APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1990, p. 275–280
0099-2240/90/010275-06$02.00/0

Vol. 56, No. 1

New Pathway for the Biodegradation of Indole in *Aspergillus niger*

AJITH V. KAMATH† AND C. S. VAIDYANATHAN*

Department of Biochemistry, Indian Institute of Science, Bangalore-560 012, India

Received 17 July 1989/Accepted 24 October 1989

Indole and its derivatives form a class of toxic recalcitrant environmental pollutants. The growth of *Aspergillus niger* was inhibited by very low concentrations (0.005 to 0.02%) of indole, even when 125- to 500-fold excess glucose was present in the medium. When 0.02% indole was added, the fungus showed a lag phase for about 30 h and the uptake of glucose was inhibited. Indole was metabolized by a new pathway via indoxyl (3-hydroxyindole), *N*-formylanthranilic acid, anthranilic acid, 2,3-dihydroxybenzoic acid, and catechol, which was further degraded by an *ortho* cleavage. The enzymes *N*-formylanthranilate deformylase, anthranilate hydroxylase, 2,3-dihydroxybenzoate decarboxylase, and catechol dioxygenase were induced by indole as early as after 5 h of growth, and their activities were demonstrated in a cell-free system.

Indole, which has a pleasant odor at low concentrations, is used in the production of cosmetics and perfumes and as flavoring agents for ice cream. Indole and its derivatives form a class of toxic recalcitrant N-heterocyclic compounds which are now considered pollutants, since they are released into the environment through cigarette smoke (17, 20, 22), coal-tar (39), and sewage (39). They are also found in many plants which are used as food (see references 1 and 39 and references cited therein). Although the observed level of indoles in general is too low to cause immediate toxicity to living systems, in cattle and goats they were reported to cause acute pulmonary edema, emphysema, lung diseases (6), hemoglobinuria, and hemolysis (21). Voacristine, a medicinally important indole alkaloid, showed cytotoxic properties in yeasts (34). The levels of indole and skatole (3-methylindole) were used as metabolic markers to screen for the incidence of bowel cancer in humans (26). Indole markedly increased the levels of dimethylnitrosamine demethylase (2, 35) and arylhydrocarbon hydroxylase in both rats and hamsters (16). It was suggested that indole is different from the well-studied inducers and might represent a new class of cytochrome P-450 inducer (35). Plant tissues exposed to indole showed low pigmentation, presumably due to the inhibition of anthraquinone biosynthesis (14). Many of the indole derivatives have been shown to be toxic and mutagenic, some of the methyl derivatives being as potent as benzo(a)pyrene in causing mutation (39). The natural occurrence of indoles and their mutagenicity have been studied extensively (39, 44).

King et al. (27) reported that in rats indole is detoxified via isatin, *N*-formylanthranilate, and anthranilate, which are excreted as sulfate and glucuronide derivatives. They also proposed a secondary pathway involving oxindole (2-hydroxyindole) and *ortho*-aminophenylacetate as intermediates. Studies from this laboratory showed that plants contain many indole compounds (see reference 30 and references cited therein). In *Jasminum grandiflorum*, indole was metabolized by indole oxygenase to give anthranilate (11); whereas, in *Tecoma stans* it was converted to either anthranil (37) or anthranilate (29). The anthranilate thus produced is further metabolized through 3-hydroxyanthranilate and *ortho*-aminophenol (38). In a tapwater bacterium, Sakamoto et al. (40) proposed a pathway via indoxyl (3-hydroxyindole), 2,3-dihydroxyindole, isatin, anthranilate, and salicylate, while in another bacterium, a direct formation of dihydroxyindole and further degradation involving *N*-carboxyanthranilate were suggested (18). A different pathway was observed in *Alcaligenes* species, in which isatin and gentisate were the intermediates (8). In anaerobic and denitrifying bacterial consortia, indole was degraded via oxindole (3, 32, 33). The pathways observed in microbes differed from each other, highlighting the diverse mechanisms which operate to degrade the same compound. Though fungi are equally efficient in detoxifying xenobiotics, so far the fate of indole in fungi has not been studied. In this study we delineate a new pathway for the degradation of indole in a versatile soil fungus, *Aspergillus niger*. We have identified all the intermediates and demonstrated many inducible enzyme activities in a cell-free system (A. V. Kamath, Ph.D. thesis, Indian Institute of Science, Bangalore, India).

MATERIALS AND METHODS

Materials. Indole, anthranilate, NADPH (reduced form), $NADP^+$, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were purchased from Sigma Chemical Co., St. Louis, Mo. 2,3-Dihydroxybenzoic acid (DHBA) was from Koch Light Laboratories, Poole, United Kingdom. Gibb's reagent (2,6-dichloroquinonechloroimide) was procured from Aldrich Chemical Co., Milwaukee, Wis. *N*-Formylanthranilate was a gift from Prema Kumar of the Organic Chemistry Department, Indian Institute of Science. All other chemicals used were of analytical grade available locally.

