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THE EFFECT OF FUNCTIONAL GROUPS ON THE INTERACTION OF AFLATOXINS B_1 AND G_1 WITH STARCH, CELLULOSE AND SEVEN CELLULOSE DERIVATIVES

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Abstract—The interactions of starch, cellulose, phosphocellulose, diethyl amino cellulose (DEAE-C), trimethylamino ethyl cellulose (TEAE-C), cellulose-acetate, carboxymethyl cellulose (cm-c), cellulose Ecteola (Ecteola-c) with aflatoxins B_1 and G_1 were studied by equilibrium dialysis. The ligands bind with starch at two sites but with ECTEOLA-C, DEAE-C and TEAE-C at one site. No significant binding to other derivatives of cellulose was observed. The chemical groups: $-CH_2-CH_2-N\equiv(CH_2-CH_3)_2$ (DEAE), $-(CH_2-CH_2-N^+\equiv(CH_2-CH_3)_3$ (TEAE), and $-CH_2-CH_2-CH_2-N^+\equiv(CH_2-CH_2-OH)_3$ (ECTEOLA), seem to aid binding. They are, however, not critical prerequisites for binding to occur since there was binding to starch in which these chemical groups are absent.

The interaction of aflatoxins with proteins [1], DNA, purines, pyrimidines and their derivatives [2–5, 7] and histones [8] have been studied. These interactions were proposed as the biochemical basis of the toxic effects of aflatoxin B_1 on these macromolecules [1–5, 12]. Further investigation to pinpoint the chemical basis of these interactions have also been reported [1, 4, 7, 12]. Studies on the interaction of aflatoxin with DNA have particularly been detailed; embracing the roles of amino groups on the macromolecule and ionic factor in the interaction [4, 6, 7].

These studies notwithstanding, the picture of the role of functional groups in the interaction of aflatoxins with macromolecules, particularly non-DNA macromolecules, remains hazy. A clear picture of the role of functional groups on macromolecules in their interaction with aflatoxins is necessary in the elucidation of the biochemical mechanism of action of aflatoxins. In this paper, therefore, we describe the effects of alteration of various chemical groups on polysaccharides on their interaction with aflatoxins. B_1 and G_1. Although, starch, cellulose and its derivatives are not targets of aflatoxin lesion in mammalian cells, we have chosen them as macromolecular models for our study because the glucose chain is basically a constant moiety in all their structures, while the functional groups are variable. Any differences in their interaction with aflatoxins, therefore, could only have been due to the variations in their functional groups.

MATERIALS AND METHODS

Aflatoxins. Aflatoxins B_1 and G_1 were purchased from Makor Biochemicals Ltd., Jerusalem, Israel.

Polysaccharides and derivatives. Cellulose, phosphocellulose, carboxymethyl-cellulose, diethylammocellulose, trimethyl-aminocellulose and ecteola-cellulose were all purchased from Solka-floc. Brown Company, 500 Fifth Avenue, New York, 36 New York. Starch was, however, purchased from Hopkin and Williams, Chadwell Heath, Essex, England.

Dialysis tubing. Visking cellophane tubing was purchased from Scientific Instrument Centre, London, U.K.

Before use, they were cleaned by rinsing on a shaking bath (Gallenkamp, U.K.) with deionized water for about 48 hr and were next stored in a 0.01 M sodium phosphate buffer pH 7.0 at a temperature of 4°.

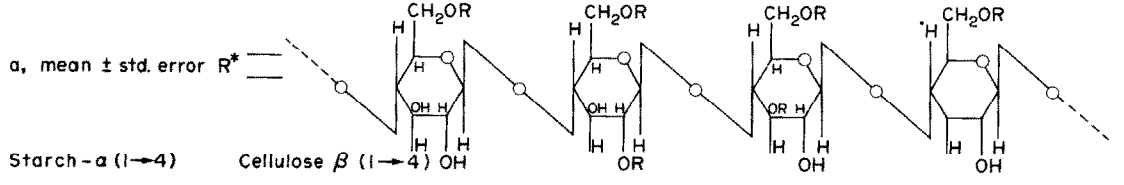
Dialysis experiment. 20 μmole ± 0.4 of each of the polysaccharides was suspended in 12 ml 0.01 M sodium phosphate buffer in pretreated Visking cellophane tubing (12 cm length × 3 cm diameter). The solutions of aflatoxin ligands were prepared to give a concentration of 80 μmole in 0.01 M sodium buffer pH 7.0 and diluted as required to give 40 μmole, 20 μmole, 10 μmole, and 5 μmole concentrations. Since the aflatoxins are not readily soluble in water, they were initially dissolved in a minimum quantity of *N*,*N*-dimethyl formamide before being diluted to volume with the buffer [1].

The cellophane tubings holding the 20 μmole suspensions of polysaccharides were placed in 15 ml buffer solutions of aflatoxins B_1 and G_1 in 50 ml glass tubes covered with cotton wool. The assemblies were rocked in a shaking bath at a frequency of 150 cycles/min at 25° ± 1 for 12 hr. Within this time interval equilibration had occured. Controls consisting of aflatoxins in buffer solutions outside and buffer solutions devoid of polysaccharides inside were included in all runs. These controls were also used to determine the degree of binding of aflatoxins to the Visking cellophane materials of the tubings. As binding to the material did not occur with any of the two aflatoxins, no corrections for dialysis tubings was required in the experimental calculation.

