Oxygen-Dependent Catabolism of Indole-3-Acetic Acid in
Bradyrhizobium japonicum

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Some strains of Bradyrhizobium japonicum have the ability to catabolize indole-3-acetic acid (IAA). Examination of this catabolism in strain 110 by in vivo experiments has revealed an enzymatic activity catalyzing the degradation of IAA and 5-hydroxy-indole-3-acetic acid. The activity requires addition of the substrates for induction and is oxygen dependent. The highest activity is obtained when the concentration of inducer is 0.2 mM. Spectrophotometric data are consistent with the suggestion that the indole ring is broken during degradation of IAA. We hypothesize that the enzyme catalyzes an oxygen-consuming opening of the indole ring analogous to the one catalyzed by tryptophan 2,3-dioxygenase. The pattern of metabolite usage by known tryptophan-auxotrophic mutants and studies of metabolites by high-performance liquid chromatography indicate that anthranilic acid is a terminal degradation product in the proposed pathway.

Infection of the soybean plant (Glycine max) with Bradyrhizobium japonicum results in the development of root nodules. It is assumed that plant hormones are involved in the development and/or maintenance of such nodules. It has been demonstrated that the root nodules of many legumes contain higher levels of auxin than do the roots (22) and that the main auxin in root nodules is indole-3-acetic acid (IAA) (5). IAA present in the nodule could be of plant origin, as plant cells in the root nodules have the enzymatic capacity to synthesize IAA from tryptophan (5), and tracer studies have shown that IAA applied to the apical bud of an intact pea plant is transported to the root nodules (1).

Both rhizobia and bradyrhizobia have the enzymatic capacity to produce IAA in culture. A mutant of B. japonicum has been isolated that accumulates IAA in culture and induces nodules that contain higher IAA levels than do nodules induced by the parent strain (10, 11). This indicates that nodule IAA can also be of bacterial origin.

When considering the catabolism of IAA, the following well-known chemical reactions of indoles and 3-substituted indoles have to be considered: (i) oxidations that alter the side chain, (ii) oxidations resulting in production of indole, dioxindole, or dioxindole compounds, (iii) opening of the pyrrole ring and further reactions, and (iv) conjugation with, for example, amino acids, carbohydrates, or other small molecules. Reactions of types i, ii, and iv have been characterized in bacteria (6, 12, 15), plants (13, 17, 18, 20, 21), and plant tissues (2, 14). Reactions of type iii, opening of the pyrrole ring and further reactions, have only been described for bacteria, e.g., Arthrobacter spp. (15), Pseudomonas spp. (19), and “a micro-organism isolated from air” (23).

We have examined the potential among strains of B. japonicum for synthesis and breakdown of IAA and have found that these strains possess, in various combinations, enzymatic activities involved in IAA metabolism; namely, enzymes catalyzing IAA formation from indole-acetideme, indole-acetamide, and indole-acetonitrile and an enzymatic activity catalyzing an oxygen-dependent degradation of IAA (16). This paper reports the results of the examination of the latter activity in strain 110 of B. japonicum.

Strains and media. All strains used in this study are presented in Table 1. Tryptophan-auxotrophic mutants were grown on minimal medium agar plates (3) (with the exclusion of vitamins and with arabinose as the carbon source and L-glutamate as the nitrogen source) supplemented with tryptophan or alternative substrates (100 μg mL⁻¹) at 28°C. Other strains were grown in yeast broth medium (4) on a rotary shaker with gentle shaking (100 rpm) at 28°C until turbidity reached an optical density at 450 nm of 0.35 to 0.45 (3 to 5 days).

Preparing cultures for in vivo experiments. We added IAA at a final concentration of 0.2 mM to a stationary liquid culture overnight to induce IAA catabolizing enzymes. The following day the culture was centrifuged at 20,000 × g for 10 min at 4°C. The pellet was washed twice in 20 mM potassium phosphate buffer, pH 7.1, suspended and centrifuged in 2/3 the initial volume, and then suspended in 1/10 the initial volume.

Detection of IAA in cultures. The disappearance of IAA in culture supernatants was determined colorimetrically. Samples (0.3 ml) were reacted with FeCl₃-HClO₄ reagent (8), and optical density at 530 nm was measured. The specific activity of the enzyme can be expressed as ΔE₅₃₀ hour⁻¹. E₅₃₀⁻¹. 10⁻¹ on the linear (initial) part of the curves, where E₅₃₀ represents the density of the parent culture and 10 is the factor by which the sample culture is concentrated.

