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The binding of aflatoxin B_1 with serum albumin

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MANY serum proteins bind small mol. wt substances for transport or other biological reactions.[1] Many of the coumarin anticoagulants are bound to serum albumin and this binding affects their pharmacokinetic properties.[2] Aflatoxin B_1 has been shown to possess haemorrhagic properties.[3,4]

It is of interest, therefore, to study the interaction of aflatoxin B_1 with serum albumin in view of the structural similarity of the toxin and synthetic coumarins[5] and also in an attempt to obtain an insight into its binding with the intracellular receptor site for anticoagulant activity. For obtaining the binding data, the method of equilibrium dialysis was employed. This method permits easy determination of protein interactions of drugs.[6]

Portions of Visking cellophane tubing of 12 cm (3 cm dia., Scientific Instrument Centre Ltd., London, U.K.) were prepared. They were cleaned by rinsing in a shaking bath (Gallenkamp, U.K.) of deionized water for at least 48 hr and were then stored in a 0·067 M sodium phosphate buffer, pH 7·4 ($I = 0{\cdot}170$) at a refrigerated temperature of 4°. Just before use, each bag was washed thoroughly with the buffer and used before it dried. For the measurement of free and bound drug, 10 ml sample of albumin was placed inside the bag and dialysed against 15 ml sample of medium containing aflatoxin B_1 (purchased from Makor Biochemicals Ltd., Jerusalem) or 4-hydroxycoumarin (purchased from Kock–Light Laboratories, Colnbrook, U.K.), in a 50 ml glass tube and covered with cotton wool. In this study we used bovine serum albumin (BSA) and human serum albumin (HSA). They were obtained from Sigma Chemical Co., U.S.A. and we considered it unnecessary to further purify the crystalline albumins. The concentration of albumin in 0·067 M sodium phosphate buffer, pH 7·4, which was used in all the experiments was 0·4 mg/ml. The solutions of the drugs were made to give a concentration 20 μM each in 0·067 M sodium phosphate buffer, pH 7·4, and diluted as required. Aflatoxin B_1 and 4-hydroxycoumarin are not readily soluble in water; therefore, they were dissolved in a minimum quantity of *N,N*-dimethylformamide and 0·01 N sodium hydroxide respectively after which each drug was diluted to volume with the buffer.

For each concentration of the drug three tubes were placed and rocked at 150 cycles/min at 25° ± 1° for 10 hr. At this time equilibration had occurred under our experimental conditions. Included in all runs were control bags which contained only buffer. Four different concentrations (5, 10, 15 and 20 μM) of each compound were used while the concentration of the protein was kept constant. The amount of free drug in equilibrium with the bound drug was estimated by measuring the concentration,

at appropriate wavelength, of either aflatoxin B_1 (362 nm) or 4-hydroxycoumarin (305 nm) according to the method of O'Reilly.[7] The amount of albumin-bound drug was then calculated by subtracting the free and unbound drug concentration from the initial concentration. Binding constants were analysed using the Scatchard equation,[8] assuming the law of mass action. Resolving the parabolic curves (Fig. 1) into linear components we have observed that aflatoxin B_1 is bound to HSA in at

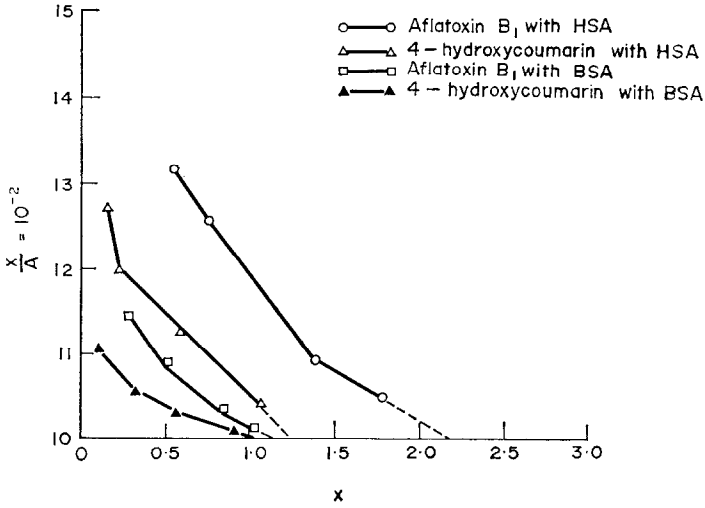

FIG. 1. Comparative Scatchard plots of the binding of aflatoxin B_1 and 4-hydroxycoumarin by HSA and BSA. Four different concentrations of each drug were used, while the concentration of the protein was kept constant. Each point is the mean of three determinations. Assuming the law of mass action, Scatchard equation may be written as $x/A = K(n - x)$ where x is the molar ratio of bound drug to albumin; A is the molar concentration of the free drug outside the dialysis bag (at equilibrium); K is the average apparent equilibrium constant for the binding at each site of the drug and albumin; and n is the average maximal number of binding sites for the drug present on the albumin molecule which is evaluated from the x-intercept.