Five different concentrations of the aflatoxins (5, 10, 20, 40 and 80 μmole) were used while the level of polysaccharides was maintained at 20 μmole. The

Table 1. Binding data of aflatoxins B_1 and G_1 to starch and 3 of the derivatives of cellulose

Polysaccharides or derivatives	R*	Association constant (K_1[a]) $M^{-1} \times 10^{-2}$		Standard free energy change (ΔF_1°) Kcal/mole		Number of binding Sites (n)[a]	
		B_1	G_1	B_1	G_1	B_1	G_1
Starch	$(-{}^{\circ}H)$	16.8 ± 0.4	13.9 ± 0.2	-4.56	-3.30	2.20	1.97
Cellulose-Ecteola	$(-CH_2-CH_2-CH_2-N^{+}\equiv(CH_2-CH_2-OH)_3)$	8.8 ± 0.2	7.5 ± 0.3	-4.04	-3.95	0.81	0.92
Cellulose-TEAE	$(-CH_2-CH_2-N^{+}\equiv(CH_2-CH_3)_3)$	8.4 ± 0.1	7.2 ± 0.1	-4.00	-3.92	1.20	0.65
Cellulose-DEAE	$(-CH_2-CH_2-N\equiv(CH_2-CH_3)_2)$	1.9 ± 0.3	1.2 ± 0.4	-3.11	-2.85	0.81	0.56



The values of K_1 and n were obtained from Figs. 1A and 1B. The intercept on the $\bar{V}/A$ axis (ordinate) is K (the association constant for the first ligand bound), while that of $\bar{V}$ axis (abscissa) is n (the average number of binding sites on each polysaccharide).

The standard free energy change for the first ligand bound (ΔF_1°) was calculated from K_1 by the general thermodynamic equation $\Delta F_1^{\circ} = RT\ 1N\ K_1$. R is the gas law constant and T is the absolute temperature.

amount of free aflatoxins in equilibrium with bound aflatoxins was estimated by measuring the concentration in Unicam S.P. 500 (Unicam Instruments, Cambridge, England) at the appropriate wavelength of either aflatoxin B_1 (360 nm) or aflatoxin G_1 (362 nm) [9, 10]. The amounts of polysaccharide-bound aflatoxin was calculated by subtracting the free and unbound ligand concentration from the initial concentration [13]. Binding constants were analysed using the scatchard equation [11], assuming the law of mass action. Other calculations to determine the free energy associations constants, average number of binding sites on polysaccharides were based on the method of R. A. O'Reilly [13–15].