Organism and growth conditions. *A. niger*, which was maintained in our laboratory (25), was used in the present study. It was grown on Byrde's synthetic medium (5) with the following modifications: glucose, 2.5%, and KNO_3, 0.25% [pH 5.5]. Indole was dissolved in a minimum amount of ethanol and added to the autoclaved medium. The medium (200 ml in 500-ml Erlenmeyer flasks) was inoculated with a heavy suspension of spores, and the fungus was grown by shaking (160 rpm) at room temperature (28°C). The growth curve of *A. niger* was measured by estimating the dry weight of the fungus. At various time points of growth, the mycelia were harvested from a set of flasks by using a Buchner funnel; they were washed with distilled water, and excess water was squeezed out by using folds of Whatman no. 3 filter paper. The mycelia were dried at 100°C for 6 to 8 h to determine the weight. For preparation of the crude

* Corresponding author.
† Present address: Department of Microbiology, The University of Texas at Austin, Austin, TX 78712.

extract, the mats were washed and used directly or stored at −20°C until use.

Isolation and identification of the metabolites. *A. niger* was grown in the medium described above, supplemented with 0.02% indole, for 45 h. The spent medium (4 liters) was separated from the mycelia and acidified to pH 2 to 3 with 1 N HCl, and the aromatic intermediates were extracted twice with peroxide-free diethyl ether. The ether layers were pooled, separated into neutral and acidic fractions, and processed as described by Kishore et al. (28). The residues were dissolved in ether and subjected to paper (Whatman no. 1) and thin-layer (silica gel G) chromatography by using the following solvent systems: A, isopropanol-ammonia-water (8:1:1); B, formic acid-water (2:98); C, benzene-ethylmethyl ketone–2% formic acid in water (90:20:20); and D, benzene-acetic acid-water (10:7:3, organic phase). The phenolic compounds were identified by spraying (i) diazotized *p*-nitroaniline followed by 10% NaOH and (ii) $FeCl_3$ reagent (28). The indole metabolites were identified by spraying Ehrlich reagent (43) and Gibb's reagent (0.05% [vol/vol] 2,6-dichloroquinonechloroimide in ethanol followed by aqueous saturated sodium bicarbonate [27]).

Estimation of anthranilate and DHBA during the growth. *A. niger* was grown in the medium supplemented with 0.02% indole. At various time periods, samples of the spent medium (25 ml) were taken out for isolating the metabolites. They were acidified and extracted into ether and evaporated to dryness, and the residues were dissolved separately in 1 ml of ethanol. A known amount (50 μl) was quantitatively applied to Whatman no. 1 filter paper and chromatographed by using solvent system B. The metabolites were located by using an UV lamp, and the spots corresponding to anthranilate and DHBA were cut out and eluted into ether under mild acidic conditions. The amounts of anthranilate and DHBA were estimated by using the methods given below. The standards (anthranilate and DHBA) were similarly treated and used for estimating the recovery, which was more than 90%.

UV and infrared spectroscopy. The metabolites were purified by preparative thin-layer chromatography on silica gel G-coated glass plates (20 cm by 20 cm) with solvent systems A and D and were further identified by their UV and infrared (IR) spectroscopic characteristics. The UV spectra of the intermediates were recorded on a Shimadzu UV 190 spectrophotometer. The IR spectra were taken in Nujoll-mull on a Perkin-Elmer model 7 IR spectrophotometer.

Preparation of cell extracts and the assay of enzymes. All operations were carried out at 0 to 4°C. The mycelia were crushed in a chilled mortar with acid-washed glass powder. The slurry was extracted with 50 mM sodium phosphate buffer, pH 7.0, passed through double-layered cheesecloth, and centrifuged at 27,000 × *g* for 20 min in a Sorvall RC 5B centrifuge. The supernatant was designated as crude extract and was used for demonstrating the enzyme activities.

All enzyme assays were carried out at room temperature (28°C), and the activities were measured under standard conditions (initial velocities, saturating conditions, etc.).

(i) *N*-formylanthranilate deformylase. This enzyme was assayed spectrophotometrically by the method of Jakoby (23) following the appearance of anthranilate. The sample cuvette, in 1 ml, contained sodium phosphate buffer (100 μmol) [pH 7.5], *N*-formylanthranilate (20 μmol), and a suitable amount of the enzyme. The reference cuvette contained only buffer and enzyme. The enzyme activity was measured by following the increase in optical density due to the formation of anthranilate. An increase of 0.15 optical density at 330 nm corresponded to formation of 100 nmol of anthranilate per ml (23).

(ii) Anthranilate hydroxylase. The reaction mixture (1 ml) contained Tris hydrochloride buffer (50 μmol) (pH 8.0), anthranilate (1.2 μmol), NADPH (1.2 μmol), and the enzyme. After incubating for 20 min, the reaction was terminated by the addition of 0.2 ml of 1 N HCl. The mixture was spun at 3,000 × *g* for 5 min, and the supernatant was extracted with 2.5 ml of ethyl acetate. A 1-ml portion of this organic phase was estimated for anthranilate by using Ehrlich reagent (43) by the procedure of Venkataraman et al. (45). Whenever a relatively large amount of NADPH was required, the reaction mixture was supplemented with the NADPH-generating system consisting of $NADP^+$ (5 μmol), glucose 6-phosphate (5 μmol), and glucose 6-phosphate dehydrogenase (500 mU). The generating system was incubated for 5 min in the buffer prior to the addition of reactants (V. Subramanian, Ph.D. thesis, Indian Institute of Science, Bangalore, India).

