Biosynthetic Origin of Aflatoxin G1: Confirmation of Sterigmatocystin and Lack of Confirmation of Aflatoxin B1 As Precursors

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The origin of aflatoxin G1 was studied using mutant strains of Aspergillus parasiticus blocked early in the pathway and by tracing 14C-labelled aflatoxin B1 (AFB1) in wild-type A. flavus and A. parasiticus strains. Sterigmatocystin (ST) was a precursor of AFB1, AFG1, and AFG2 in the four mutants examined. The identity of AFG1 was confirmed by mass spectrometry. No evidence for conversion of AFB1 to AFG1 was found. A rigorously controlled study of conversions of radioactivity based on preparative thin-layer chromatography of aflatoxins demonstrated that low levels of aflatoxin interconversions previously reported in the literature might actually be artifacts.

INTRODUCTION

The aflatoxins are a family of potent mycotoxins produced by certain strains of the common moulds Aspergillus flavus and A. parasiticus. Aflatoxin contamination of foods and feeds presents an important toxicological hazard (Goldblatt, 1969; Betina, 1984). The major naturally occurring aflatoxins are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Most toxigenic strains of A. parasiticus produce AFB1, AFB2, AFG1, and AFG2 while toxicogenic strains of A. flavus produce only AFB1 and AFB2. No strains that produce the G aflatoxins without the B aflatoxins have been isolated from nature or from laboratory mutants (Klich & Pitt, 1985; Bennett & Deutsch, 1986); however, a number of strains that produce AFB2 and little or no AFB1 are known (Dutton et al., 1985).

Considerable research has been done on aflatoxin biosynthesis (Maggon et al., 1977; Heathcote & Hibbert, 1978; Steyn, 1980; Applebaum & Marth, 1981; Bennett & Christensen, 1983). Known aflatoxin precursors include five anthraquinones (norsolorinic acid, averantin, averufin, averufin and versicolorin A) and the xanthones sterigmatocystin (ST) and O-methylsterigmatocystin (Bhatnagar et al., 1987; McCormick et al., 1987) with the general sequence, acetate→anthraquinones→xanthones→aflatoxins. Biotransformation experiments with blocked mutants, radiotracer studies with 14C-labelled precursors and stable isotope studies with 13C-labelled precursors have emphasized the biosynthetic origin of AFB1, while the biosynthetic origins of AFB2, AFG1 and AFG2 have received less attention. It is generally assumed that AFB1 is a precursor of the other aflatoxins (Thomas, 1965; Biollaz et al., 1970), and several reviews include Figures illustrating an AFB1 to AFG1 conversion (Berger & Jadot, 1976; Heathcote & Hibbert, 1978; Bu'Lock, 1979).

Experimental verification of the role of AFB1 as the precursor of AFG1 is based on two studies. Maggon & Venkitasubramanian (1973) used [14C]AFB1 as a substrate for in vitro incubation with a cell-free homogenate of A. parasiticus and reported recovery of low levels of 14C label in AFB2, AFG1 and AFG2; Heathcote et al. (1976) reported similar results using an in

Abbreviations: AFB1, B1, G1, G2, aflatoxins B1, B2, G1 and G2; ST, sterigmatocystin; GM, growth medium; LSRM, low-sugar replacement medium; EMW, ethanol/methanol/water.
vivo system with wild-type A. flavus. However, recent work in our laboratory raises questions about the metabolic origin of AFG1. Using a mutant blocked in the aflatoxin pathway at versicolorin A we were unable to biotransform AFB1 or AFB2 into AFG1 or AFG2 (Floyd et al., 1987).

In this report we re-examine the final stages of aflatoxin biosynthesis by doing rigorously controlled experiments on biotransformation of ST, AFB1, and AFG1, with four blocked mutants, as well as by tracing [14C]AFB1 in wild-type A. flavus and A. parasiticus.

