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SHORT COMMUNICATIONS

Investigations of the nature of the binding of aflatoxin B_1 with DNA

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STUDIES on the biochemical action of the carcinogen aflatoxin B_1, both *in vitro* and *in vivo*, revealed that the toxin inhibited DNA-directed RNA synthesis and DNA synthesis.[1–4] Clifford and Rees[5,6] proposed that these inhibitions were a consequence of the toxin interacting with the deoxyribonucleic acid (DNA). They demonstrated that mixing aflatoxins with DNA resulted in difference spectra. The production of a difference spectrum indicates that molecular interaction has occurred but it does not give a measure of the degree of interaction, nor does it give information of the type of binding. In the present study the nature of the binding of aflatoxin B_1 with DNA has been further examined.

Crude aflatoxin was a gift from the Medical Research Council Toxicology Unit, Carshalton. The toxins were separated as described by Clifford and Rees.[6] DNA type 1, sodium salt, highly polymerized from calf thymus, was obtained from the Sigma (London) Chemical Co., (London, S.W.6), as were the deoxyguanosine, deoxyadenosine and thymidine.

A solution of calf thymus DNA (0·4 mg/ml) was prepared in 0·04 M Tris-HCl pH 7·9. Varying quantities of aflatoxin B_1 were dissolved in 10 ml of this solution. Aliquots were reserved for analysis and the remainder (7 ml) centrifuged for 17 hr at 50,000 rpm in a Spinco preparative ultracentrifuge together with a DNA control. The supernatants were removed and the gel-like pellets were washed once with aqueous alcohol (1 H_2O:3 ethyl alcohol).

Fibres were drawn from the gel-like pellets by methods similar to those described previously.[7] Control fibres of DNA were prepared in a similar way. X-ray diffraction patterns were obtained as described by Langridge *et al.*[7] No alteration in X-ray diffraction was detected between the preparations from the DNA alone and those from the DNA/aflatoxin B_1 mixtures.

In order to determine the quantities of DNA and aflatoxin B_1 the samples of the uncentrifuged mixtures and the supernatants were measured optically for toxin and DNA concentrations in an SP 500 spectrophotometer. For the X-ray studies the pellets used were those obtained from centrifugation of a solution containing 2 mg of aflatoxin B_1 and 4 mg DNA. It was found that all the DNA was precipitated and 21% of the aflatoxin B_1. Even when varying quantities of the toxin were mixed with a constant DNA solution, a more or less constant percentage of the toxin (20–30%) was precipitated with the DNA (Table 1).

It therefore was hoped to determine the quantity of aflatoxin B_1 interacting with DNA by obtaining a series of difference spectra. The difference spectra were studied using a Cary Model 14 (serial no. 273)

TABLE 1. COMPOSITION OF PELLETS FOLLOWING CENTRIFUGATION OF DNA/AFLATOXIN B_1 MIXTURES

Quantity of aflatoxin B_1 in original mixture (mg)	Quantity of aflatoxin B_1 present in pellet (mg)
1·4	0·30
0·7	0·21
0·35	0·09
0·175	0·05

The conditions of centrifugation are as described in the text. All the DNA present in the mixture prior to centrifugation (2·8 mg) was present in the pellet. The results are of a typical experiment.

recording spectrophotometer with a 1 cm optical path length. Aflatoxin B_1, DNA and the nucleosides were dissolved in 0·01 M Tris-HCl, pH 7·4, containing 0·01 M NaCl. One ml of the solutions being studied were put in each half of the split cells and final concentrations are given in the Results section. First with a constant concentration of aflatoxin B_1 (64 μM) the DNA concentration was varied up to the highest concentration that could be achieved without gel formation (0·3 mM to 1·99 mM DNA-P). Secondly, a solution of the highest concentration of DNA was mixed with solutions of decreasing aflatoxin B_1 concentration to give final concentrations of 1·99 mM DNA-P with 64, 32, 16 μM aflatoxin B_1. The difference spectra of these mixtures were measured and the results are given in