(iii) DHBA decarboxylase. This enzyme was assayed by following the disappearance of DHBA as described earlier (25). The assay mixture (1 ml) contained sodium acetate buffer (50 μmol) (pH 5.2), DHBA (2 μmol), and the enzyme. It was incubated for 15 min, and the reaction was terminated by the addition of 1 ml of 15% trichloroacetic acid. The precipitated protein was removed by centrifugation at 3,000 × *g* for 5 min, and the supernatant was diluted to 6 ml with double-distilled water. The decrease in the optical density at 316 nm was measured (25).

(iv) Catechol dioxygenase. The reaction mixture (1 ml) contained Tris hydrochloride buffer (50 μmol) (pH 7.5), catechol (200 nmol), and the enzyme. After incubation for 10 min, the reaction was stopped by adding 0.1 ml of 50% trichloroacetic acid and the precipitated protein was removed by centrifugation at 3,000 × *g* for 5 min. The catechol remaining was estimated by the method of Nair and Vaidyanathan (36).

Replacement studies. *A. niger* was grown for 40 h in 200 ml of medium supplemented with 0.02% indole. The mycelial felts were thoroughly washed with sterile double-distilled water and distributed into a set of flasks, each containing 50 ml of basal medium (Byrde's medium without glucose) and 0.1% of the metabolite of interest. The flasks were placed on a rotory shaker for 6 to 8 h, and the products in the spent media were identified by paper and thin-layer chromatography by using the solvent systems given above.

Other methods. Indole was estimated by using 4-dimethylaminobenzaldehyde reagent (43). For this, the spent medium was extracted under mild alkaline conditions and estimation was done as previously described (29). Anthranilate was estimated by the procedure described for arylamine by Venkataraman et al. (45), and DHBA was measured colorimetrically by the method of Nair and Vaidyanathan for *ortho* dihydroxy compounds (36). Glucose was estimated by the method of Somogyi (41), and protein was measured by the method of Lowry et al. (31), using bovine serum albumin as the standard.

RESULTS

Effect of different concentrations of indole on growth. *A. niger* was grown on modified Byrde's medium supplemented with different concentrations of indole (Fig. 1). The growth curve of the fungus did not show a lag phase when the medium contained only glucose; it reached the stationary phase by 70 h. However, when a low amount of indole

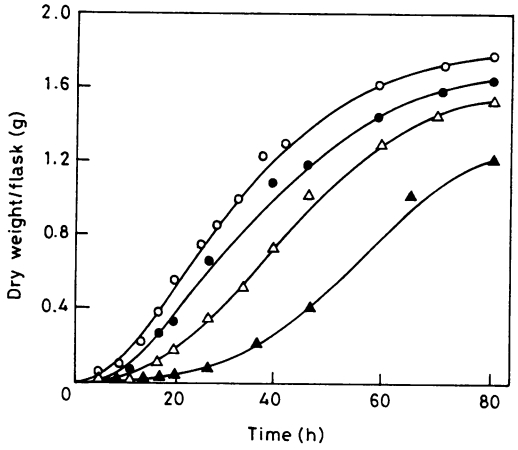

FIG. 1. Inhibition of the growth of *A. niger* by different concentrations of indole. The fungus was grown in the medium containing glucose alone (○); glucose plus 0.005% indole (●); glucose plus 0.01% indole (△); and glucose plus 0.02% indole (▲). The mycelia were processed to obtain the dry weight as described in the text.

(0.005%) was present along with glucose, there was a lag in the growth curve which became more pronounced when indole concentration was increased to 0.01 and 0.02%. At 0.02% indole, the lag phase was observed until 30 h and by the end of 80 h, the fungus grew only to an extent of 65% compared with the control set (Fig. 1). These experiments suggested that indole probably inhibited the growth, and the fungus resumed growth when indole was slowly degraded.

Utilization of glucose and indole during the growth. When *A. niger* was grown in the medium containing glucose alone (the control set), a continuous utilization of glucose was observed and by 60 h, there was a 90% depletion of glucose (Fig. 2). This pattern of glucose uptake corresponded well with its increase in growth. When indole was present along with glucose, there was a decrease in glucose uptake in the initial stages of growth. After about 26 h, both glucose utilization and growth increased. A very small amount of indole was degraded in the initial stages of growth, but 90% of it had disappeared by 80 h.