Induction studies. To investigate the induction of IAA-catabolizing activity, stationary liquid cultures of strain 110 were amended with one of the following 15 indole compounds (of analytical grade): indole, indole-carboxylic acid, indole-aldehyde, indole-acetamide, indole-acetonitrile, indole-acetaldehyde, indole-ethanol, tryptamine, indole-propanoic acid, indole-butanoic acid, indole-pyruvic acid, indole-lactic acid, tryptophan, 5-hydroxy-indole-3-acetic acid (5-OH-IAA), and IAA. The indole compounds were added to final concentrations of 0.2 mM, and the cultures were then incubated overnight.
TABLE 1. B. japonicum strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>110</td>
<td>spc-4</td>
<td>9</td>
</tr>
<tr>
<td>TA 1</td>
<td>azi rif str trpB</td>
<td>24</td>
</tr>
<tr>
<td>TA 3</td>
<td>azi rif str trpE</td>
<td>24</td>
</tr>
<tr>
<td>TA 5</td>
<td>azi rif str trpD</td>
<td>24</td>
</tr>
<tr>
<td>TA 11</td>
<td>azi rif str trpC</td>
<td>24</td>
</tr>
</tbody>
</table>

* The trp genes listed code for the following enzyme activities: trpB, tryptophan synthetase B; trpE, anthranilate synthetase; trpD, anthranilate-5-phosphoribosyl pyrophosphate phosphoribosyl transferase; trpC, indole glycerol phosphate synthase.

The following day, the cultures were prepared for in vivo experiments and IAA was added as a substrate to a concentration of 10.2 mM. At different times, samples were taken and assayed for the disappearance of IAA. From these experiments we have demonstrated that only IAA and 5'-OH-IAA are successful in inducing IAA degradation activity. To investigate whether the concentration of inducer has any influence on IAA-catabolizing activity, a culture was divided and induced overnight with different concentrations of IAA in each of the resulting cultures. From these experiments, we clearly demonstrated that the highest specific activity was obtained at 0.2 mM IAA (Fig. 1).

Substrate studies. The same 15 indole compounds were tested as substrates for the IAA degradation activity by adding one of the compounds at a final concentration of 0.2 mM to an IAA-induced culture. As the colorimetric method could not be applied for detection of all the compounds, the absorption changes of the culture supernatants were monitored by scanning from 200 to 450 nm in a Unicam SP 800B spectrophotometer. Changes in absorption patterns were found only in cultures with IAA or 5'-OH-IAA added, indicating that these two compounds are the only substrates for the activity. The characteristic spectrum from an indole ring, with a shoulder at 288 nm (25), disappeared in the IAA culture, indicating that the indole structure is broken in the degradative process. Oxygen dependence studies. We demonstrated that the metabolism of IAA is oxygen dependent by in vivo experiments with cultures induced by IAA and then incubated in a normal atmosphere or in an N₂ atmosphere. IAA disappeared only from the normal-atmosphere culture. Metabolic studies. The oxygen dependence and the disappearance of the characteristic indole absorption spectrum when IAA is degraded have led us to suggest that IAA is

![FIG. 1. Specific activity of the IAA-degrading activity in relation to concentration of inducer (IAA). Data are means of double measurements in two independent experiments.](http://jb.asm.org/)

![FIG. 2. Elution profile from culture supernatant of strain 110. Peak 2.43 originates from the phosphate buffer, and peak 8.51 is IAA. The other peaks, all more polar than IAA, arise from IAA. Peak 6.62 has retention time similar to that of anthranilic acid. Detection wavelength was 260 nm.](http://jb.asm.org/)
catabolized by an oxidative opening of the pyrrole ring in a reaction analogous to the one catalyzed by tryptophan 2,3-dioxygenase (7). The terminal degradation product could then be anthranilic acid. To examine this, we used tryptophan-auxotrophic mutants of *B. japonicum* (24). If IAA is metabolized to anthranilic acid, one would expect that strain TA 3 would grow when the growth medium was supplied with IAA instead of tryptophan, while the other mutants would fail to do so (Table 2).

We also studied the metabolism of IAA in *B. japonicum* by the use of reverse-phase high performance liquid chromatography (HPLC). The equipment for these analyses was obtained from Spectra-Physics and consisted of an SP 8800 ternary pump with solvent programming and an SP 4290 integrator with recorder. Samples were introduced via a sample injector from Rheodyne with a 50-μl sample loop. In addition, a wavelength detector from Applied Biosystems was attached to the system. The assay was performed on a 20-cm Spherisorb ODS1 C18 column (particle size, 5 μm) with an isocratic run of 40% methanol (Licrosolv, gradient grade; Merck)-0.5% acetic acid (analytical grade). The solvent was continuously ventilated with helium and flowed at 1 ml·min⁻¹. The run time was 10 min. Pressure was about 2,150 psi.