**METHODS**

**Organisms.** The wild-type strain of A. parasiticus was SU-1 (ATCC 56775); the wild-type strain of A. flavus was SRRC 2002, isolated by L. S. Lee from aflatoxin-contaminated cottonseed. Four anthraquinone-accumulating mutants of A. parasiticus were used: a norsolorinic acid-accumulating mutant, nor-1 (ATCC 24690); an averufin-accumulating mutant, avr-1 (ATCC 24551); a versicolorin-A-accumulating mutant, ver-1 (ATCC 36537); and an averantin-accumulating double mutant, avn-1 ver-1 (ATCC 56774).

**Media and culture conditions.** Stock cultures were maintained on potato dextrose agar (Difco) containing 0.5% yeast extract. Synthetic growth medium (GM) was modified by substituting sucrose for glucose but otherwise was formulated according to Adye & Mateles (1964). The low-sugar replacement medium (LSRM) was also formulated according to Adye & Mateles (1964). Approximately 10³ spores were used to inoculate 100 ml GM. Cultures were incubated at 27 °C in the dark on a gyratory shaker (model G76, New Brunswick) operating at 180–200 r.p.m.

**Biotransformation experiments.** Wild-type or mutant strains were grown for 48 h at 27 °C on the shaker in GM. Resultant mycelial pellets were harvested on cheesecloth and rinsed with LSRM. Pellets were weighed on sterile, tared paper and 1 g (wt wt) was added to 9.8 ml LSRM. For biotransformation studies, 555 nmol unlabelled ST, AFB1, AFB2, AFG1, or AFG2, or 2 and 4 nmol [14C]AFB1, dissolved in 200 µl acetone were added to the 9.8 ml LSRM cultures.

Controls consisted of adding 200 µl acetone without aflatoxin; in some controls, mycelia were also omitted. After transfer, cultures were incubated for an additional 24 h, then extracted.

**Extraction of metabolites.** Extraction procedures were essentially those used by Lee et al. (1976). Briefly, entire cultures (mycelium plus culture filtrate) were first extracted with aqueous acetone (70%, v/v). Mycelial pellets were then removed by filtration and the culture filtrate was extracted twice with chloroform in a separating funnel. The pooled, non-aqueous fractions from entire cultures were filtered through sodium sulphate, then concentrated in a Buchi rotary evaporator or air-dried in a chemical hood. Dried samples were resuspended in 1 ml chloroform for subsequent TLC. For preparative TLC the resuspended samples were concentrated to approximately 500 µl under nitrogen.

**Quantification of metabolites.** Initial TLC on silica gel G plates in ether/methanol/water (EMW) (96:3:1, by vol.) was done to obtain a visual estimate of the appropriate amount of extract to spot for densitometric readings, as recommended in the Official Methods of the American Oil Chemists' Society (Walker, 1983). Prescored 250-µm-thick silica gel G plates (20 x 20 cm) (Analtech) were spotted, developed in EMW, and scanned for fluorescent materials using a Shimadzu model CSW 9-10 recording densitometer; excitation was at 360 nm for aflatoxins and at 540 nm for ST. The quantities of aflatoxins and ST were calculated from the respective integrated areas of standards run on the same plate.

**Preparative TLC.** Samples were concentrated under nitrogen to approximately 500 µl and then streaked at the origin of 500-µm-thick Absorbosil Plus One (Alltech) silica gel plates (20 x 20 cm) which were poured in the laboratory. A separate spot of mixed aflatoxin standards was placed adjacent to each streak; plates were developed in EMW. Zones of sample corresponding to the RF values of aflatoxin standards were visualized by examining the plate under low-wattage UV and then scraped from the plate. Aflatoxins were extracted from the silica with acetone through a sintered glass filter. A. parasiticus SU-1 culture extracts alone and with added ST were used to establish preparative TLC conditions and to determine the expected RF zones for aflatoxins and ST. The methods were standardized further by analyzing the extracts again by TLC. In the samples containing [14C], zones corresponding to the RF values of aflatoxins were scraped directly into vials of scintillation fluid for counting radioactivity.