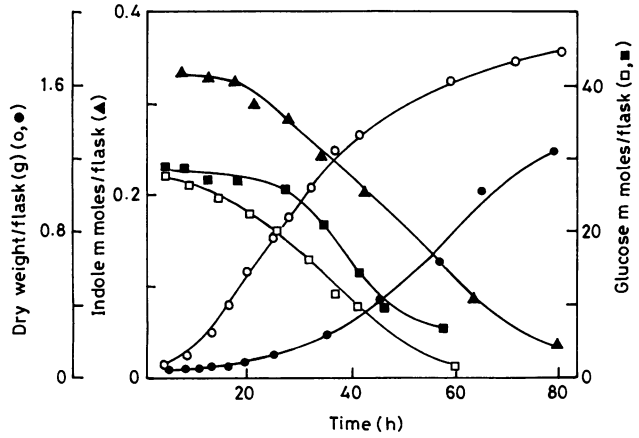

FIG. 2. Utilization of glucose and indole during the growth of *A. niger*. The growth was measured as dry weight in the presence of glucose alone (○) and glucose plus indole (●). The amount of glucose in the control set (□) and in the set supplemented with indole (■) were measured; ▲ represents the amount of indole in the experimental set. The methods used are given in the text.

TABLE 1. R_f values of the authentic compounds and metabolites isolated from the spent medium of *A. niger*

Compound	Solvent systems used[a]							
	A		B		C		D	
	(a)	(i)	(a)	(i)	(a)	(i)	(a)	(i)
N-Formylanthranilate	0.55	0.55	0.66	0.65	0.32	0.31		
Anthranilate	0.44	0.44	0.80	0.79	0.56	0.56	0.65	0.65
DHBA	0.38	0.38	0.54	0.55	0.28	0.28	0.61	0.61
Catechol	0.41	0.41	0.81	0.80	0.54	0.54	0.87	0.88

[a] Solvent systems used are given in the text. (a), Authentic; (i), isolated.

Identification of the intermediates. The metabolites were extracted from the spent medium and subjected to paper chromatography. Table 1 shows the R_f values of the metabolites in four different solvent systems. The intermediates were also identified by cochromatography with authentic compounds, specific color reactions, fluorescence, UV, and IR spectra. These compounds were identified as *N*-formylanthranilate, anthranilate, DHBA, and catechol; the properties of the isolated metabolites corresponded well with those of authentic samples. *N*-Formylanthranilate (λ_{max} = 306 nm) gave a yellow color with Ehrlich and Gibb's reagents (slow reactions [27]) and had a blue fluorescence. It was also identified by two-dimensional paper chromatography with solvent system A in the first dimension and butanol-acetic acid-water (4:1:1) in the second dimension and by converting it into anthranilate by refluxing with 1 N HCl for 15 min (27). Anthranilate (λ_{max} = 330 nm) gave similar color reactions, which appeared immediately and had an intense blue fluorescence. It gave IR bands at 1,480, 1,700, and 3,000 cm^{-1}. DHBA had a λ_{max} at 316 nm and gave IR bands at 1,480, 1,700, and 3,500 cm^{-1}. Catechol (λ_{max} = 276 nm) gave a dark brown color with $FeCl_3$ reagent; its IR bands were positioned at 1,480, 3,000, and 3,500 cm^{-1}. Similar results were obtained when authentic samples were scanned. In addition to these metabolites, the spent medium had a blue tinge which could be extracted into chloroform (15). This compound was identified as indigo (λ_{max} = 600 nm), formed by the oxidation of indoxyl.

The levels of two of the metabolites (Fig. 3), anthranilate and DHBA, were measured in the spent medium by the procedures described. Anthranilate appeared in the medium as early as after 5 h of growth and reached a concentration of 24 μmol per flask by 24 h, after which its concentration decreased to 17 μmol per flask by 70 h. In contrast, DHBA was observed in the medium rather late (25 h), and its concentration gradually increased to 102 μmol per flask.

Enzymes of the pathway and their profiles during growth. The induction of the enzymes in the pathway for degrading indole was examined by measuring the specific activities of the enzymes in the mycelia obtained by growing for 35 h on glucose and indole (Table 2). *N*-Formylanthranilate deformylase, anthranilate hydroxylase, DHBA decarboxylase, and catechol dioxygenase were induced to an extent of 30-, 516-, 278-, and 50-fold, respectively, when the medium contained indole (0.02%). The specific activities of the enzymes in mycelia grown on glucose alone (control set) were very low (Table 2). Attempts to demonstrate the activities of indole hydroxylase and indoxyl dioxygenase under a variety of conditions were unsuccessful.

The time courses of induction of *N*-formylanthranilate deformylase, anthranilate hydroxylase, DHBA decarboxylase, and catechol dioxygenase were studied by measuring

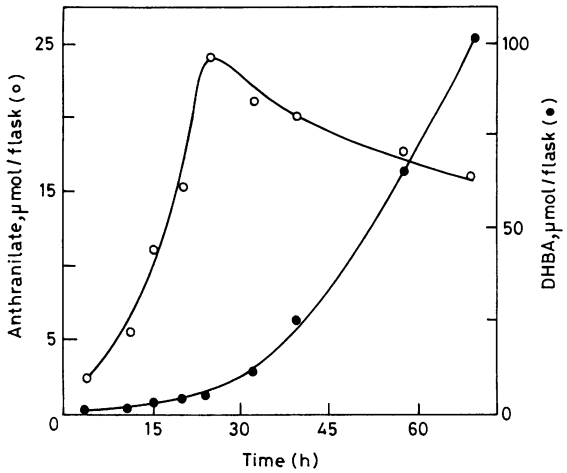

FIG. 3. Time course for the accumulation of anthranilate and DHBA during the growth period.

