Indole-3-acetic Acid Biosynthesis
in Cultured Crown Gall Cells

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(Received October 1, 1986)

Abstract
Metabolism of tryptophan and tryptamine and biosynthesis of indole-3-acetic acid (IAA) in cultured sunflower crown gall cells (strain name CG) induced by the Agrobacterium tumefaciens ATCC 4452 were examined. Either from tryptophan (Trp) or tryptamine (TNH2) the largest accumulations of indole-3-pyruvic acid (IPyrA) were detected among seven indolic derivatives studied. CG cells also converted Trp and TNH2 into indole-3-acetamide (IAAmd), but the conversion from TNH2 was more efficient than that from Trp. On the other hand, Trp and TNH2 were found to be converted to each other. Results presented in this paper suggests the probability that the CG cells possess two pathways to biosynthesize IAA from Trp, that is, the one is the natural pathway in plants from Trp via IPyrA and IAAld, and the other is from Trp or via IAAmd. And TNH2 was able to be a precursor of IAA, after a conversion to Trp.

Introduction
Crown gall tumors which are induced by Agrobacterium tumefaciens or genetic tumors of tumor-prone hybrids have been believed to produce IAA in the tumor tissues and the autonomous synthesis of IAA also has been believed to allow the tumor tissues to proliferate without limitation (Wood, 1972). In fact, crown gall tissues contained a large amount of IAA even when they were cultured in aseptic conditions (Atsumi and Hayashi, 1978, Henderson and Bonner, 1952, Kulescha and Gautheret, 1948). Although many reports have been published on productions of IAA in the plant tissues (Schneider and Wightman, 1974, Truelsen, 1973, Gibson et al, 1972, Sherwin and Purves, 1969, etc), there are a few studies on the production of IAA in plant tumor tissues (Rausch et al, 1985, Liu et al, 1978, Cheng, 1972, Henderson and Bonner, 1952, Kulescha and Gautheret, 1948). And in the recent two of them tumor-prone hybrid tissues were reported to produce IAA from 14C-3-Trp, and the rate of conversion was also reported to reach at a high rate of 35%. Although the IAA in the tumor-prone hybrids was reported to be synthesized from Trp via IPyrA, that of a crown gall induced by A. tumefaciens has not yet re-

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ported.

On the induction of crown gall tumors it has been revealed that transfer and maintenance of peculiar tumorigenic portions of a large T-plasmid from A. tumefaciens to plant cells is the essential of the crown gall disease (Schilperoort et al., 1978). And it is probable that the transferred regions of T-plasmid (T-DNA) carries the genetic information on the IAA biosynthesis in crown gall cells. Recently Liu and Kado (1979) reported that A. tumefaciens C58 could produce IAA from Trp in the presence of virulent T-plasmid. Therefore, they concluded that IAA production was regulated by the same plasmid which was attributed to the crown gall disease. The T-DNA, however, was reported to carry codes for tryptophan 2-monoxygenase into tobacco crown gall cells (Van Onckel et al., 1986). This means that the biosynthetic pathway of IAA coded by the transferred T-DNA is from Trp via IAAmd.

In higher plants IAA is believed to be synthesized from Trp via IPyrA and IAAld. And the pathway from Trp via TNH2 and IAAld is one byways. Also IAAmd was regarded not to be intermediate in the IAA biosynthesis in plants naturally (Sembdner et al., 1980), but it was found not to be harmful to CG cell (Atsumi, 1980). It is interesting to understand the synthesis of IAA in crown gall cells maintained in culture for a long period.

In this paper, IAA production from Trp and TNH2 was examined in cultured crown gall cells derived from a malignant sunflower tumor tissue. And also six indole derivatives related Trp and TNH2 were evaluated as intermediary metabolites in the biosynthesis of IAA.

**Materials and Methods**

Cultured crown gall cells (strain CG) was derived from a crown gall tissue induced on a stem of sunflower (Helianthus annuus cv. Giant Russian) by infecting A. tumefaciens ATCC 4452 at in 1960. Hence they have been cultured without auxin and cytokinin. CG cells were suspension-cultured in 150 ml of a liquid Murashige-Skoog medium without auxin and cytokinin in 500ml Sakaguchi flasks on a reciprocal shaker (85 rpm) at 26 ± 1°C in the dark. The cells were transferred every 7 days with 30 ml of inoculum to 120 ml of fresh culture medium, after adjusting the cell density to give an initial cell density of 1 ml pcv per 10 ml of cultures. Growth of cultures was monitored by measuring pcv in milliliter cells per 10 ml of cultures, after centrifugation at 1500rpm (3400g) for 15 minutes and weighing fresh weight in gram of cells per 10 ml of culture. The cells proliferated till the day 15 or 16 logarithmically, and then stopped their cell divisions and the culture entered into the stationary stage.