**Confirmation of identity of AFG1.** Ten separate cultures (1 g wet wt mycelium) of the double mutant avn-1 ver-1, grown for 48 h in GM, were incubated for 24 h in LSRM supplemented with 555 nmol ST. Chloroform extracts of the cultures were fractionated into individual aflatoxins by preparative TLC. The purity of each recovered AFG1 sample was assessed by TLC.

The pooled, purified AFG1 was analysed by HPLC using a Waters model 480 high pressure liquid chromatograph equipped with a 300 x 4 mm (inside diameter) normal phase silica gel column (10 µm particle size) by the method of the American Oil Chemists' Society (Walker, 1983). Further, the AFG1 was identified by MS with a Finnigan MAT quadrupole mass spectrometer.
Table 1. Production of aflatoxins by wild-type and blocked mutants of A. parasiticus with and without ST

Mycelia were grown in GM for 48 h at 27 °C on a gyratory shaker, after which 1 g (wt) of mycelium was transferred to 10 ml LSRM with or without 555 nmol ST and incubated for an additional 24 h. Results are means of triplicate samples; SD values are given in parentheses. ND, None detected; tr., <1 nmol (g mycelium)-1.

<table>
<thead>
<tr>
<th>Strain</th>
<th>ST</th>
<th>B₁</th>
<th>B₂</th>
<th>G₁</th>
<th>G₂</th>
<th>ST recovered [nmol (g mycelium)-1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (medium only)</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>500 (106)</td>
</tr>
<tr>
<td>nor-1</td>
<td>– tr.</td>
<td>ND</td>
<td>tr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ 449 (27)</td>
<td>ND</td>
<td>15 (3)</td>
<td>0·5 (0·25)</td>
<td>96 (22)</td>
<td></td>
</tr>
<tr>
<td>avr-1 ver-1</td>
<td>– ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ 439 (56)</td>
<td>ND</td>
<td>10 (3)</td>
<td>2·8 (0·9)</td>
<td>71 (16)</td>
<td></td>
</tr>
<tr>
<td>ver-1</td>
<td>– 2 (1)</td>
<td>ND</td>
<td>tr.</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ 417 (9)</td>
<td>ND</td>
<td>19 (4)</td>
<td>1·0 (0·85)</td>
<td>219 (94)</td>
<td></td>
</tr>
<tr>
<td>SU-1 (Wild-type)</td>
<td>– 577 (29)</td>
<td>8 (2)</td>
<td>397 (10)</td>
<td>11(2)</td>
<td>287 (76)</td>
<td></td>
</tr>
</tbody>
</table>

Quantification of radioactive label. Radioactivity was determined by liquid scintillation counting in a Beckmann LS 1800 scintillation counter interfaced to a Hewlett Packard 26171A printer to record c.p.m., efficiency of 14C detection and d.p.m. 14C standards were run for every 50 sample counts, and both silica gel and solvent controls were included. The scintillation fluid for non-aqueous samples was Kodak ‘Ready to Use I’; for aqueous extracts Kodak ‘Ready to Use II was used.

Autoradiography. Developed TLC plates from radioactive tracer studies were marked under longwave UV light, then covered with an unexposed X-OMAT AR film (Eastman Kodak) and placed into a cassette type exposure holder (Kodak). Exposure time at room temperature was 5 weeks. In some experiments, TLC plates were first sprayed with Enhance, then exposed at −10 °C for 5–10 d. All films were developed according to manufacturer’s protocol with GBX developer and fixer (Kodak). The developed autoradiogram was interpreted by superimposing the film onto the corresponding TLC plate.

Chemicals and solvents. All the chemicals used were of American Chemical Society quality. Aflatoxin standards, [14C]AFB₁ [uniformly ring-labelled, specific activity 50 Ci mol-1 (1·85 TBq mol-1)] and ST were purchased from Sigma.