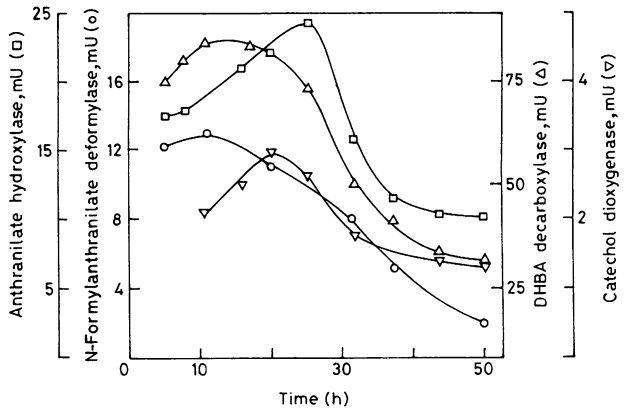

FIG. 4. Profiles of the specific activities of *N*-formylanthranilate deformylase, anthranilate hydroxylase, DHBA decarboxylase, and catechol dioxygenase induced by indole during the growth.

their specific activities in crude extracts of mycelia grown on glucose and 0.02% indole (Fig. 4). These enzymes were induced as early as 5 h, and their specific activities were high during the initial stages of growth. All the enzymes (except the deformylase) had maximum specific activities in the first 15 to 25 h of growth; they later decreased to about 20 to 40% of their maximum by 45 to 50 h.

Mode of ring cleavage. The possibility of an *ortho* ring cleavage of catechol to form *cis-cis* muconate and subsequently β-ketoadipate was tested by the Rothera reaction as described by Eaton and Ribbons (12). *A. niger* was grown for 40 h in the medium (200 ml) containing indole (0.02%); the mycelium was thoroughly washed with sterile double-distilled water and transferred to a conical flask in 50 ml of 50 mM sodium phosphate buffer, pH 7.0, containing 0.1% catechol. After incubation for 60 min, the mycelial felt was separated and solid ammonium sulfate was added to 1 ml of the buffer to saturate the solution; 2 drops of an aqueous solution of sodium nitroferricyanide were added, followed by ammonium hydroxide, which was layered over the mixture. A positive reaction was indicated by the development of a deep purple color in the upper layer after 5 to 10 min.

DISCUSSION

This is the first report on the degradation of indole by a fungus. *A. niger* did not grow when indole was used as the sole source of carbon or nitrogen. However, it cometabolized indole when glucose and nitrate were present in the medium. When indole was added to the medium containing glucose, there was a decrease in the growth of *A. niger* (Fig. 1). Although the concentration of glucose was about 125- to 500-fold in excess compared with indole, there was an inhibition of growth and the fungus did not utilize glucose, the abundant primary carbon source. In another set of experiments, when indole (0.02%) was added to the mycelium which was in log phase (medium contained only glucose), an immediate cessation in the growth for almost 12 h was observed (data not presented). These results suggested that indole probably inhibited the uptake or utilization of glucose. When the enzymes for degrading indole were induced (see discussion below), the level of indole decreased, which in turn resulted in the resumption of growth. This hypothesis finds favor from the observation that indole was metabolized very slowly until about 25 h, during which time glucose was not metabolized (Fig. 2). When indole in the medium was degraded, the inhibition was relieved and an increase in glucose uptake and growth was seen. It might also be possible that indole repressed the glucose transport or inhibited the glycolytic enzymes, causing the observed decrease in growth. A similar observation was made in the case of *Aspergillus nidulans*, in which caffeine was shown to inhibit at the level of glucose uptake (4). The growth of *A. niger* on other carbon sources, such as sucrose and maltose, was decreased by an extent of 15 to 35% when indole (0.02%) was also present in the medium.

TABLE 2. Specific activities of the enzymes in cell extracts of *A. niger* grown on glucose and glucose plus indole

Enzyme	Sp act[a] (mU)		Fold increase
	Glucose	Glucose + indole	
N-Formylanthranilate deformylase	0.40	12.0	30
Anthranilate hydroxylase	0.05	25.8	516
DHBA decarboxylase	0.28	78.0	278
Catechol dioxygenase	0.05	2.5	50

[a] Activity is defined as micromoles of product formed or substrate which disappeared per minute. Specific activity is expressed as units per milligram of protein.

By using various techniques, we have identified the possible metabolites of indole degradation. These compounds were indoxyl, *N*-formylanthranilate, anthranilate, DHBA, and catechol. Identification of the products in the replacement studies carried out with the plausible metabolites suggested that the compounds mentioned above were the intermediates. Many of the enzymes in this pathway have been demonstrated in cell extracts. With these results, we propose a new pathway for the biodegradation of indole in *A. niger* via indoxyl → *N*-formylanthranilate→anthranilate→DHBA→catechol (Fig. 5).