As precursors of IAA DL-(3-14C)-Trp (New England Nuclear, 1.78 GBq per mmol) or (2-14C)-TNH2 (New England Nuclear, 1.91 MBq per mmol) was aseptically added to 12-day-old cultures, which were in the late log stage.
As precursors of IAA \textit{dL-3-}^{14}\text{C-TNH}_2 were ca. 2 \mu Ci \left(7.4 \times 10^4 \text{ Bq}\right) and ca. 1 \mu Ci \left(3.7 \times 10^4 \text{ Bq}\right) for 25 g fresh weight cells, respectively. After incubating for a few hours or one day, the cultures were filtrated by suction. Cells collected on a filter-paper were rinsed with distilled water and excess water was removed by suction. Then the cells were subjected to extraction. Collected cells were suspended in 3 times of volumes of cold ethanol and divided into nine portions. One portion was used to determine the total radioactivities uptaken by the cells and the rests were used to determine the amounts of radioactivities incorporated into the seven indole derivatives, and TNH_2 when \textit{^{14}C-Trp} was added as a precursor to the culture or Trp when \textit{^{14}C-TNH}_2 was used. After homogenizing with a blender, each cell suspension was further ground with a mortar and a pestle to determine the total incorporation of the labeled compounds by the cells. Radioactivities in 1 ml of the homogenate mixed with 20 ml of a dioxane scintillation cocktail were measured with a scintillation counter (LS-9000, Beckman). To extract indole derivatives metabolized from \textit{^{14}C-Trp} or \textit{^{14}C-TNH}_2, on the other hand, ca. 25 mg of one of the nine authentic indole derivatives as a carrier was added to the cell suspension prior to homogenization. After homogenizing the suspension as mentioned above, the homogenate was filtered by suction and residues were re-extracted with 80% ethanol further 3 times. Combined extracts were concentrated to eliminate ethanol in vacuo. Obtained aqueous extract was subjected to further purification described below.

\textit{Extraction of acidic and neutral indole derivatives.} The aqueous extracts were acidified with dil H_2SO_4 (ca. 3.6 n) and extracted two times with an equal volume of ethyl acetate. Ethyl acetate fractions were combined and after dehydration with anhydrous sodium sulfate washed twice with a half volume of NaHCO_3 saturated solution. Neutral indole derivatives, that is, IAAld, IAAMD, indoleacetaldoxime (IAAox), indoleacetonitril (IAN) and tryptophol (TOH) remained in the ethyl acetate phase were obtained after dehydration as mentioned before (Fig. 1). The aqueous phases were then acidified with dil \textit{H}_2\text{SO}_4 and extracted twice with a half volume of ethyl acetate. In combined ethyl acetate phases acidic indole derivatives were found (Fig. 1). Extracted acidic and neutral indole derivatives were subjected to purification with thin layer chromatography (TLC), and then some of them were further purified by recrystallization. Trp and TNH_2 were remained in the aqueous extracts, even when the alkaliﬁed aqueous extracts were shaken with ethyl acetate.

\textit{Extraction of tryptophan and tryptamine.} The aqueous extracts were applied to a column of Dowex 1 \times 4 (50 - 100 mesh, 3 ml, OAc"-form), and then the column was eluted with 100 ml of distilled water. As the absorption of Trp and TNH_2 in the Dowex resin were extremely weak, they were found in the elutes. The elutes were then applied to a column of Amberlite IRC-50 (6 ml, H"-form),
**Fig. 1.** The procedure for the extraction of acidic, neutral and basic indole derivatives from cultured crown gall cells. A table under the figure represents extraction efficiencies for nine indole derivatives. A part of IAAmd is extracted in the acidic and basic fractions, but the main portions are extracted in the neutral fraction. Trp and TNH$_2$ are remained in the aqueous fraction throughout the procedure. In the table, (−) indicates the absence of chemicals in the corresponding fractions, (+) indicates a trace of chemicals is detected, (+++) indicates the almost portions presented in the corresponding fractions, and (+) and (++) indicate not-negligible portions of chemicals are detected.

