Lack of Mutagenicity of Products Formed by Ammoniation of Gossypol

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ABSTRACT

Gossypol distributed on an inert gel carrier was treated in a pressurized ammoniation chamber used to detoxify aflatoxin-contaminated cottonseed meal. Ames tests for mutagenicity were performed on untreated gossypol and on fractions eluted sequentially from the gel. Nonammoniated gossypol was not mutagenic nor were any of the products formed from gossypol by ammoniation.

INTRODUCTION

Cottonseed meal produced from seed infected with *Aspergillus flavus* can contain aflatoxins. In addition to their mutagenicity (1), aflatoxins are toxic to domestic livestock and poultry (2). Norred et al. (3) have shown that ammoniation reduces the toxic effects of aflatoxins. Ames test results indicate a reduction in mutagenic potential of aflatoxin-derived products (4), both from a system where toxin contaminated cottonseed meal was ammoniated (5) and in a model system in which the toxin was ammoniated in the same equipment and under the same conditions but in the absence of meal (6).

Cottonseed meal derived from traditional varieties of glanded cottonseed contains gossypol, a unique polyphenolic bi-naphthyl aldehyde. The biological activity of gossypol is well known (7). In large doses it is a cellular, vascular, and nervous system toxin, causing inflammation in tissues and sometimes even necrosis (8). The present study was undertaken to determine the mutagenic potential of the products formed by ammoniation of gossypol in the same system used for ammoniating aflatoxin β_1 in the absence of meal (9). Even though gossypol alone is not mutagenic (10, 11), it is of value to determine the mutagenic potential of any of the reaction products of ammonia with gossypol.

MATERIALS AND METHODS

Fraction Preparation

Approximately 2 g of a gossypol-acetic acid complex was partitioned between diethyl ether and water; the acidic water layer was discarded, and the ether extract was evaporated in a vacuum oven and crystalline gossypol recovered. Five hundred mg of this gossypol was dissolved in acetone and distributed by pipette onto ca 15 g of silica gel H. After drying overnight in a vacuum oven, the gel was divided into two portions. Each portion was transferred to a square of fine mesh nylon cloth secured as a small bag fastened with a twist of stainless steel wire. The subsequent ammoniation of gossypol distributed on this inert carrier was identical to that previously reported for ammoniation of aflatoxin β_1 (11). Treatment was for 30 min, 40 psig at 100 C.

After air-drying, the gel was transferred to a 150 ml coarse (60 μm pore) fritted-glass filter funnel. Solubles were eluted sequentially with cyclohexane, benzene, methylene chloride, acetone and methanol. Elution with methanol was continued until the gel was visibly clear of pigmented material. From 1 to 2 l of each solvent was used. Solvent volume was decreased by evaporation under reduced pressure. Concentrated extracts were transferred to tared beakers, residual solvents were removed at room temperature in a vacuum oven and the extract weight determined.

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Mutagenicity Assay

Salmonella typhimurium strains TA98 and TA100 were obtained from B.N. Ames, University of California, Berkeley. Cultures were maintained and grown as described by McCann et al. (12). S-9 hepatic homogenate (from phenobarbital-induced rats) was purchased from Litton Bionetics (Kensington, Maryland) or prepared as described by McCann et al. (12) using livers from rats induced with arochlor 1254. The mutagenicity assay was conducted as described by Ames et al. (13) on gossypol ammoniation products and on suitable controls dissolved in dimethyl sulfoxide and tested according to the soft agar incorporation protocol with or without incubation with rat liver S-9 mix.

RESULTS AND DISCUSSION

Weights on fractions from the ammoniated gossypol complex indicated complete recovery of material fractionated. Apparently ammoniation did not produce volatiles from gossypol.

Results of the Salmonella/microsome mutagenicity test on the various ammoniation fractions are summarized in Table I. Testing of all fractions at two levels indicated little mutagenic difference suggesting that the increases of colonies over control levels for TA100 probably are not a result of mutagenesis but rather incorporation of nutrients into the plate allowing increased background bacterial growth. In no case was a two-fold increase observed; thus, all samples can be regarded as non-mutagenic for TA100. For TA98 no increases over control levels of revertants were found for any of the extracts tested. From these results the products of ammoniated gossypol are non-mutagenic in the Ames test. It may be noted, however, that the methylene chloride extract was slightly toxic to strain TA100 when tested in the absence of S-9 mix. In the presence of S-9 mix the tox-

TABLE I

Mutagenicity of Gossypol and Gossypol Ammoniation Products Separated by Solubility

Sample	Amount tested µg/plate	Histidine revertants/plate			
		Strain			
		98	98+S9 mix	100	100+S9 mix[a]
Ammoniated gossypol					
Cyclohexane eluant	20	3	33	132	448
	500	9	38	474	528
Benzene eluant	20	10	46	202	500
	500	28	47	568	504
Methylene chloride eluant	20	–	39	54	352
	500	20	35	68	460
Acetone eluant	20	10	40	220	600
	500	15	72	157	540
Methanol eluant	20	–	35	110	516
	500	72	57	284	452
Non-ammoniated gossypol	20	–	51	–	416
	500	21	40	81	424
DMSO		15	48	238	316

[a]Aflatoxin B_1 tested at 0.1 µg/plate produced 1672 revertants (14).

icity was lost, indicating that a toxic product was destroyed by the cytochrome P450 oxidation process. This product may be residual gossypol, because a similar loss of toxicity was found for non-ammoniated gossypol.

For aflatoxin related materials, there was a positive correlation between mutagenicity and toxicity (14). Compounds that were mutagenic were also toxic in the chick embryo test, and those with reduced mutagenicity were non-toxic. It has been shown previously that gossypol alone is not mutagenic (9,10), a result confirmed in the present study. It also would appear that there is a lack of mutagenicity of products formed by ammoniation of gossypol. These results should allay any fears that ammoniation could form gossypol-derived products harmful to animals ingesting feed made from ammoniated cottonseed products. Moreover, these results may help explain the encouraging reports of feeding studies using cottonseed meal following ammoniation (Waldrup, P.W., personal communication, and 15).

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