In bacterial systems, unhydroxylated aromatic compounds are degraded by initial double hydroxylation (9, 19). Gibson and co-workers (15) showed that in bacteria, indole was converted by a dioxygenation reaction to indole *cis*-2,3-dihydroxy 2,3-dihydrodiol. By a nonenzymatic dehydration reaction, this compound gave unstable indoxyl, which

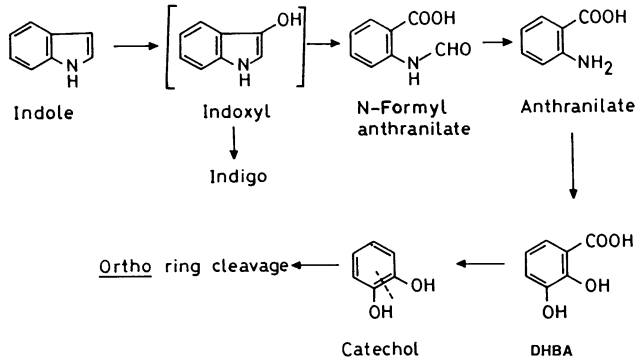

FIG. 5. Proposed pathway for the degradation of indole in *A. niger*. Indoxyl is a proposed intermediate.

oxidized in the presence of air to indigo. This reaction was demonstrated by using cloned naphthalene dioxygenase and other dioxygenases (15). In fact, the nonspecific double hydroxylation of indole to give indoxyl and then indigo has been used for the assay of some dioxygenases (13, 24). Hence, the indoxyl which was suggested to be an intermediate in bacterial indole degradation (40) might have arisen by such a mechanism, rather than by a monooxygenation reaction. However, in anaerobic bacteria, single hydroxylation to oxindole has been observed (3, 32), although this enzyme has not been demonstrated in a cell-free system. In fungi and other higher organisms, monohydroxylation of aromatic compounds is prevalent (10, 42); similarly, in *A. niger*, indole was monohydroxylated at the C-3 position, which is known to be the most reactive carbon. The product (indoxyl), being unstable, dimerized in the presence of air to indigo and gave a blue tinge to the growth medium. The formation of indigo was clearly observed when transformations were carried out with *A. niger* immobilized on various inert matrices (unpublished results).

By using radioactive indole, King et al. (27) showed that in animals it was hydroxylated at the C-3, C-2, C-5, C-6, and C-7 positions. However, in *A. niger*, no other hydroxy indoles were detected. Reactions converting indole to indoxyl could not be shown in cell extracts. Our efforts to show this indole oxygenase activity by using a variety of conditions, such as assaying at different stages of growth, in cytosolic and microsomal fractions, and using various cofactors, thiol compounds, metal ions, and stabilizers, were unsuccessful. By carrying out oxygen-uptake studies, Sakamoto et al. (40) proposed indoxyl, dihydroxyindole, and isatin as the metabolites during the bacterial degradation of indole, whereas another group suggested a direct double hydroxylation of indole (18). So far, this initial hydroxylation reaction has not been shown in cell extracts in any system. In *A. niger*, indoxyl was formed by monooxygenation and it was further converted to *N*-formylanthranilate by a dioxygenase reaction. Our attempts to demonstrate this indoxyl dioxygenase activity were unsuccessful because of the unstable nature of the substrate. Unlike in microbes and animals, in plants indole was directly cleaved by indole oxygenase/indole oxidase to *N*-formylaminobenzaldehyde, which was further converted to either anthranil (37) or anthranilate by the same enzyme (29).

All the enzymes demonstrated in the above pathway were found in the cytosolic fraction (105,000 × *g* supernatant) and were induced by indole (Table 2). *N*-Formylanthranilate deformylase showed high activity and gave rise to anthranilate. The anthranilate was further converted to DHBA by oxidative deamination and hydroxylation reactions catalyzed by NADPH-dependent anthranilate hydroxylase. In the bacterial pathway, anthranilate was directly converted to either salicylate (40) or gentisate (8), whereas in plants it was converted to 3-hydroxyanthranilate and *o*-aminophenol (38). Anthranilate did not inhibit the growth of *A. niger* (25) and other members of this genus (7). Therefore, indole or the metabolite preceding anthranilate might have caused the observed inhibition of growth of the fungus.

From the spent medium, two key intermediates, namely, anthranilate and DHBA, were isolated during various phases of growth, and this showed a typical precursor-product relationship (Fig. 3). Anthranilate was degraded to DHBA by anthranilate hydroxylase, which was further converted to catechol. The later reaction was catalyzed by DHBA decarboxylase. This enzyme, found only in fungal systems, was recently purified from *A. niger* (25). Catechol dioxygenase converted catechol to *cis-cis* muconate by an *ortho* cleavage. The variation in the specific activities of these four enzymes (Table 2) might be attributed to the presence of endogenous inhibitors (25). It was interesting to note that these enzymes (Fig. 4) were induced as early as after 5 h of growth and the specific activities were maximum by about 15 to 20 h, after which they decreased, presumably due to the synthesis of other cellular proteins.

