PREPARATION OF LABELED AFLATOXIN B_1 FROM ACETATE-1, 2-^{14}C.

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ABSTRACT

Aflatoxin B_1-^{14}C was produced by resting cell cultures of Aspergillus flavus (NRRL 3145) from a sodium acetate - 1, 2-^{14}C precursor. A specific activity of 455 uCi/uM was determined for the purified aflatoxin B_1-^{14}C.

The production of ^{14}C-labeled aflatoxin with high specific activities by resting cell cultures of Aspergillus parasiticus (ATCC 15517) from acetate-1-^{14}C has been determined (4). Studies dealing with the biosynthesis of aflatoxins (1) have disclosed that aflatoxin B_1 produced by A. parasiticus from acetate-1-^{14}C or acetate-2-^{14}C result in the incorporation of ^{14}C into 9 or 7 of the 16 ring carbon positions, respectively.

Labeled aflatoxin B_1 is useful in investigations dealing with metabolism and tissue distribution (7) because aflatoxin B_1 is the most toxic form (6) and is generally produced in greater quantities than the other major toxins (2). It would be advantageous to utilize aflatoxin B_1-^{14}C with ^{14}C in all of the 16 ring positions because of higher specific activities obtainable and greater reliability in metabolism, excretion, and tissue distribution studies. The present report describes the isolation and characterization of such a toxin.

Resting cell cultures of A. flavus (NRRL 3145) were prepared according to Hsieh and Mateles (4). The resting medium, containing 30 g of glucose per liter, was inoculated with 1.5 g (wet weight) of washed mold pellets per 10 ml of medium contained in rubber-stoppered 50 ml baffled Erlenmeyer flasks. The rubber stoppers

were equipped with tubing connected from a positive pressure diaphram type aquarium pump (Metaframe Aquarium Products, Maywood, New Jersey) to a barium hydroxide trap system to collect the expired $^{14}CO_2$ produced by the resting culture. The air flow was adjusted to 1 ml per 4.5 sec.

Sodium acetate-1, 2-^{14}C (specific activity 55.2 mCi/mM, New England Nuclear Corporation, Boston, Massachusetts) was combined with 955.5 u moles of unlabeled sodium acetate to yield 960 u moles contained in 2.4 ml of sterile distilled water. The acetate was added to the resting culture at a rate of 0.4 ml per addition at regular intervals for 20 hours. The filtered resting medium was extracted three times with equal volumes of chloroform and the extracts pooled and concentrated. The concentrate was applied to silica gel GHR (Brinkman Instruments, Westbury, New York) thin-layer plates and developed in benzene-methanol-acetic acid (18:1:1). Fluorescing spots corresponding to aflatoxin B_1 were removed and eluted with chloroform-methanol (9:1). Subsequent thin-layer chromatography in chloroform-acetone (9:1) yielded pure aflatoxin B_1.

The extract was dissolved in benzene-acetonitrile (98:2) and the optical density was compared to known standards at 350 nm (5).

The radioactivity of 1 ug of aflatoxin B_1 in toluene-based scintillation fluid was measured with a packard TriCarb model 2405 liquid scintillation spectrometer.

Sixty ug of aflatoxin B_1 with a specific activity of 455 uCi/uM were produced from five resting cell cultures. Under similar growth conditions, other workers (4) reported the production of aflatoxins (80% aflatoxin B_1) from acetate-1-^{14}C with specific activities of about 200 uCi/uM. Our results are in general agreement since the double carbon label in the acetate precursor contributes to an increase in the specific activity of aflatoxin B_1-^{14}C by a factor of approximately 2.

The efficiency of acetate-1,2-^{14}C incorporation expressed as relative isotopic content (RIC) and percentage of incorporation (PI) were calculated to be 8.2 and

6.9 respectively, according to the following equations:

$$RIC = A_2/A_1$$

Where A_2 and A_1 are the specific activities of the product and precursor, respectively, expressed in mCi/mM, and

$$PI = (100)(RIC)(X)/(F)$$

where X and F are the amounts of product and precursor, respectively, expressed in u moles.(4). Both values are higher than those previously reported for acetate-1-^{14}C(4) and reflect the influence of the additional carbon label in the precursor as well as the performance of the strain of A. flavus used in this study.

The concentration of aflatoxin B_1-^{14}C produced was relatively low. Hsieh and Mateles (3,4) reported higher levels of aflatoxin produced under similar conditions, and concluded that higher initial concentration of glucose resulted in greater yields of aflatoxin. The difference may be attributable to the species of microorganism used in the presently reported study.

LITERATURE CITED

1. Biollaz, M., Buchi, G., and Milne, G. J. Amer. Chem. Soc. 92:105 (1970).
2. Diener, U. L. and Davis, N. D. "Aflatoxin Formation by Aspergillus flavus" Goldblatt, L. A., Academic Press, N. Y., 1969.
3. Hsieh, D. P. H. and Mateles, R. I. Biochim. Biophys. Acta. 208:482 (1970).
4. Hsieh, D. P. H. and Mateles, R. I. Appl. Microbiol. 22:79 (1971).
5. Rodricks, J. V. and Stoloff, L. J. Assoc. Offic. Anal. Chem. 53:92 (1970).
6. Wogan, G. N. Bacteriol. Rev. 30:460 (1966).
7. Wogan, G. N., Edwards, G. S. and Shank, R. C. Cancer Res. 27:1729 (1967).

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