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# Effect of Aldehyde Trapping Agents on Ethylene and Aflatoxin Biogenesis in *Aspergillus parasiticus*

Arun Sharma,* S. R. Padwal-Desai, and G. B. Nadkarni

Dimedone, an aldehyde trapping agent, was found to stimulate ethylene evolution while suppressing aflatoxin biogenesis in *Aspergillus parasiticus*. Dimedone did not seem to affect the growth of the fungus. Potassium metabisulfite, another aldehyde trapping agent, was also found to be an effective inhibitor of aflatoxin biogenesis. Unlike dimedone, however, this compound inhibited the growth of the fungus. Stimulation of ethylene evolution by aldehyde trapping agents shows that ethanol is not the precursor of ethylene in fungi.

The mechanisms that trigger biosynthesis of secondary metabolites are still obscure. Ethylene has been shown to inhibit the biogenesis of aflatoxin and other secondary metabolites (Sharma et al., 1985; Sharma and Padwal-Desai, 1986). Though L-methionine is the precursor of ethylene in plants, the precursor of ethylene in microbes is yet to be established. Ethanol was earlier presumed to be the precursor of microbial ethylene (Abeles, 1973). However, recently $\alpha$-ketoglutaric acid was shown to be converted to ethylene in a cell-free preparation of *Penicillium digitatum* (Fukuda et al., 1986). Aldehyde trapping agents could be employed to test the earlier hypothesis. Acetaldehyde is the known precursor of ethanol. Therefore, if ethanol were the precursor of ethylene, aldehyde trapping agents should suppress the synthesis of ethylene and hence enhance aflatoxin formation in cultures. The validity of this presumption has been tested in the present report using dimedone (5,5-dimethyl-1,3-cyclohexanedione) and metabisulfite, the two well-known aldehyde trapping agents.

Food Technology & Enzyme Engineering Division, Bhabha Atomic Research Centre, Bombay 400 085, India.

## EXPERIMENTAL SECTION

**Organism and Culture Conditions.** Aflatoxin-producing strains of *Aspergillus parasiticus* NRRL 3145, NRRL 2999, and ATCC 15517 were used in these studies. The cultures were maintained on potato dextrose agar (Difco Laboratories, Detroit, MI), and regular transfer to fresh slants was carried out at 1-month intervals.

**Preparation of Spore Suspension.** The recultivation of stock cultures of the above fungi and the technique of preparation of spore suspension have been described earlier (Sharma et al., 1984). A synthetic growth medium containing 20% (w/v) of glucose and salts was used. For inoculations, 1-mL aliquots of the spore suspension containing $10^5$ spores were added to the flasks containing 50 mL of growth medium.

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Table I. Effect of Dimedone on Growth and Aflatoxin Formation in *A. parasiticus* NRRL 3145[a]

| dimedone concn, mM | growth, mg | aflatoxin, μg |
|---|---|---|
| 0 (control) | 700 ± 49 | 160 ± 50 |
| 0.2 | 750 ± 83 | 77.5 ± 30 |
| 0.10 | 710 ± 55 | 22.5 ± 80 |
| 0.20 | 770 ± 80 | 2.2 ± 0.3 |

[a] Conditions: inoculum, ca. $10^5$ spores; shake culture (150 rpm); incubation 6 days at ambient temperature (28–30 °C); growth measured as dry weight and total aflatoxin estimated as outlined in the text. Readings are the averages of at least three replicates.

**Measurement of Growth.** Mycelial dry weight was determined by filtering the contents of the flask through Whatman No. 541 filter paper, drying at 85 °C for 24 h, weighing, and taking the average of at least three replicates.

**Extraction and Estimation of Aflatoxin.** Extraction of aflatoxin, purification, and estimation using minicolumns were carried out as described earlier (Sharma et al., 1985) using a fluorotoxin meter (Velasco fluorotoxin meter; Neotec Instruments Inc., Silver Spring, MD).

