Interactions of Aflatoxin with Histones and DNA

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Summary. The interactions of aflatoxin B₁ with certain histone fractions and DNA were investigated by means of viscosity measurements and equilibrium dialysis.

Two main histone fractions (F₂b and F₁), both lysine-rich, were examined after treatment with the toxin. Fraction 2b and 1 differ in amino acid composition and behave differently, in regard to gross conformation, in the presence of electrolytes. Aflatoxin increased the viscosity of fraction 2b but affected the viscosity of fraction 1 only slightly. Equilibrium dialysis experiments showed that aflatoxin was bound to both histone fractions.

Aflatoxin also increased the viscosity of DNA and equilibrium dialysis showed that 1 molecule of the toxin was bound to approximately every 5 nucleotides of the nucleic acid.

Binding constants for the aflatoxin complexes were calculated as 1000 for F₂b, 700 for F₁, and 5500 for DNA.

The biological implications of these data, in regard to the effect aflatoxin has on the information-transcription process, are discussed.

Histones have been implicated in the control mechanism of the transcription process. They have been shown to inhibit DNA synthesis in vitro as well as DNA-dependent RNA synthesis (7, 12). Qualitative changes in the base composition of newly synthesized RNA in the presence of histone have also been reported. Whereas interaction of carcinogens with nucleic acids have been studied extensively, little is known of the effects these compounds have on histone. Ts'o and Lu (22), although using rather heterogeneous preparations of histone, reported binding of aromatic hydrocarbons and steroid hormones. In view of the possible role histones play, in regard to gene action, it seemed worthwhile to examine the interactions of histone and aflatoxin, the carcinogenic metabolite of Aspergillus flavus. Aflatoxin inhibits amino acid incorporation by liver slices and amino acid activation by liver and Escherichia coli (17, 18).

Gablik's et al. (5), reported that in Chang liver cell cultures treated with aflatoxin, protein and RNA levels were lowered whereas DNA levels remained relatively constant. Others reported inhibition of DNA synthesis by aflatoxin in human lung cell cultures (11). Binding of aflatoxin to DNA has now been reported (3, 19).

Aflatoxin also produces a number of toxic effects in plant tissues. Chlorophyll development in seedlings of Lepidium sativum and cotyledons of cottonseed is inhibited by the toxin (1, 16). Aflatoxin also inhibits mitosis and causes fragmentation and bridging of chromosomes in roots of Vicia faba (13). Black and Altschul (1) reported inhibition by aflatoxin of gibberellin-induced lipase formation in cotyledon. Furthermore it was observed that under certain conditions aflatoxin could stimulate protein synthesis in cotyledons and exhibited similar activity to that of gibberellic acid (10).

The present paper describes direct effects of aflatoxin upon 2 histone fractions, comparative experiments with DNA, and discusses the implications of the experimental results in regard to the effects of aflatoxin on living cells.

Materials and Methods

Viscosity was measured with Cannon-Ubbelohde type viscometers at 28.0°C (±0.03°C). The relationship (t/\( t₀ \)) of the solution and \( t₀ \) = flow time of the solvent, was used to determine the specific viscosity. Solvent flow times were 192.67 and 191.18 seconds for DNA and histone experiments respectively.

Binding of aflatoxin to histone and DNA (highly polymerized calf thymus DNA from Worthington Biochemical Corp.) was determined directly by equilibrium dialysis experiments. Aqueous solutions (2.0 ml) of 2 main fractions of purified calf
thymus histone (F2b or F1) were placed inside dialysis tubing in small plastic screw-top tubes. These solutions were equilibrated with an equal volume of water containing various concentrations of aflatoxin B1. Experiments with DNA were conducted in the same manner as for histone using distilled water or 0.2 M NaCl as solvents. All mixtures were equilibrated 112 hours at 5°C. A rocking dialysis platform was used to assure proper mixing. Concentrations of aflatoxin were determined spectrophotometrically at 363 nm (6,15). A standard solution of aflatoxin was prepared in ethanol and aliquots diluted with 2.0 ml of solvent to give the desired concentration (5-25 µg/ml). Although aflatoxin is quite hydrophobic, once the toxin was dissolved in ethanol, dilution with water or salt solution did not significantly lower the extinction coefficient except at high concentrations. The toxin solutions remained clear indicating that no aggregation occurred. Only concentrations exhibiting a coefficient near that reported (εM = 22,000) were used. All controls contained a volume of ethanol equal to that of the toxin treatment.

The association constants (K) for the aflatoxin complexes were determined from the relationship

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\text{slope} = \frac{1}{r \text{Max. } K}
\]

Concentrations of aflatoxin both inside and outside the dialysis tubing were determined and reciprocal plots of 1/r (where r = the number of moles of toxin bound per mole of substrate) were plotted against 1/c (where c = molar concentration of free aflatoxin). The maximum number of moles of toxin bound per mole of histone or DNA (rMax.) was determined by extrapolation of this plot to the y axis. The slope of the plot was determined and the relationship solved for the association constant (K).

Molecular weights of 14,000 and 21,000 were used for histone fraction 2b and 1, respectively (9,20). The molecular weight of DNA as determined from viscosity measurements was 3.5 × 10^6 (4).

Protein concentrations were determined by the biuret-Folin reaction (14). The diphenylamine reaction was used to determine DNA concentrations (2).