RESULTS

The role of ST as a precursor of aflatoxins was examined in four mutants blocked in the aflatoxin pathway prior to ST. The effect of exogenous ST on aflatoxin production by wild-type A. parasiticus was also examined (Table 1). Three of the mutants produced aflatoxins in the absence of added ST: nor-1 and ver-1 produced trace to low levels of aflatoxin [2 nmol (g mycelium)-1] while avr-1 produced 253 nmol AFB₁ (g mycelium)-1. Only the averantin-accumulating double mutant avr-1 ver-1 was entirely blocked, producing no detectable aflatoxins under the conditions of these experiments. In the presence of ST, all four mutants produced similar levels of AFB₁ [417–488 nmol (g mycelium)-1], AFG₁ [8–19 nmol (g mycelium)-1] and AFG₂ [0·5–2·8 nmol (g mycelium)-1]. No detectable AFB₂ was produced by any of the mutant strains in the presence or absence of ST. When exogenous ST was added to wild-type A. parasiticus SU-1, the level of total aflatoxin increased and shifted from production of all four aflatoxins to production of predominately AFB₁ and AFG₁.

Previous identification of ST as a precursor of AFG₁ was based solely on TLC assay (Singh & Hsieh, 1977; Floyd et al., 1987). We verified the precursor status of ST with respect to AFG₁ by analysing a pooled sample of AFG₁ isolated after biotransformation of ST by the totally blocked double mutant avr-1 ver-1. HPLC analysis yielded a peak with the same retention time (8·4 min) as that of an authentic standard of AFG₁. The identity of AFG₁ was confirmed by MS. The
Table 2. Recovery of aflatoxins B$_1$, B$_2$, G$_1$ and G$_2$ after incubation in replacement medium with and without wild-type and mutant mycelia of A. parasiticus

Mycelia were grown for 48 h in GM; 1 g (wet wt) was transferred to 10 ml LSRM and incubated with 555 nmol aflatoxin; in controls aflatoxins were incubated in LSRM alone. Results are means of triplicate samples; SD values are given in parentheses. ND, None detected.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Aflatoxin added (555 nmol)</th>
<th>Aflatoxin recovered (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B$_1$</td>
<td>B$_2$</td>
</tr>
<tr>
<td>Control (no mycelium)</td>
<td>None</td>
<td>ND</td>
</tr>
<tr>
<td>SU-1 (Wild-type)</td>
<td>None</td>
<td>1930 (83)</td>
</tr>
<tr>
<td>avn-l ver-l (Averantin mutant)</td>
<td>None</td>
<td>1200 (240)</td>
</tr>
<tr>
<td>AFB$_1$</td>
<td>446 (45)</td>
<td>ND</td>
</tr>
<tr>
<td>AFG$_1$</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

In an attempt to demonstrate interconversion of AFB$_1$ to AFG$_1$, individual samples of AFB$_1$ and AFG$_1$ were added separately both to strain SU-1 and to the avn-l ver-l double mutant (Table 2). Since the avn-l ver-l double mutant makes no detectable aflatoxins, recovery of aflatoxins other than the aflatoxin presented to the mutant would be evidence for metabolic conversion. When AFB$_1$ and AFG$_1$ were incubated with the avn-l ver-l double mutant, or in controls lacking mycelia, they were recovered in good yields (AFB$_1$, 52 and 80%; AFG$_1$, 44 and 75%, respectively). Low levels of AFB$_2$ (13 nmol) were detected, suggesting a possible biotransformation of AFB$_1$ to AFB$_2$; however, there was no evidence of an AFB$_1$ to AFG$_1$ biotransformation. In general, the incubation of exogenous aflatoxins with the wild-type strain SU-1 resulted in a simple additive recovery over control values. No increase in AFG$_1$ was observed with added AFB$_1$, although production of AFG$_2$ was higher. When AFG$_1$ was added to the wild-type, recovery of AFB$_1$ was lower and neither AFB$_2$ nor AFG$_2$ were detected.

