Biological Reactive Intermediates of Bisfuranoid Mycotoxins

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Introduction

Bisfuranoid mycotoxins are a family of toxic fungal metabolites that contain in their molecules a characteristic dihydrobisfuran ring structure, such as aflatoxins B₁, G₁, M₁, versicolorin A, and sterigmatocystin (Fig. 1).

Fig. 1. Structures of some bisfuranoid mycotoxins.

Among these, Aflatoxin B₁ (AFB₁) and sterigmatocystin (ST) are well known potent hepatocarcinogens in some laboratory animals (11). AFB₁ was designated by IARC as a human carcinogen in 1987 (12) and is considered by the agency as an etiological factor of primary liver cancer in some African and Asian countries, such as Mozambique, Swaziland, Kenya, Thailand, Taiwan, and China.

Occurrence

The bisfuranoid mycotoxins are biogenetically related to AFB₁, a decaketides derived from 10 acetate units (8). In the pathway of aflatoxin biosynthesis, one acetyl CoA primer and 9 malonyl CoA units undergo a head-to-tail assembly to form an C₁₄ anthraquinone with a C₆ side chain. The C₆ side chain then undergoes deacetylation and cyclization to form the characteristic dihydrobisfuran ring of versicolorin A (VA). The anthraquinone moiety of VA is then converted to xanthone to form sterigmatocystin. The xanthone finally is converted to coumarin to form AFB₁. AFB₁ and ST has been found to occur in corn, peanut, cottonseed, and other calory-rich commodities (15). The occurrence of aflatoxins and related bisfuranoid mycotoxins in food commodities has been a worldwide concern.

Mutagenicity and Carcinogenicity

Ames *Salmonella typhimurium* microsomal/mutagenicity assays on the intermediates of the aflatoxin pathway have revealed that only the compounds containing the dihydrobisfuran ring are mutagens (24). The structure required for the mutagenicity has been determined to be the vinyl ether double bond of the terminal furan ring. The derivatives of the bisfuranoid mycotoxins with this double bond saturated, such as AFB₂, G₂, and versicolorin C, are not mutagenic in the Ames test.

The potent mutagenicity of bisfuranoid mycotoxins suggests that they are potential genetic carcinogens. Hepatocarcinogenicity of AFB₁, ST, and VA has been demonstrated in laboratory animals, especially the rat and rainbow trout (7, 11).

Biological Reactive Intermediates

The mechanism of toxic action of AFB₁ has been extensively studied. It is now established that AFB₁ is a procarcinogen that requires metabolic activation to exert toxic effects (13, 19). The active form of AFB₁ has been identified as the 8,9-oxide of AFB₁, or AFB₁ epoxide (AFBO) (5, 13). Being a strong electrophile, AFBO reacts readily with the nucleophiles in DNA, RNA, protein, and reduced glutathione. The formation of AFB-DNA adducts (ADA) has been associated with the carcinogenicity of AFB₁, whereas the removal of AFBO from tissues by conjugation with glutathione as mediated by the glutathione S-transferase has been associated

with the animal resistance to the carcinogenicity (1, 2, 14). Thus the large difference in species susceptibility to the carcinogenicity of AFB is attributed to the relative abundance of activation and deactivation enzymes. A recent in vitro study (3) has revealed that human livers are one half as active as the rat livers in activation but are less than one tenth as active in deactivation, suggesting that humans could be more than five times as sensitive as rats to the carcinogenicity of AFB₁. In comparison, mice are twice as active as rats in activation but 100 times as active in deactivation, suggesting that mice could be 50 times more resistant to the carcinogenicity of AFB, consistent with in vivo experimental observations (22).

In addition to epoxidation, the primary metabolism of AFB₁ also includes hydroxylation to AFM₁ and AFQ₁, demethylation to AFP₁, and reduction to AFL (9). These primary metabolites, whose structures are shown in Fig. 2, all contain the vinyl ether double bond in the dihydrobisfuran ring moiety, and therefore can be epoxidated and activated to form mutagenic species (23). However, the mutagenic potency of AFB₁ is much greater than that of its metabolites, indicating that the coumarin structure in AFB₁ is the optimal structure for the biological activity of

Fig. 2. Primary metabolites of AFB₁

bisfuranoid compounds. When a susceptible animal such as rat is given AFB₁, the hepatocellular DNA would be modified to a large extent by the epoxide of AFB₁ and to a small extent by the epoxides of AFB₁ metabolites (4). The principal adduct, 8,9-dihydro-9-hydroxy-(N7-guanyl) AFB₁, accounts for more than 80% of the total aflatoxin-DNA adducts.

The appropriate enzymes that catalyze the formation of the various AFB₁ metabolites have been largely isolated and characterized. The epoxidase that activates AFB₁ is localized both in the endoplasmic reticulum and the nuclear membrane (18, 20). The principal enzyme involved in the bioactivation of aflatoxins in human liver has been identified as cytochrome P-450_{NF}, the nifedipine oxidase (17).

Under physiological conditions, AFBO is readily hydrolyzed, enzymatically or spontaneously, to form a dihydrodiol metabolite of AFB₁. The latter undergoes a ring opening rearrangement to form a dialdehyde intermediate. The carbonyl group of the dialdehyde then forms a Schiff base with the primary NH₂ group of lysine in albumin to form a stable structure. The pathways for the formation of DNA and albumin adducts of AFB₁ from AFBO is shown in Fig. 3.

Fig. 3. Formation of DNA and albumin adducts of AFB₁ from AFBO

DNA and Albumin Adducts as Markers for Exposure Assessment

Active work has been going on in recent years in the use of DNA and albumin adducts of AFB₁ as markers to assess individual exposure to AFB₁ to confirm the correlation between aflatoxin and human liver cancer. Two recent limited studies by Garner et al. (6) and Hsieh et al. (10), both using the enzyme-linked immunosorbent assay (ELISA) method, have revealed that most of the hepatoma patients in Czechoslovakia (7 out of 8) and Taiwan (9 out of 9) that were monitored had the ADA in their livers. These results however need confirmation by HPLC because the ELISA used in these studies may not be completely specific.

Similarly, in a recent study (20), peripheral blood samples obtained from individuals from Thailand and Kenya, where AF exposure is likely high, and from France, where AF exposure is likely low, were assayed for AFB₁-albumin adducts by ELISA and HPLC methods. Levels of 7 to 338 pg AF/mg albumin were observed in the former two countries while no adducts were detected in samples from France. The exposure assessment at an individual level using DNA and protein adducts of AFB₁ appears very promising. The data obtained so far are consistent with the theory that aflatoxin is involved in the etiology of human liver cancer.

Studies on the DNA binding of ST have revealed that it is also activated by epoxidation at the vinyl ether double bond of the dihydrobisfuran ring moiety and forms N7 guanine adducts with DNA (16). It is likely that the mode of action of all bisfuranoid compounds is similar to that of AFB₁ and that they are all genetic carcinogens. The difference in the relative mutagenic potency of various bisfuranoid mycotoxins makes this family of compounds useful in the study of quantitative structure-activity relationships.

Summary

Based on the mode of action of AFB₁ and the activities of its biologically active intermediates, one may conclude that:

1. The mode of toxic action of the bisfuranoid mycotoxin is through epoxidation of the vinyl ether double bond of their dihydrobisfuran functionality.
2. The DNA and plasma albumin adducts formed *in vivo* may be useful in the molecular dosimetry of these environmental carcinogens.

3. Monitoring of these adducts of AFB₁ in biological samples so far indicates that aflatoxin is likely involved in the etiology of human liver cancer.

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