**Fig. 2.** The procedure for extraction and separation of tryptophan and tryptamine with ion exchange resin columns. Dowex 1 × 4 (HCOO$^-$-form) column scarcely adsorbs Trp and TNH$_2$, but Amberlite IRC-50 (H$^+$-form) and IR-120B (H$^+$-form) columns adsorb TNH$_2$ and Trp, respectively.
and the column was washed with 100ml of distilled water. TNH₂ was absorbed in the column and Trp was found in the water elutes. Absorbed TNH₂ was eluted with 80ml of 4N acetic acid. On the other hand, water elutes from IRC-50 column were further applied to a column of Amberlite IR-120B (3ml, H⁺-form). After washing the column with 200ml of distilled water, absorbed Trp was eluted with 100ml of 2N NH₄OH. Each elute containing Trp or TNH₂ was concentrated to dryness in vacuo (Fig. 2).

TNH₂ was recrystallized from a solution in 2N HCl, and Trp was recrystallized from an ethanol solution. After washed with chloroform, each of them was further purified with cellulose-TLC, and then recrystallized.

**Conditions of TLC to isolate indole derivatives, and tryptophan and tryptamine.** G type silica gel thin layer (PK6F, Whatmann Inc.) and micro crystalline cellulose thin layer (Avicel, Asahi-Kasei Co.) were used. More than eighty combinations of above two types of thin layer and solvent systems were examined and five of them were used in this experience (Table 1). Separation of the two acidic indole derivatives, that is IAA and IPyrA, were carried out with two combinations. IAA moved on the PK6F plate with a

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>Acidic</th>
<th>Neutral</th>
<th>Basic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IAA</td>
<td>IPyrA</td>
<td>IAAId</td>
</tr>
<tr>
<td>G type silica gel (PK6F)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylacetate-Ethanol 95:5</td>
<td>0.47</td>
<td>&lt;0.20</td>
<td>0.76</td>
</tr>
<tr>
<td>Chloroform-Ethanol 8:2</td>
<td>0.37</td>
<td>&lt;0.07</td>
<td>0.80</td>
</tr>
<tr>
<td>Dichloromethane (multiple)</td>
<td>NM</td>
<td>NM</td>
<td>0.33</td>
</tr>
</tbody>
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Micro crystalline cellulose (Avicel)

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<th>SYSTEM</th>
<th>Acidic</th>
<th>Neutral</th>
<th>Basic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene-Acetic acid-Water 8:3:5 (lower layer)</td>
<td>0.78</td>
<td>0.37</td>
<td>0.56</td>
</tr>
<tr>
<td>n-Butanol-Acetic acid-Water 63:10:27</td>
<td>0.88</td>
<td>0.77</td>
<td>0.87</td>
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<th>Neutral</th>
<th>Basic</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Butanol-Acetic acid-Water 63:10:27</td>
<td>0.90</td>
<td>0.84</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Table 1. Chromatographic separation of nine indole derivatives including tryptophan and tryptamine. In this table six systems with G type silicagel plates, micro crystalline cellulose plates and filterpapers are shown with Rf values. NM in the table indicates the corresponding chemical did not move from the streaked line and a symbol "<" means the Rf value are less than the indication.
solvent system of ethylacetate-ethanol (95:5, Rf value 0.47), but not IPyrA. IPyrA, on the other hand, moved slightly on the Avicel plate with a solvent system of benzeneacetic acid-ethanol (lower layer of 8:3:5 mixture, Rf value 0.37), but IAA advanced rapidly (Rf value 0.78).

Separation of the five neutral indole derivatives were carried out on the PK6F plates. IAAmd and TOH could be separable with the solvent system of ethylacetate-ethanol (95:5) from the other three. The Rf values of IAAmd and TOH were 0.30 and 0.63, respectively. With a solvent system of chloroform-ethanol (8:2), IAAox was separable from the others. The Rf value of IAAox was 0.71, and distinct from IAAld, IAAmd, IAN and TOH, of which Rf values were 0.80, 0.46, 0.78 and 0.64, respectively. IAAld and IAN were separated with multiple ascending development with a simple solvent system of dichloromethane, and their Rf values were 0.33 and 0.41, respectively.