Since it is possible that exogenous aflatoxins were not taken up by mycelia in replacement cultures, exogenous [${}^{14}$C]AFB$_1$ (4 nmol) was fed to wild-type A. parasiticus before incubation for 24 h. The distribution of ${}^{14}$C label was then measured before and after extraction. When separate samples of culture filtrates and mycelia were placed directly into scintillation fluid, all the original label was recovered (Table 3). When the entire culture (medium plus mycelia) was extracted, 71% of the label was recovered in the chloroform-soluble fraction. When the mycelia were separated from the culture filtrate and washed, 2% of the label was associated with the water-soluble mycelial fraction and 16% with the chloroform-soluble mycelial fraction, indicating probable uptake of 0.6 nmol [${}^{14}$C]AFB$_1$. An autoradiogram of a TLC plate of chromatographed mycelial extract indicated that the majority of the ${}^{14}$C label recovered was associated with a spot corresponding to AFB$_1$ (not shown).

Since published reports of conversion of AFB$_1$ into other aflatoxins were based on recovery of ${}^{14}$C label in biotransformation experiments with wild-type strains, we studied the metabolic conversion of exogenous [${}^{14}$C]AFB$_1$ added to both wild-type A. parasiticus (SU-1) and wild-type A. flavus (SRRC 2002). In addition, [${}^{14}$C]AFB$_1$ was incubated in LSRM in the absence of mycelia. All cultures were extracted and subjected to preparative TLC according to the aforementioned procedures. The TLC plate was divided into zones corresponding to the chromatographic mobilities of the different aflatoxins and entire silica gel fractions were...
Table 3. Recovery of $^{14}$C label from mycelia of wild-type A. parasiticus incubated with exogenous $[^{14}$C]AFB$_1$

Strain SU-1 was grown for 48 h in GM; 1 g (wet wt) mycelium was transferred to 10 ml LSRM and incubated with 4 nmol $[^{14}$C]AFB$_1$ for 24 h. Data are means of four independent observations; SD values are given in parentheses.

<table>
<thead>
<tr>
<th>Fraction used for scintillation counting</th>
<th>$10^{-3} \times$ d.p.m.</th>
<th>%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unextracted culture filtrate†</td>
<td>357 (10)</td>
<td>83</td>
</tr>
<tr>
<td>Unextracted mycelium‡</td>
<td>100 (5)</td>
<td>23</td>
</tr>
<tr>
<td>Chloroform extract of culture filtrate plus mycelium</td>
<td>308 (15)</td>
<td>71</td>
</tr>
<tr>
<td>Chloroform extract of mycelium§</td>
<td>68.8 (13)</td>
<td>16</td>
</tr>
<tr>
<td>Water extract of mycelium∥</td>
<td>9.3 (3)</td>
<td>2</td>
</tr>
</tbody>
</table>

* (d.p.m. recovered/d.p.m. introduced) $\times$ 100.
† Samples of culture filtrate and mycelia placed directly into the scintillation vial.
‡ Both culture filtrate and mycelium extracted into aqueous acetone and partitioned into chloroform.
§ Mycelia separated from culture filtrate, washed, extracted with aqueous acetone and partitioned into chloroform.
∥ Aqueous phase of §.

Table 4. Recovery of $^{14}$C label from preparative TLC plates of chloroform extracts after addition of $[^{14}$C]AFB$_1$ to wild-type mycelia of A. parasiticus and A. flavus, and to controls without mycelia

Mycelia were grown for 48 h in GM; 1 g (wet wt) was transferred to LSRM and incubated for 24 h with $[^{14}$C]AFB$_1$, [4 nmol (431 $\times$ 10$^3$ d.p.m.) added to control and A. parasiticus; 2 nmol (215 $\times$ 10$^3$ d.p.m.) added to A. flavus]. For A. parasiticus and A. flavus, results are means of two independent observations; SD values are given in parentheses. In controls, $[^{14}$C]AFB$_1$ was incubated in LSRM alone.