least two sites whereas the binding site on BSA is not greater than one. The number of binding sites of 4-hydroxycoumarin on either HSA or BSA is, also, not much greater than one. This agrees with the previous finding of O'Reilly.[9] We have assumed that the albumins have not been denatured under the present experimental conditions. The fact that the Scatchard profiles were parabolic is an indication that the binding sites of aflatoxin B_1 on the HSA are not equivalent. Therefore, the apparent association (equilibrium) constants for the binding at each site of the drug may not be identical.

Some of the differences in the anticoagulant activities of aflatoxin B_1 and 4-hydroxycoumarin in some animal species[10] may be due to differences in binding with intracellular proteins. It would appear that multiple factors may be involved in the interaction of aflatoxin with albumin, in view of the variation in the binding of the drug with the protein from human and bovine species. Compounds containing 4-hydroxycoumarin ring systems have been shown to be bound to serum albumins[2] and recent studies have indicated that the introduction of a hydrophobic group to a coumarin compound greatly increases its anticoagulant action and its binding to albumin.[9] Aflatoxin B_1 has been shown to be much more effective than 4-hydroxycoumarin in prolonging the blood clotting time of rats.[4] In this connection, the comparative effective doses were 56×10^{-6} mM aflatoxin B_1 to 93×10^{-3} mM 4-hydroxycoumarin. The addition of furan ring systems to the central 5-methoxycoumarin moiety in aflatoxin B_1 may result in a decrease or increase in the polarity of the compound. Further experiments are, however, in progress to determine the extent to which the binding of 5-methoxycoumarin with serum albumin, if any, is affected by the enlargement of its configuration.

Department of Biochemistry,
University of Ibadan,
Ibadan,
Western State,
Nigeria

OLUMBE BASSIR
ENITAN A. BABABUNMI

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Methylation of exogenous 3,4-dihydroxyphenylalanine (L-dopa)—Effects on methyl group metabolism*

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WHEN ANIMALS are given 3,4-dihydroxyphenylalanine (L-dopa) in doses (100 mg/kg, i.p.) similar to those used to treat patients with Parkinson's disease, a major portion of the administered catechol amino acid is *O*-methylated to form 3-*O*-methyldopa (OMD) and other methylated products.[1] In the process, the concentrations of the methyl donor, *S*-adenosylmethionine (SAM), in brain and adrenal fall markedly.[2] Single doses of SAM do not affect its concentration in liver. However, after chronic L-dopa treatment, hepatic SAM levels are acutely lowered by further doses.[3]

The possibility that the methylation of large amounts of exogenous L-dopa might ultimately deplete the body of free methionine has been suggested.[1,4] If dietary methionine provided the only source of methyl groups for SAM synthesis, a 70-kg human taking 6–10 g L-dopa per day and consuming proteins containing 1–2 g methionine should become methionine deficient. However, even after prolonged L-dopa administration, the percentage of the drug that is excreted in the urine as methylated product remains very high.[5]

This paper presents evidence that methionine concentrations in serum and tissue do not fall in animals treated chronically with sufficient L-dopa to depress hepatic SAM. Hence, sources of methyl groups other than dietary methionine must be available to the body during L-dopa therapy.

Sprague–Dawley male rats (Charles River Laboratories, Wilmington, Mass.) weighing 150, 200 or 300 g were housed five per cage and exposed to light (Vita-Lite, Duro-Test Corp., North Bergen, N.J.) between 9 a.m. and 9 p.m. daily. They had free access to Purina laboratory rat chow and water. The animals received intraperitoneal injections of L-dopa (100 mg/kg) or its diluent alone (0·05 N HCl) and were decapitated at the times described. Serum and the various tissues were frozen on dry ice until assayed; the muscle specimen was taken from the thigh.

All chemicals were of the highest purity commercially available. L-Dopa was a gift of the Hoffmann-La Roche Co. (Nutley, N.J.); yeast *t*-RNA and methyl-^{14}C-L-methionine (50 μCi/m-mole) were purchased from Schwarz–Mann Co. (Orangeburg, N.Y.).

SAM concentrations were assayed according to the method of Baldessarini and Kopin,[6] and the results expressed as percentages of the control values.

A modification of the *t*-RNA loading assays of Holley and Chambers was used for the methionine assay.[7] Limiting amounts of crude yeast *t*-RNA were incubated with saturating amounts of ^{14}C-methionine (100 pmoles) and the unlabeled methionine present in 10 μl of the sample to be analyzed.

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