In conclusion, the present study showed that *A. niger* efficiently cometabolized indole. The degradation pathway observed in this fungus was different from the pathways observed so far in other organisms.

ACKNOWLEDGMENTS

We thank N. Appaji Rao for his keen interest and constructive criticism, P. N. Vishwanathan (Industrial Toxicology Research Institute, Lucknow, India), and L. A. Damani (University of Manchester) for their help and suggestions.

A. V. K. is grateful to the Department of Atomic Energy, Government of India, for a senior research fellowship.

LITERATURE CITED

1. **Acheson, R. M., and J. O. Nwankwo.** 1984. The metabolism of some 1-hydroxylated indoles in the rat. Xenobiotica **14**:877–883.
2. **Arcos, J. C., S. C. Myers, B. J. Newburger, and M. F. Argus.** 1980. Comparative effects of indole and acetonitrile derivatives on dimethylnitrosamine demethylase and arylhydrocarbon hydroxylase activities. Cancer Lett. **9**:161–167.
3. **Berry, D. F., E. L. Madsen, and J.-M. Bollag.** 1987. Conversion of indole to oxindole under methanogenic conditions. Appl. Environ. Microbiol. **53**:180–182.
4. **Buchanan, R. L., and D. F. Lewis.** 1984. Caffeine inhibition of aflatoxin synthesis: probable site of action. Appl. Environ. Microbiol. **47**:1216–1220.
5. **Byrde, R. J. W., J. F. Harris, and D. Woodcock.** 1956. The metabolism of ω-(2-naphthyloxy)n-alkyl carboxylic acids by *Aspergillus niger*. Biochem. J. **64**:154–160.
6. **Carlson, J. R., M. T. Yokoyama, and E. O. Dickson.** 1972. Induction of pulmonary edema and emphysema in cattle and goats with 3-methylindole. Science **176**:298–299.
7. **Chipley, J. R., and N. Uraih.** 1980. Inhibition of *Aspergillus* growth and aflatoxin release by derivatives of benzoic acid. Appl. Environ. Microbiol. **40**:352–357.
8. **Claus, G., and H. J. Kutzner.** 1983. Degradation of indole by *Alcaligenes* sp. Syst. Appl. Microbiol. **4**:169–180.
9. **Dagley, S.** 1986. Biochemistry of aromatic hydrocarbon degradation in pseudomonads, p. 527–555. *In* N. L. Ornston (ed.), The bacteria, vol. 10. Academic Press, Inc., New York.
10. **Dodge, R. H., C. E. Cerniglia, and D. T. Gibson.** 1979. Fungal metabolism of biphenyl. Biochem. J. **178**:223–230.
11. **Divakar, N. G., V. Subramanian, M. Sugumaran, and C. S.**