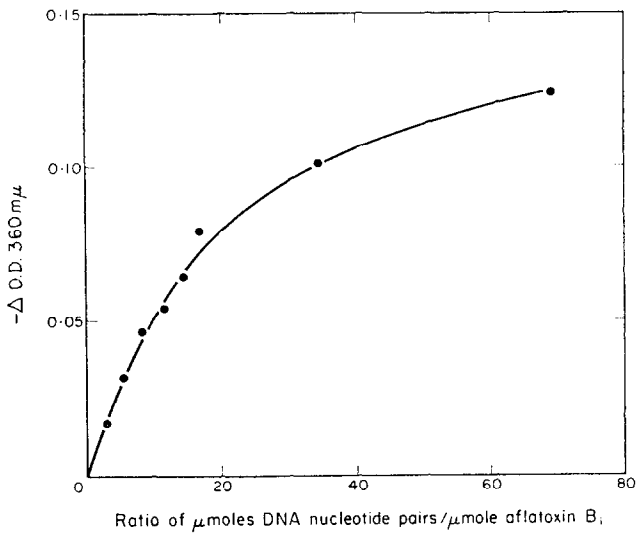

FIG. 1. The points at 360 mμ taken from a series of difference spectra of aflatoxin B_1/DNA mixtures.

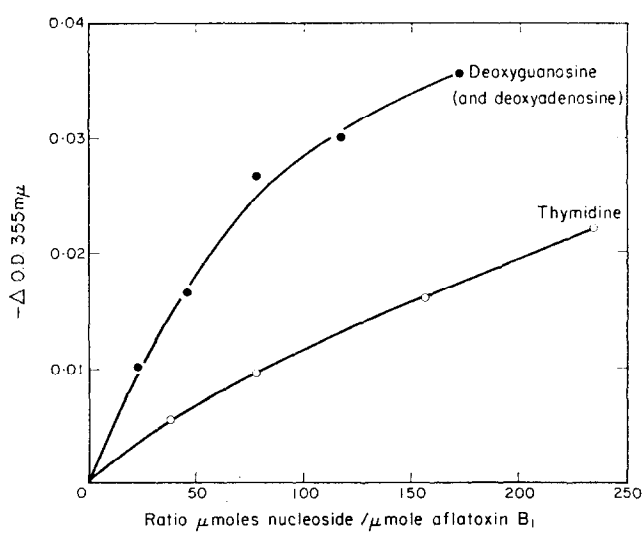

FIG. 2. The points at 360 mμ taken from a series of difference spectra of aflatoxin B_1/deoxyadenosine of thymidine mixtures.

Fig. 1 in which the ΔO.D. at 360 mμ is plotted against the ratio of the μmoles of DNA nucleotide pairs/μmole aflatoxin B_1. The figure is that of a shallow curve with no evidence of a break. As previously it had been found that solutions of nucleosides mixed with aflatoxin B_1 gave difference spectra,[8] similar curves were prepared using deoxyguanosine and thymidine, as shown in Fig. 2. Deoxyadenosine gave a curve identical with that obtained with deoxyguanosine. The curves are similar to those obtained with DNA but higher concentrations of the nucleosides were required for a similar ΔO.D. 360 mμ.

A number of drugs and antibiotics which inhibit DNA-dependent nucleic acid synthesis have been found to complex with DNA and thereby alter its X-ray diffraction pattern.[9–11] In contrast the X-ray diffraction studies from DNA/aflatoxin B_1 pellets did not reveal any alterations in the geometry of the pattern. Thus it must be concluded that aflatoxin B_1 neither intercalates in the DNA helix, nor does it disturb packing. In view of this finding it is of interest to consider what could be the nature of the binding of aflatoxin B_1 to DNA.

As previous studies[8] showed that the aflatoxin B_1/DNA complex was split on a Sephadex G-50 column and that the melting temperature of the complex was the same as that of the uncomplexed DNA the toxin cannot be bound by a covalent bond. Furthermore in the present investigation it was found that varying the concentration of aflatoxin in the DNA/aflatoxin B_1 mixtures did not alter the percentage precipitated which emphasises the weakness of the binding of the toxin.

The absence of a break in the curve obtained from the difference spectra of the DNA/aflatoxin B_1 mixtures indicates that there is no stoichiometry in the reaction. The shallow curve that was obtained suggests that the toxin and the DNA formed a continuously dissociating system which would be in accord with the other evidence of their weak binding. It is of interest that the nucleosides gave curves similar to that of DNA which suggests that their binding also is weak. As may be seen from the figures more nucleoside than DNA was required to be mixed with the toxin to produce the same ΔO.D. 350 mμ. If this *in vitro* interaction of DNA and aflatoxin B_1 reflects the type of reaction which the toxin enters into *in vivo* it may be of considerable significance, notwithstanding the weakness of the linkage, that such a marked inhibition in nucleic acid synthesis occurs.[6] Szent-Gyorgyi[12] has previously considered the possibility that weak interactions involving macromolecules could produce profound effects in biological systems and Mason[13] has implicated a role for such interactions in carcinogenesis.

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