<table>
<thead>
<tr>
<th>$R_F$ of preparative silica gel zone*</th>
<th>Description of zone</th>
<th>Control (no mycelium)</th>
<th>Wild-type A. parasiticus</th>
<th>Wild-type A. flavus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^{-3} \times$ d.p.m.</td>
<td>%</td>
<td>$10^{-3} \times$ d.p.m.</td>
<td>%</td>
</tr>
<tr>
<td>0-0.05</td>
<td>Origin</td>
<td>1.9</td>
<td>0.4</td>
<td>5.1 (0.5)</td>
</tr>
<tr>
<td>0.05–0.18</td>
<td>Below G$_2$</td>
<td>10.1</td>
<td>2.3</td>
<td>3.6 (0.3)</td>
</tr>
<tr>
<td>0.18–0.22</td>
<td>G$_2$</td>
<td>3.0</td>
<td>0.7</td>
<td>3.5 (6.4)</td>
</tr>
<tr>
<td>0.22–0.25</td>
<td>G$_1$</td>
<td>12.0</td>
<td>2.8</td>
<td>8.8 (0.9)</td>
</tr>
<tr>
<td>0.25–0.26</td>
<td>B$_3$</td>
<td>3.0</td>
<td>0.7</td>
<td>6.4 (0.9)</td>
</tr>
<tr>
<td>0.26–0.36</td>
<td>B$_1$</td>
<td>47.0</td>
<td>10.9</td>
<td>95.0 (2.9)</td>
</tr>
<tr>
<td>0.36–1.0</td>
<td>Above B$_1$</td>
<td>5.9</td>
<td>1.4</td>
<td>–</td>
</tr>
<tr>
<td>Total $^{14}$C label recovered</td>
<td>19.2</td>
<td>26.3</td>
<td>33.1</td>
<td></td>
</tr>
</tbody>
</table>

* Developing solvent was ethanol/methanol/water (96:3:1, by vol.).

scraped into scintillation vials and counted. Total d.p.m. recovered (Table 4) from cultures containing mycelia ranged from 26–32%; only 18% of the label was recovered from medium alone. With each species most of the label was associated with the zone corresponding to AFB$_1$. The level of label associated with AFB$_2$, AFG$_1$ and AFG$_2$ was similar for controls lacking mycelia and for in vivo studies.

 Autoradiograms prepared from chromatographed control extracts of $[^{14}$C]AFB$_1$ incubated with LSRM showed smearing of radioactivity from the origin to an $R_F$ corresponding to AFB$_1$. Extracts from wild-type A. flavus (SRRC 2002) showed similar smearing on autoradiograms.
DISCUSSION

Most published studies on aflatoxin biosynthesis have utilized isotopic labels to measure the incorporation of label from putative precursors into AFB₁ (Heathcote & Hibbert, 1978; Steyn, 1980; Bennett & Christensen, 1983). We examined the biosynthetic origin of AFG₁ using in vivo (whole cell) biotransformations with mutant strains that were blocked in the aflatoxin pathway but were derived from wild-type A. parasiticus that originally produced both B and G aflatoxins. Analysis of end-product aflatoxins by fluorodensitometric procedures permitted reproducible quantification of aflatoxin levels as low as 2-5 ng.

The four characterized anthraquinone-accumulating mutants, nor-I, avr-I, ver-I and aun-I ver-I are all blocked in aflatoxin biosynthesis prior to ST. In feeding studies with ST, after 24 h incubation, all four blocked mutants produced AFB₁, AFG₁ and AFG₂, but no detectable AFB₂. Similar results for 24 h incubations were reported by Floyd et al. (1987) for avr-I, ver-I and aun-I ver-I; when incubations with ST were continued for 96 h AFB₂ was detected. Floyd et al. (1987) used autoclaved mycelia as controls; in this study living mycelia without ST were used. The controls with living mycelia demonstrated that the nor-I, avr-I and ver-I mutants are 'leaky'; i.e. they produce low levels of aflatoxin in the absence of ST. The strain aun-I ver-I is a double mutant, isolated after nitrosoguanidine treatment of ver-I (Bennett et al., 1980). This double mutant strain has never produced detectable aflatoxins in our laboratory and is therefore the mutant of choice for studying the late stages of the aflatoxin pathway.