Cultures of strain 110 were induced overnight. The following day, the cultures were prepared for in vivo experiments, and IAA was added as a substrate at a final concentration of 0.2 mM. At different times, 100-μl samples of the culture supernatants were centrifuged through a 0.22-μm Durapore filter (Millipore) and then analyzed by HPLC. Elution profiles of culture supernatants (Fig. 2) show the appearance of five peaks (apart from the peaks originating from IAA and from buffer), representing compounds more polar than IAA. A control culture of strain 110 without the addition of IAA did not accumulate any detectable compounds, indicating that the peaks arise from metabolism of IAA.

By collection and rechromatography of peak 1, we detected the appearance of a new peak identical to that of anthranilic acid in both retention time and spectrum (Fig. 3). No other peaks changed retention time upon rechromatography.

**Discussion.** Our experiments have revealed the existence of an IAA degradation pathway in *B. japonicum* 110. The catabolic activity is inducible by IAA or 5′OH-IAA, with...
Since we see as many as five intermediates by means of HPLC, it is likely that IAA is initially metabolized by modifications of the side chain or by oxidations resulting in the production of oxindole or dioxindole compounds. However, we have no evidence for this, and we do not know whether some of the IAA is conjugated. Unfortunately, we see no IAA disappearance in cell extracts, which makes it impossible to design any in vitro assays for detection of the enzymatic activity.

In our future work, we will try to purify and identify metabolic intermediates in this degenerative process. The accumulations of the intermediates are very minor, so we hope to create new bacterial strains mutated in genes coding for the different enzymes involved in the catabolism of IAA. The mutants would make possible the identification of intermediates by spectroscopic methods (e.g., infrared and nuclear magnetic resonance spectroscopy), as we expect that some of the intermediates would accumulate.

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REFERENCES


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**FIG. 4.** Hypothesis for the catabolism of IAA by oxidative opening of the pyrrole ring. This pathway has also been suggested for "a micro-organism isolated from air" (23).

These compounds serving as substrates. By monitoring IAA degradation by UV absorption, we see that the characteristic spectrum from the indole ring disappears. Splitting of the indole ring has been described for bacteria only. For *Pseudomonas* spp., it has been suggested that IAA is catabolized by modifications of the side chain followed by a hydrolytic opening of the pyrrole ring. Salicylic acid and catechol have been identified as degradation products (19). In *Arthrobacter* spp., catechol and pyrogallol have been identified as degradation products (15). For "a micro-organism isolated from air" (23), it has been suggested that IAA is degraded by an oxidative opening of the indole ring in a reaction analogous to the one catalyzed by tryptophan 2,3-dioxygenase. *O*-Formaminobenzoyl-acetic acid and anthranilic acid have been identified as degradation products.

When IAA is degraded in *B. japonicum*, the process is oxygen dependent, and absorption from the indole ring disappears; this indicates that an oxygen-consuming opening of the indole ring could take place. Experiments with tryptophan-auxotrophic mutants (Table 2) and studies of a metabolite by HPLC (Fig. 3) indicate that anthranilic acid is a degradation product. We therefore hypothesize that IAA in *B. japonicum* can be degraded in the same way as that suggested for "the micro-organism isolated from air" (23) (Fig. 4), indicating that they could be related species.
Bradyrhizobial indoleacetic acid metabolism and its 
significance for root nodule development, p. 151–152. In R. 
Palacios and D. P. S. Verma (ed.), Molecular genetics of 
plant-microbe interactions. APS Press, St. Paul, Minn.

Nonhebel, H. M., and R. S. Bandurski. 1984. Oxidation of 
indole-3-acetic acid and oxindole-3-acetic acid to 2,3-dihydro-7-
hydroxy-2-oxo-1H-indole-3-acetic acid-7'-O-β-d-glucopyranoside in 

Indole-3-acetic acid catabolism in Zea mays seedlings. Meta-
bolic conversion of oxindole-3-acetic acid to 7-hydroxy-2-oxin-
dole-3-acetic acid 7'-O-β-d-glucopyranoside. J. Biol. Chem. 

Proctor, M. H. 1958. Bacterial dissimilation of indoleacetic acid: 
a new route of breakdown of the indole nucleus. Nature 
(London) 181:1345.

acid, an indole-3-acetic acid catabolite in Zea mays. Plant 
Physiol. 71:211–213.

Sandberg, G., A. Crozier, and A. Ernstsen. 1987. Indole-3-acetic 
acid and related compounds, p. 169–301. In L. Rivier and A. 
Crozier (ed.), Principles and practice of plant hormone analysis. 

Thimann, K. V. 1936. On the physiology of the formation of 
514.

Tsubokura, S., Y. Sakamoto, and K. Ichihara. 1961. The Bacterial 

Wells, S. E., and L. D. Kuykendall. 1983. Tryptophan auxo-