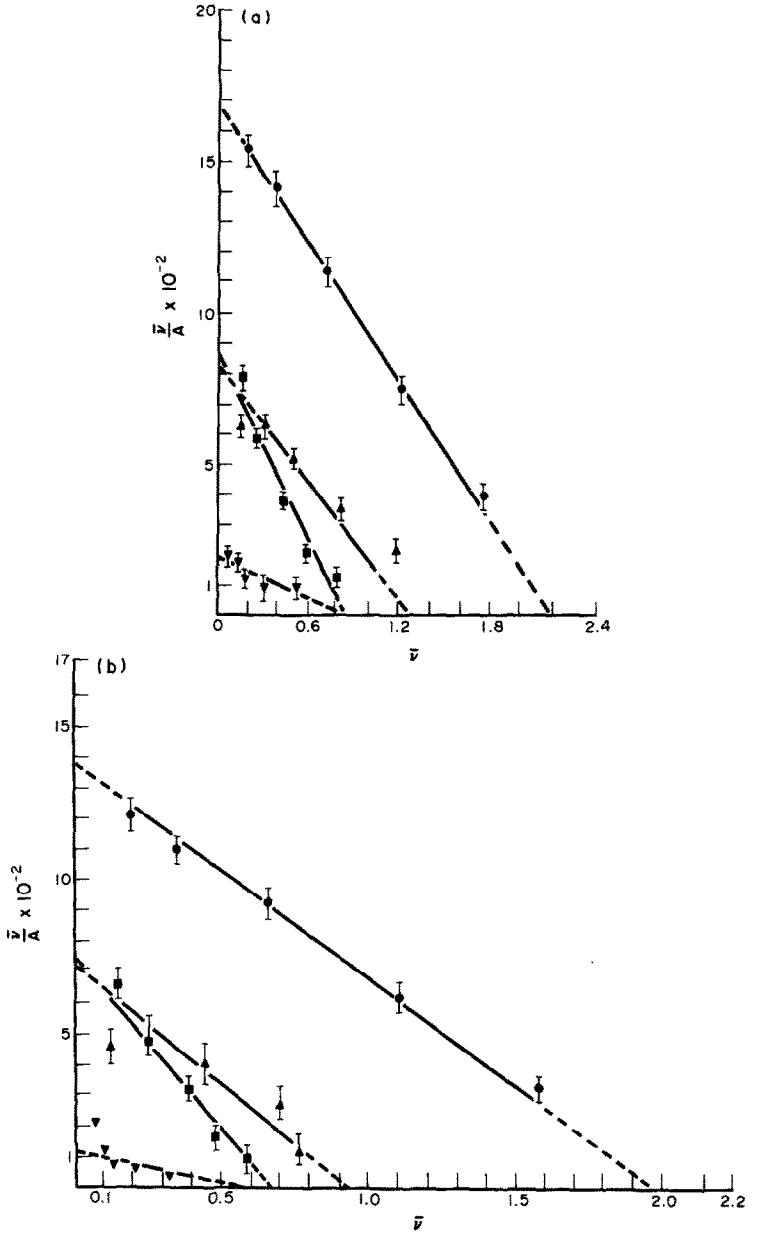


Fig. 1(a) (aflatoxin B_1) and Fig. 1(b) (aflatoxin G_1)—Scatchard plot of data for binding of aflatoxins B_1 and G_1 to starch and three derivatives of cellulose—ECTEOLA-cellulose (ECTEOLA-C), DEAE-cellulose, (DEAE-C) and TEAE-cellulose, (TEAE-C). Five different concentrations of aflatoxins B_1 and G_1 (5 μmole, 10 μmole, 20 mole, 40 μmole, 80 μmole) were interacted with 20 μmole of each of the polysaccharides. Scatchard equation for the law of Mass action, $\bar{V}/A = Kn\text{-}K\bar{V}$, was made use of: where $\bar{V}$ is the molar ratio of bound aflatoxins B_1 and C_1 to polysaccharides. A is the molar concentration of free aflatoxins G_1 and B_1 at equilibrium. K is the average association constant for the binding at each site and n is the average number of binding sites on a polysaccharide molecule. For each polysaccharide, $\bar{V}$ and $\bar{V}/A$ and their standard errors at each concentration studied were calculated. The regression line was calculated for each set of data by the method of least squares. Each point on the graph is the mean of five determinations. Starch. ●——●; ECTEOLA-cellulose, ▲——▲; TEAE-cellulose, ■——■; DEAE-cellulose ▼——▼; Mean ± S.E., ⊕. The values of $\bar{V}/A$ plotted against V give a straight line when the binding sites are independent and equivalent.

RESULTS

In the binding experiment, aflatoxins B_1 and G_1 were found to bind to starch, diethylaminocellulose (DEAE-C), triethylamino-cellulose (TEAE-C) and ECTEOLA-cellulose. No binding was observed between the aflatoxins and cellulose, phosphocellulose, carboxymethyl cellulose and cellulose acetate.

The binding data for the four polysaccharides which bind aflatoxins B_1 and G_1 are shown in Table 1. From this table, it is apparent that starch has an average number of binding sites of two for aflatoxins B_1 and G_1 while the other polysaccharides have one binding site. Also, the standard free energies for the binding of aflatoxins B_1 and G_1 to the polysaccharides seem to be highest in starch and least in DEAE-cellulose (Starch > ECTEOLA-cellulose > TEAE-cellulose > DEAE-cellulose). Aflatoxin B_1 seems to bind more strongly to any of the polysaccharides than aflatoxin G_1.

Figures 1(a) and 1(b) show a typical Scatchard plot for binding sites that are independent and equivalent (i.e. non-cooperative type of binding).

DISCUSSION

The significant difference between the binding properties of starch compared with those of cellulose, show that the —OH groups on the glucose units which make up the two polysaccharides do not play critical roles in the binding interaction. This should be expected because of the hydrophilic nature of the —OH groups [14–17]. Since, in addition, starch and cellulose have similar primary chemical structures, it would appear that the secondary and tertiary structures of the two polysaccharides play a decisive role in this difference in their binding interactions.

The results for the binding of the aflatoxins to cellulose and its derivatives show a marked variation based on different chemical groups substituted in the basic chemical structures of cellulose. Thus, cellulose and its derivatives-carboxymethyl cellulose, phosphocellulose and cellulose acetate did not bind the aflatoxin while the amino derivatives of cellulose-Ecteola-cellulose, TEAE-cellulose, and DEAE-cellulose bound the aflatoxins. The presence of an amino group seems to induce binding ability in cellulose. The ability of amino groups to aid the binding of aflatoxins to macromolecules has previously been reported [5]. The increase in the number of methylaminoethyl chemical group from 2 in DEAE-cellulose to 3 in TEAE-cellulose seems to have increased the binding ability of the latter. This increase in the binding ability was probably due to an increase in the hydrophobic nature of the macromolecule [13, 14, 18]. The effect of increase in the number of methylamino ethyl groups seems less significant beyond 3. This is apparent in the similarity between the binding data of TEAE-cellulose and ECTEOLA-cellulose.

On the whole, the binding data of aflatoxins B_1 and G_1 are similar for any particular polysaccharide. This trend seems to conform to the belief that the critical structure in the binding of aflatoxins is the 5-methoxycoumarin moiety [1, 15] which is present in aflatoxins B_1 as well as G_1.

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