Purification of Trp and TNH2 was carried out on the Avicel plates with a solvent system of n-butanol-acetic acid-water (63:10:27). The Rf values of Trp and TNH2 were 0.16 and 0.37, respectively.

Measurement of radioactivity. After purification by TLC, isolated indole derivatives except IAAld and IAN were recrystallized. Small crystals were collected with needle points and weighed to get efficiency of their recovery. As IAAld and IAN could not be recrystallized by ordinary procedures, they were re-chromatographed in the same manner as the first development. Extracted IAAld or IAN from their chromatograms was weighed after absorption to glass fiber filters (type GF/F, Whatmann Inc.). After mixing ethanol solutions of recrystallized indole derivatives with a toluen scintillator or dipping glass fiber filters absorbed IAAld or IAN in the scintillator, radioactivities of each indole derivatives were measured with a liquid scintillation counter. Counts of the radioactivities were processed by the data reduction system of the LS-9000 and the corrected counts in dpm were obtained.

Chemicals. IAAld was liberated from an alkalined solution (pH 8.0 with NaHCO3) of IAAld-Na bisulphite (Sigma Chemical Co., USA) by extraction with CH2Cl2. TOH, IAAmd, IPyrA and IAN were purchased from Sigma Chemical Co., IAA and Trp were from Wako Pure Chemical Industries, Ltd. and TNH2 was from Nakarai Chemicals, Ltd. Synthesis of IAAox from IAAld was described before (Atsumi, 1980).

Results

Radioactivities of 14C-Trp incorporated in the CG cell were 9.9×104 dpm per gram cells during a incubation period of 24 hours and those of 14C-TNH2 were 12×104 dpm per gram cells (Table 2). There were not observed any significant differences between the incorporations of 14C-Trp and 14C-TNH2. Practically radioactivities of Trp incorporated, however, were nearly a half of that of 14C-TNH2, because a half of 14C-Trp was expected to be D-tryptophan. Al-
though the incubation periods had little influence for the incorporation of the radioactivities, since the incorporated radioactivities for a short period of five hours were more than one third of those during 27 hours, the incorporations of $^{14}$C-TNH$_2$ were more efficient than $^{14}$C-Trp in any cases (Table 3).

Authentic indole derivatives added as carriers to the cell extracts were recovered generally in efficiency ranging between ten and thirty percent of the amounts of their annexing. However, the extraction of TOH from cells incubated with $^{14}$C-Trp for 24 hours gave an efficiency less than ten percent, and that of IAAox with $^{14}$C-TNH$_2$ gave an efficiency reaching to 40 percent (Table 2). In any cases the losses of each substances might be caused by decompositions during the development of TLC or arise from the recrystallization procedures and other extraction procedures.

After incubation for 24 hours, IAA, IPyrA, IAAld, IAAmd, IAAox, TOH and TNH$_2$ were detected as metabolites of $^{14}$C-Trp. Among them IPyrA was the major metabolite and IAA, IAAox and TOH were the secondary in the amount of radioactivities. Although IPyrA was also the major metabolite of $^{14}$C-TNH$_2$, IAAmd was the next metabolite to IPyrA in amounts of radioactivities, and the radioactivities of the recovered IAAmd were nine times as much as that from $^{14}$C-Trp and one-third of IPyrA from $^{14}$C-TNH$_2$.

Table 2. The conversions from DL-(3-$^{14}$C)-tryptophan and (2-$^{14}$C)-tryptamine in cultured crown gall cells. 25 grams of CG cells were incubated for 24 hours with 2 $\mu$Ci of $^{14}$C-Trp or 1 $\mu$Ci of $^{14}$C-TNH$_2$. After incubation, cells were extracted and metabolites were purified with the corresponding authentic cold carriers. The recovered carrier were weighed to estimate the recovery in percentage to initial amounts of the carriers. A symbol ND indicates that any radioactivities of the corresponding substances were not detected.