**Results and Discussion**

In regard to the 2 histone fractions tested, the toxin increased the viscosity of F2b strongly (fig 1A) whereas it affected the viscosity of F1 very little (fig 1B). From figure 1A it can be seen that the viscosity curve of aflatoxin-treated histone had the same features as the curve for the pure aqueous histone but the viscosity values were much higher. At a concentration of 0.5 % histone the reduced specific viscosity was 0.39 dl/g for the aqueous solution whereas in the presence of aflatoxin the value was increased to 0.80 dl/g. At concentrations below 0.5 %, the reduced specific viscosity in the presence of the toxin increased with dilution exponentially as did the untreated solutions. Equilibrium dialysis experiments showed that the toxin was bound to both histone fractions (fig 2). Histone fractions F1 and F2b are both lysine-rich. However, F1 has a higher lysine and proline content than F2b. Contrary to F2b, the fraction 1 remains in a disordered conformation even in the presence of electrolytes (8). Approximately 35 molecules of the toxin were bound per molecule of F2b. The association constant for the aflatoxin-F2b complex was 1000. An association constant of 700 was calculated for the aflatoxin-F1 complex with about 65 molecules of the toxin bound per molecule of histone.

**Fig. 1A.** (left) Effect of aflatoxin on the viscosity of histone fraction 2b. **Fig. 1B.** (right) Effect of aflatoxin on the viscosity of histone fraction 1. One ml of a 1 % aqueous solution of histone was incubated for 2 hours at 5°C with 100 µg of aflatoxin. ○ = Control; ● = Aflatoxin-treated.
These studies indicate that aflatoxin affects in some specific way the gross conformation of certain histone fractions. The increase in viscosity of F2b could be explained by a stretching effect on the protein or, alternatively, by aggregation. Because the number of molecules of aflatoxin bound to F2b is relatively small, i.e. 35 (fig 2), aggregation could possibly occur by staggered junction of the fibrous macromolecules aligning them in a coplanar arrangement. Optical rotatory dispersion measurements indicated that the secondary structure of the histone fraction 2b was not significantly altered by the toxin.

In the presence of aflatoxin the viscosity of DNA was greatly increased (fig 3). This indicated that the asymmetry of the DNA molecule was possibly increased due to binding of the toxin. Since stretching of the double-stranded DNA molecule seems highly unlikely, a staggered linear aggregation similar to that postulated for histone would seem to be more probable. Equilibrium dialysis experiments showed that 1800 molecules of the toxin were bound to each molecule of DNA (fig 4). An association constant (K) of 5500 was calculated for the DNA-aflatoxin complex. When expressed in terms of nucleotide residues, 1 molecule of aflatoxin was bound per each 5 nucleotides. Studies of the melting profiles of DNA in the presence of aflatoxin revealed no differences from untreated DNA. Both DNA preparations, in 0.2 M NaCl, exhibited a Tm of 89°. However, the toxin showed an 8- to 9-fold greater affinity for denatured DNA than for the native form. Also when experiments were conducted in distilled water, approximately 6 times as much aflatoxin was bound per mole of native DNA as when 0.2 M NaCl was used as solvent, although the association constant was lowered to 1500. The affinity of aflatoxin to DNA appears to be related to the conformation of the DNA molecule.
No effect of aflatoxin on the viscosity of RNA was observed. Equilibrium dialysis experiments showed that the toxin was readily bound to RNA although the conformation of this molecule apparently remains unaffected.

Although the gross conformation of DNA may be affected, the melting profile of aflatoxin-treated DNA indicated that the secondary structure is not altered by the toxin. Aflatoxin showed a much greater affinity for the coil form of DNA than for the helix form, however. Ts'o (23) on the basis of binding studies, concluded that when carcinogens enter a living cell the compounds are more likely to interact with DNA than with anything else; that interactions of these compounds will lead to uncoiling of DNA; and that interaction with uncoiled DNA is most probable. Aflatoxin, under our experimental conditions, appears not to act entirely in accordance with Ts'o's predictions. The notable exceptions are that aflatoxin appears to have no effect on the secondary structure of DNA and that binding forces for the histone, when the molecular weights are considered, are as great or greater than those for native DNA.

It remains speculative as to whether these findings are related to the mode of action of aflatoxin. However, in view of our present knowledge on the general affects of this toxin on cells, it is conceivable that aflatoxin exerts its influence at the information-transcription level. Indeed, others have reported the effects of aflatoxin on RNA metabolism in vivo and it has been suggested that these effects could occur by direct interaction with DNA thereby interfering with such processes as DNA replication and DNA-dependent RNA synthesis (3, 19).

Alternatively aflatoxin could affect the information-transcription process indirectly by affecting the stability of the nucleoprotein complex. It has already been pointed out that a conformational change of either DNA or histone could reduce their affinity in the nucleohistone complex (21). Aflatoxin causes conformational changes in both DNA and specific histones. It is conceivable that at low concentrations aflatoxin does affect the stability of the nucleohistone complex, that consequently previously repressed DNA template is derepressed, and finally, increased concentrations of the toxin again inhibit DNA activity by direct interaction. A mechanism of this sort could explain the concentration-dependent effects of aflatoxin on protein synthesis (10).

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Literature Cited