Vaidyanathan. 1979. Indole oxygenase from the leaves of *Jasminum grandiflorum*. Plant Sci. Lett. **15**:177–181.
12. **Eaton, R. W., and D. W. Ribbons.** 1982. Metabolism of dibutylphthalate and phthalate by *Micrococcus* sp. strain 12B. J. Bacteriol. **151**:48–57.
13. **Eaton, R. W., and K. N. Timmis.** 1986. Characterization of a plasmid-specified pathway for catabolism of isopropylbenzene in *Pseudomonas putida* RE204. J. Bacteriol. **168**:123–131.
14. **El-Shagi, H., U. Schulte, and M. H. Zenk.** 1984. Specific inhibition of anthraquinone formation by amino compounds in *Morinda* cell cultures. Naturwissenschaften **71**:267.
15. **Ensley, B. D., B. J. Ratzkin, T. D. Osslund, M. J. Simon, L. P. Wackett, and D. T. Gibson.** 1983. Expression of naphthalene oxidation genes in *Escherichia coli* results in the biosynthesis of indigo. Science **222**:167–169.
16. **Evarts, R. P., and M. H. Mostafa.** 1981. Effects of indole and tryptophan on cytochrome P-450, dimethylnitrosamine demethylase and arylhydrocarbon hydroxylase activities. Biochem. Pharmacol. **30**:517–522.
17. **Florin, I., L. Rutberg, M. Curvall, and C. R. Enzell.** 1980. Screening of tobacco smoke constituents for mutagenicity using the Ame's test. Toxicology **18**:219–232.
18. **Fujioka, M., and H. Wada.** 1968. The bacterial oxidation of indole. Biochim. Biophys. Acta **158**:70–78.
19. **Gibson, D. T., and V. Subramanian.** 1984. Microbial degradation of aromatic hydrocarbons, p. 181–252. *In* D. T. Gibson (ed.), Microbial degradation of aromatic compounds. Marcel-Dekker, Inc., New York.
20. **Grob, K., and J. A. Voellmin.** 1970. GC-MS analysis of the semi-volatiles of cigarette smoke. J. Chromatogr. Sci. **8**:218–220.
21. **Hammond, A. C., J. R. Carlson, and R. C. Breeze.** 1980. Indole toxicity in cattle. Vet. Rec. **107**:344–346.
22. **Ishiguro, S., and S. Sugawara.** 1978. Comparison of smoke components in the semi-volatile phase from lamina and midrib cigarette of flue-cured tobacco leaves. Agric. Biol. Chem. **42**:1527–1531.
23. **Jakoby, W. B.** 1954. Kynurenine formamidase from *Neurospora*. J. Biol. Chem. **207**:657–663.
24. **Jenkins, R. O., and H. Dalton.** 1985. The use of indole as a spectrophotometric assay substrate for toluene dioxygenase. FEMS Microbiol. Lett. **30**:227–231.
25. **Kamath, A. V., D. Dasgupta, and C. S. Vaidyanathan.** 1987. Enzyme-catalysed non-oxidative decarboxylation of aromatic acids. I. Purification and spectroscopic properties of 2,3-dihydroxybenzoic acid decarboxylase from *Aspergillus niger*. Biochem. Biophys. Res. Commun. **145**:586–595.
26. **Karlin, D. A., A. J. Mastromarino, R. D. Jones, J. R. Stroehlein, and O. Lorentz.** 1985. Fecal skatole and indole and breath methane and hydrogen in patients with large bowel polyps or cancer. J. Cancer Res. Clin. Oncol. **109**:135–141.
27. **King, L. J., D. V. Parke, and R. T. Williams.** 1966. The metabolism of [2-^{14}C]indole in the rat. Biochem. J. **98**:266–277.
28. **Kishore, G., M. Sugumaran, and C. S. Vaidyanathan.** 1976. Metabolism of DL-(+)-phenylalanine by *Aspergillus niger*. J. Bacteriol. **128**:182–191.
29. **Kunapuli, S. P., and C. S. Vaidyanathan.** 1983. Purification and characterization of a new indole oxygenase from the leaves of *Tecoma stans*. Plant Physiol. **71**:19–23.
30. **Kunapuli, S. P., and C. S. Vaidyanathan.** 1984. Indolic compounds in the leaves of *Tecoma stans*. Phytochemistry **23**: 1826–1827.
31. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
32. **Madsen, E. L., and J.-M. Bollag.** 1989. Pathway of indole metabolism by a denitrifying microbial community. Arch. Microbiol. **151**:71–76.
33. **Madsen, E. L., A. J. Francis., and J.-M Bollag.** 1988. Environmental factors affecting indole metabolism under anaerobic conditions. Appl. Environ. Microbiol. **54**:74–78.
34. **Melo, A. A., C. B. Querol, A. T. Henriques, and J. A. P. Henriques.** 1986. Cytostatic, cytotoxic and mutagenic effects of voacristine, an indole alkaloid in wild type and repair-deficient yeasts. Mutat. Res. **171**:17–24.
35. **Mostafa, M. H., M. Ruchirawat, and E. K. Weisburger.** 1981. Comparative studies on the effects of various microsomal enzyme inducers on the *N*-demethylation of dimethylnitrosamine. Biochem. Pharmacol. **30**:2007–2011.
36. **Nair, P. M., and C. S. Vaidyanathan.** 1964. A colorimetric method for determination of pyrocatechol and related substances. Anal. Biochem. **7**:315–321.
37. **Nair, P. M., and C. S. Vaidyanathan.** 1964. An indole oxidase isolated from the leaves of *Tecoma stans*. Biochim. Biophys. Acta **81**:496–506.
38. **Nair, P. M., and C. S. Vaidyanathan.** 1966. Anthranilic acid oxidase system of *Tecoma stans*. II. Studies on the preparation of a purified anthranilic acid oxidase system and separation into different components. Phytochemistry **3**:513–523.
39. **Ochiai, M., K. Wakabayashi, T. Sugimura, and M. Nagao.** 1986. Mutagenicities of indole and 30 derivatives after nitrite treatment. Mutat. Res. **172**:189–197.
40. **Sakamoto, Y., M. Uchida, and H. Ichihara.** 1953. The bacterial decomposition of indole. I. Studies on its metabolic pathway by successive adaptation. Med. J. Osaka Univ. **3**:477–486.
41. **Somogyi, M.** 1952. Note on sugar determination. J. Biol. Chem. **195**:19–23.
42. **Subramanian, V., M. Sugumaran, and C. S. Vaidyanathan.** 1978. Double hydroxylation reactions in microorganisms. J. Indian Inst. Sci. **60**:143–178.
43. **Stanley, A. R., and R. S. Spray.** 1941. The photoelectric determination of indole in bacterial cultures. J. Bacteriol. **41**: 251–257.
44. **Vance, W. A., H. S. Okamoto, and Y. Y. Wang.** 1986. Structure-activity relationship of nitro and methyl-nitro derivatives of indoline, indole, indazole, and benzimidazole in *Salmonella typhimurium*. Mutat. Res. **173**:169–176.
45. **Venkataraman, A., P. R. Venkataraman, and H. B. Lewis.** 1948. The metabolism of *p*-aminosalicylic acid in the rabbit. J. Biol. Chem. **173**:641–651.