan	0.17	0.16	6.4×10^{-8}
^{14}C -indole	0.67	0.65	5.4×10^{-6}
^{14}C -tryptamine	0.91	0.69	1.0×10^{-5}

account the endogenous, non-radioactive precursors, these rates represent minimal rather than the absolute endogenous fluxes. Nonetheless, these results support the earlier conclusion of Winter (6) and Thimann and Grochowska (5) that tryptophan is normally not a direct precursor of IAA in plants, rather tryptamine and/or indole are direct precursors of IAA. In contrast, studies which show tryptophan conversion to IAA are too numerous to recount here [see (3) for a summary], but it is not possible to rule out conversion by small numbers of epiphytic bacteria or other artifacts mentioned above even though some precautions were taken.

Although the data reported here do not resolve the problem of how IAA is synthesized, they cast some doubt on tryptophan as a direct precursor of IAA, at least in this crown gall tissue. They favor tryptamine and indole, and they illustrate a system which could be very useful in elucidating the pathway of IAA synthesis in higher plants.

This represents part of a project which was discontinued in this laboratory in May 1971.

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