**Estimation of Ethylene.** Ethylene was estimated by a gas chromatographic method (Sharma et al., 1985). The flasks (100-mL capacity) containing 50 mL of synthetic glucose salt medium were inoculated with 1-mL aliquots of spore suspension ($10^5$ spores) of each of the fungal strains listed above. The flasks were subsequently incubated on a rotary shaker (150 rpm) at ambient temperature (28–30 °C) for 72 h. A measured amount of the headspace gases was withdrawn by syringe. The headspace gases were analyzed for ethylene in a gas chromatograph. Standard ethylene was obtained from Matheson Gas Products, Rutherford, NJ. The operating conditions were as follows: column, Porapak T (183 × 0.3 cm); carrier gas, nitrogen; flow rate, 30 mL $min^{-1}$; temperatures, column 60 °C, injection port 110 °C, flame ionization detector 120 °C.

**Experiments with Dimedone.** Dimedone (5,5-dimethyl-1,3-cyclohexanedione, molecular weight 140.18) was procured from British Drug House, Poole, England. The stock solution of dimedone (10 mM) in methanol–water (1:1, v/v) was sterilized by passing through a membrane filter (Millipore 0.45 μm, 25 mm). Measured aliquots of 0.1, 0.5, and 1 mL of dimedone solution (10 mM) were added to the experimental flasks containing the growth medium (50 mL) prior to inoculation with the fungal spores, giving the final concentrations of 0.02, 0.1 and 0.2 mM, respectively.

**Experiments with Bisulfite.** Potassium metabisulfite (BDH, Poole, England) solution (10 mM, aqueous) was also sterilized by passing through a membrane filter (Millipore 0.45 μm, 25 mm), and measured aliquots of 0.1, 0.5, and 1 mL were added to the experimental flasks containing the growth medium prior to inoculation with the fungal spores.

## RESULTS AND DISCUSSION

Table I shows the effect of dimedone on the growth and aflatoxin biogenesis in the control and dimedone-treated cultures of *Aspergillus parasiticus* NRRL 3145. Without affecting the growth of the fungus, dimedone showed remarkable inhibition of aflatoxin biosynthesis. An increased inhibition of aflatoxin biogenesis was observed with an increase in the dimedone concentration in the medium. Biogenesis of aflatoxin was almost completely inhibited at 0.2 mM level.

Table II shows the effect of dimedone on the growth and ethylene and aflatoxin biogenesis in the three aflatoxin-producing strains. Both the growth and aflatoxin yields were in general low in these cultures because of oxygen limiting conditions induced by capping of the flasks for the purpose of retention of ethylene. Dimedone-treated cultures apparently showed more growth as compared to the controls. The maximum increase in the growth was observed in case of *A. parasiticus* NRRL 2999, followed by the strains NRRL 3145 and ATCC 15517. Dimedone-treated cultures also showed a 20-fold increase in ethylene evolution in the case of *A. parasiticus* ATCC 15517 followed by a 15-fold increase in the case of strain NRRL 2999 and a 12-fold increase in the strain NRRL 3145. Aflatoxin biogenesis by all the three strains was inhibited in the presence of dimedone. The maximal reduction in aflatoxin yield was observed in *A. parasiticus* ATCC 15517 followed by the strains NRRL 2999 and NRRL 3145. Dimedone-stimulated ethylene evolution was maximum in *A. parasiticus* ATCC 15517, which also showed the highest reduction in aflatoxin production.

The results presented above are thus at variance with the presumption that ethanol was the precursor of ethylene in fungi (Abeles, 1973). The blocking of ethanol pathway of the fungus by acetaldehyde trapping reagents could presumably shunt pyruvate to the tricarboxylic acid cycle to supply α-ketoglutarate for ethylene biogenesis (Fukuda et al., 1986). The inhibition of aflatoxin biogenesis in the cultures concurrent with the stimulation of ethylene biogenesis upon treatment with dimedone confirms our earlier observations that ethylene is involved in suppressing aflatoxin biogenesis (Sharma et al., 1985).