<table>
<thead>
<tr>
<th>Total uptake</th>
<th>Acidic</th>
<th>Neutral</th>
<th>Basic</th>
</tr>
</thead>
<tbody>
<tr>
<td>(dpm/g cell)</td>
<td>IAA</td>
<td>IPyrA</td>
<td>IAAld</td>
</tr>
<tr>
<td>$^{14}$C-Trp</td>
<td>9.9 $\times 10^4$</td>
<td>19.9</td>
<td>17.7</td>
</tr>
<tr>
<td>% Recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>290</td>
<td>1570</td>
<td>97</td>
</tr>
<tr>
<td>(dpm/g cell)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C-TNH$_2$</td>
<td>12 $\times 10^4$</td>
<td>26.3</td>
<td>11.3</td>
</tr>
<tr>
<td>% Recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>420</td>
<td>1860</td>
<td>140</td>
</tr>
</tbody>
</table>
The third metabolite from $^{14}$C-TNH$_2$ was IAA on the incorporation of radioactivities. On the other hand, radioactivities incorporated into TOH from $^{14}$C-Trp was three times as much as that from $^{14}$C-TNH$_2$. CG cells formed TNH$_2$ from $^{14}$C-Trp, and also Trp from $^{14}$C-TNH$_2$. But the incorporation into IAN of radioactivities was not detected from both $^{14}$C-Trp and $^{14}$C-TNH$_2$. Conversions to IAA and IPyRA occurred from either precursors (Table 2).

In a case of a short incubation period of five hours, incorporations of radioactivities into IPyRA occurred more efficiently than those of a long period of 27 hours from each of the two precursors. Furthermore the incorporations of the precursors in the shorter incubation period were almost one-third of those in the longer incubation period as mentioned above (Table 3).

Table 3. The comparison between the two incubation periods in the conversion of $^\text{DL}$-($3$-$^{14}$C)-Trp and ($2$-$^{14}$C)-TNH$_2$. Incorporated radioactivities were represented with the recovery of the authentic cold carrier added just before the extraction.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Incubation Period</th>
<th>Total uptake (dpm/g cell)</th>
<th>Activity [dpm/g cell]</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}$C-Trp</td>
<td>27</td>
<td>$10.1 \times 10^4$</td>
<td>1690 / 10.8</td>
<td>124 / 30.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>$3.9 \times 10^4$</td>
<td>4870 / 8.7</td>
<td>71 / 19.6</td>
</tr>
<tr>
<td>$^{14}$C-TNH$_2$</td>
<td>27</td>
<td>$18.8 \times 10^4$</td>
<td>3380 / 5.0</td>
<td>677 / 4.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>$6.4 \times 10^4$</td>
<td>5430 / 12.2</td>
<td>155 / 17.7</td>
</tr>
</tbody>
</table>

**Discussions**

Transformation of plant cells with infection of A. *tumefaciens* causes 'crown gall disease' with a formation of tumors which grow in aseptic culture without auxin and cytokinin. This phenomenon is the result of the transfer, stable integration and expression of the T-DNA into plant nuclear DNA (Gheysen et al, 1985). And T-DNA was also reported to bear the auxin biosynthesizing genes ($tms$) (Black et al, 1986). Gene $tms$-2 (or iaaH) of T-DNA, was shown to code for an indoleacetamide hydrolase which converted IAAm into IAA (Schröder et al, 1984, Thomashow et al, 1984) and gene $tms$-1 (or iaaM), was shown to code for tryptophan 2-monooxygenase which converted Trp into IAAm (Van Onckelen et al, 1985 and 1986). Therefore these enzymes can be expected to catalyze the conversion of Trp to IAA via
IAAm. However, it is doubtful whether IAAm occurs naturally in plants, because indolepyruvic acid pathway, which is from Trp to IAA via IPyRA, is generally believed to be the predominant route of IAA biosynthesis in higher plants (Sembdner et al., 1980).

In this work, CG cells derived from *H. annuus* were also found to have the activities to convert Trp to IAAm, in spite of the maintenance in vitro culture for a long years. As well as Trp, however, IAAm was formed from TNH₂ in CG cells. This result requires a conversion of TNH₂ to Trp, or a direct conversion of TNH₂ to IAAm. But the occurrence of IAAm from TNH₂ was more efficiently than that from Trp, although IAA was formed from both Trp and TNH₂ in almost the same efficiency. This difference in the efficiency is seemed to suggest that the occurrence of IAAm from ¹⁴C-TNH₂ was not through Trp from TNH₂ because of the insignificant conversions to Trp from TNH₂ in a short or a long incubation periods. However, there is no evidence which provide the existence of enzymes catalyzing the direct conversion of TNH₂ to IAAm.