The production of AFG₁ from ST has been reported before (Singh & Hsieh, 1977; Floyd et al., 1987). In those studies, AFG₁ was identified by TLC assay and biotransformation was effected by the 'leaky' ver-I mutant. We have verified the role of ST as a precursor of AFG₁. The 'non-leaky' aun-I ver-I double mutant was incubated with ST; the resultant AFG₁ was purified and confirmed by HPLC using fluorescent detection and by MS.

The mutant bioconversion strategy was then attempted in order to confirm the role of AFB₁ as the precursor of AFG₁. Exogenous unlabelled AFB₁ and AFG₁ were presented to replacement cultures of the aun-I ver-I double mutant, and after 24 h incubation cultures were extracted (Table 2). No interconversion of AFB₁ to AFG₁ or AFG₂ was found, nor did AFG₁ demonstrate interconversion in this study. There was evidence for a slight conversion (2%) of AFB₁ to AFB₂ but this would have to be established by further studies and could be attributed to streaking. This confirms the lack of AFB₁ to AFG₁ bioconversion reported by Floyd et al. (1987) using ver-I.

It is possible that aflatoxins are not permeable through the fungal cell wall and hence not available at the biosynthetic site. To test this hypothesis we measured the fungal uptake of [14C]AFB₁, and found that 16% of the label was associated with the chloroform-soluble portion of the mycelia, indicating uptake of [14C]AFB₁. To our knowledge this is the first report of movement of AFB₁ from the outside to the inside of the cell. It is well-known that aflatoxins synthesized in the mycelium readily cross the cell wall into the culture medium (Shih & Marth, 1973). Therefore, our inability to biotransform exogenous aflatoxins cannot be dismissed as a mere problem of permeability.

The two published reports concerning AFB₁ to AFG₁ conversions utilized [14C]AFB₁. Incorporation of label into aflatoxin was measured after preparative TLC. Maggon & Venkitasubramanian (1973) used cell-free homogenates and reported incorporation of label from [14C]AFB₁ into AFB₂ (0.8%), AFG₁ (2.9%) and AFG₂ (0.8%). Heathcote et al. (1976) used whole cell biotransformations with wild-type A. parasiticus. Five separate trials were reported: label from [14C]AFB₁ was recovered in AFB₂ (0.5-3.5%), AFG₁ (0.3-3.7%) and AFG₂ (0.6-1.8%). In our studies, two wild-type strains were selected for biotransformations. Controls consisted of exogenous [14C]AFB₁ incubated and extracted from LSRM in the absence of mycelium. In our controls, recovery of radioactivity from preparative TLC zones corresponding with AFB₂ was 0.7%; with AFG₁, 2.6%; and with AFG₂, 0.7%. These control values were within the range of recovery of label for our incubations with mycelia and with the values given by Maggon & Venkitasubramanian (1973). At one end of the range, the incorporation values reported by Heathcote et al. (1976) are higher. In our experiments autoradiography revealed smearing of radioactivity on the preparative TLC plates. We conclude that the low levels of
radioactivity found in aflatoxins other than AFB₁ after preparative TLC can represent artifacts of the method rather than metabolic interconversion.

In summary, ST serves as a precursor of both B and G aflatoxins. No AFB₁ to AFG₁ conversion could be demonstrated either in feeding studies with a blocked mutant or in radiotracer studies with wild-type strains. These results suggest independent pathways from ST to the B and G aflatoxins. AFB₁ is not an intermediate, but rather an end-product of the aflatoxin biosynthetic pathway.

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