As can be seen in Table III, even potassium metabisulfite was effective in inhibiting aflatoxin biogenesis in cultures. Unlike dimedone, the growth of the organism was affected by bisulfite. A concentration of 0.2 mM completely inhibited the growth of the organism. The observed inhibition of growth by bisulfite could be at-

Table II. Effect of Dimedone on Growth and Ethylene and Aflatoxin Biogenesis by the Three Strains of Aflatoxin-Producing Fungi[a]

| strain | | growth, mg | inc, % | ethylene, μmol | inc, fold | aflatoxin, μg | dec, % |
|---|---|---|---|---|---|---|---|
| ATCC 15517 | A | 548 ± 39 | | 8.9 ± 3.25 | | 16.0 ± 1.6 | |
| | B | 630 ● 45 | 14.9 | 182.7 ± 20.30 | 20.5 | 9.5 ± 1.0 | 40.0 |
| NRRL 3145 | A | 335 ± 25 | | 16.24 ± 4.06 | | 20.0 ± 2.0 | |
| | B | 404 ± 29 | 20.6 | 203.0 ± 10.15 | 12.5 | 13.5 ± 1.5 | 32.5 |
| NRRL 2999 | A | 379 ± 30 | | 12.9 ± 2.84 | | 10.0 ± 1.2 | |
| | B | 496 ± 36 | 30.8 | 203.0 ± 16.24 | 15.7 | 7.0 ± 0.8 | 30.0 |

[a] Key: A, without dimedone; B, with 0.02 mM dimedone in the medium. Conditions: incubation, 72 h; others as in Table I. The culture flasks were capped with silicon rubber septa for the retention of ethylene. For the estimation of ethylene, a known aliquot of standard ethylene gas was injected into the gas chromatograph and the peak height was noted. From the peak heights obtained from the ethylene in the headspace of flasks, the quantity of total ethylene was calculated, taking into account the volume of the flask. The readings are the averages of at least three replicates.

**Table III. Effect of Potassium Metabisulfite on the Growth and Aflatoxin Biosynthesis in the Cultures of *A. parasiticus* NRRL 3145[a]**

| potassium bisulfite concn, mM | growth, mg | aflatoxin, μg |
|---|---|---|
| 0 | 700 ± 49 | 160 ± 50 |
| 0.02 | 660 ± 42 | 75.0 ± 50 |
| 0.10 | 500 ± 36 | 6.0 ± 0.5 |
| 0.20 | ng | nd |

[a] Culture conditions were as in Table I. Key: ng, no growth; nd, not detected. A 10 mM aqueous solution of potassium metabisulfite was sterilized by passage through a Millipore filter and used in different concentrations during the experiment. The readings are the averages of three replicates.

tributed to its nonspecific addition reactions with C=C and C=O groups and the formation of α-hydroxy sulfonic acids (Joslyn and Braverman, 1954). In an earlier study potassium sulfite, but not bisulfite, was reported to be inhibitory to aflatoxin biosynthesis (Davis and Diener, 1967). Higher concentrations of bisulfite are also known to degrade aflatoxin (Doyle and Marth, 1978; Moerck et al., 1980). The ability of bisulfite to cause inhibition of aflatoxin biosynthesis as well as degradation could offer an effective method to control aflatoxin in stored commodities. The use of bisulfite is permitted in foods (Roberts and McWeeny, 1972). This compound has also been used for changing yeast fermentation from alcohol to glycerol (West et al., 1968).

## CONCLUSIONS

Ethylene has been found to inhibit aflatoxin biogenesis (Sharma et al., 1985). In microbes immediate precursor of ethylene has not been confirmed. Earlier works presumed it to be ethanol. However, the present study using aldehyde trapping agents clearly shows that ethanol is not the precursor of ethylene in fungi. α-Ketoglutaric acid was recently found to be converted to ethylene in a cell-free system by Fukuda et al. (1986). This study indirectly supports their findings. Antitoxigenic properties of aldehyde trapping agents could be exploited for aflatoxin-free storage of agricultural and food commodities.

**Registry No.** $K_2S_2O_5$, 16731-55-8; ethylene, 74-85-1; dimedone, 126-81-8.

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