On the other hand, the accumulation of a large amount of radioactivities into IPyRA from TNH₂ requires the efficient conversion of TNH₂ to Typ and the turnover in Trp pools to be at a high rate. The apparent efficiency of the formation of IPyRA from TNH₂ was higher than that from Trp (Table 2 and 3). There can be two interpretations of this phenomenon. The one is that ¹⁴C-Trp used in this work was a mixture of D- and L-trypophan. Being the practical uptake of l-(¹⁴C)-Trp to be a half of the measured radioactivities, the efficiency of the conversion to IAA or IPyRA from Trp was higher than that from TNH₂. And the other is that l-¹⁴C-Trp converted from ¹⁴C-TNH₂ was supplied to Trp pools at a low but steady rate. Biosynthesis of Trp has well been known as a typical example of negative feed back control systems, and the size of Trp pools in cells are expected to be small. Under such situation, exogenously applied Trp should be consumed rapidly, and the most of the incorporated labels of ¹⁴C-Trp appeared in its metabolites during initial periods of the incubation, and disappeared or decreased in the following incubation (Table 3). On the other hand, if labelled Trp has been steady and efficiently supplied from ¹⁴C-TNH₂, the specific radioactivities of Trp pools might increase as the results of the decrease of biosynthesis of Trp by the negative feed back control. Finally, increased labels from ¹⁴C-TNH₂ are observed to be incorporated in the metabolites of Trp.

In any case, without an assumption that the incorporated ¹⁴C-TNH₂ in CG cells could not be converted into L-Trp efficiently, it might arouse a deep antagonism between the results that among the seven indolic metabolites of TNH₂ CG cells accumulated the largest and the second largest amounts of radioactivities in fractions of IPyRA and IAAm, respectively.

Previously the author reported that one of auxin-heterotrophic mutants derived
from CG cells were arrested its auxin biosynthetic pathway between IPyrA and IAALd, and could not utilize IPyrA as a precursor of IAA. But the mutant cells could grow with excess amounts of Trp, TNH₂ or IAAMd supplemented exogenously (Atsumi, 1980). These observations suggest CG cells or their mutant cells really possess the activities of tryptophan decarboxylases and tryptamine oxidases to produce IAALd, which could be converted into IAA by aldehyde dehydrogenases (or aldehyde oxidases). And also it might be sure that CG cells and their mutants could catalize the hydration of IAAMd and produce IAA from it. However, these enzyme systems were not seemed to operate precisely in CG cells and the mutants cells, because without an enough supplement of one of Trp, TNH₂ and IAAMd the mutant cells could not grow.

In this work, it was ensured that CG cells catalize the transamination, decarboxylation and 2-monooxygeation of Trp. CG cells might biosynthesize IAA predominantly from Trp via IPyrA and IAALd, because IPyrA was produced superiorly among the indole derivatives examined, and because the mutants were defective in tryptophan transaminase. Tryptophan 2-monooxygease and tryptamine hydrolase coded by T-DNA might operated more than fifteen years ago when CG cells were initiated. However, the natural biosynthetic pathway of IAA via IPyrA and IAALd has took up its old position in IAA biosynthesis during the following period of culture in vitro.

Acknowledgement

The author makes a greatful acknowledgement for Prof. T. Hayashi (Univ. Ibaragi) to allow an opportunity to study on this theme. The author also wishes to thank Prof. T. Yamada (Hyogo Univ. Teacher Education) for a critical discussion on this work.

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Indole-3-acetic acid biosynthesis in cultured crown gall cells


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培養クラウンゴール細胞における
インドール-3-酢酸の生合成の研究

渥 美 茂 明

邦文要旨
Agrobacterium tumefaciens (ATCC 4452) を接種して得たクラウンゴールに由来する培養細胞（株名: CG）におけるトリプトファン（Trp）とトリプタミン（TNH₂）の代謝と，インドール-3-酢酸の生合成を調べる実験を行った。Trp，TNH₂のいずれからもインドール-3-ピルビン酸（IPyrA）が最も多量に生成した。またインドール-3-アセトアミド（IAAmd）がTrpと，TNH₂からも生成したが，TNH₂からの生成量はTrpからのものに比べ約9倍も多量であった。これらの結果から，CG株は通常の植物が持つTrpからIPyrAを経るIAAの生合成経路を持つほかに，腫瘍化DNA（T-DNA）に由来するTrpからIAAmdを経てIAAを生成する経路を有することが示された。