

Effect of Aflatoxins on ATPase Activity of Rat Liver Mitochondria

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ATPase activity, which is localised in the inner mitochondrial membrane and is highly sensitive to the integrity of that membrane, is diminished by inhibitors of oxidative phosphorylation and stimulated by uncoupling agents. Several respiratory chain inhibitors have been shown to diminish uncoupler stimulated ATPase activity (WEINER and LARDY, 1974). Aflatoxins, present in several mold-infested food and feed materials, are toxic to a large number of animal species and have been found to inhibit and uncouple oxidative phosphorylation (RAMACHANDRA PAI et al. 1974 a). Reports on the effect of aflatoxin B₁ on ATPase activity are inconsistent (CLIFFORD and REES, 1967; BABABUNMI and BASSIR, 1972). Hence a detailed study was undertaken to see the effect of aflatoxin B₁, M₁ and G₁ on the ATPase activity and uncoupler-stimulated ATPase activity of rat liver mitochondria.

Methods

Isolation of mitochondria and preparation of aflatoxins were as described earlier (RAMACHANDRA PAI et al. 1974 a, 1974 b). ATPase activity was measured by the method of LARDY and WELLMAN. Aflatoxins were added dissolved in dimethylsulphoxide and 2,4-dinitrophenol (DNP) in ethanol. The same amount of solvents were added to control experiments. All the results presented are the mean \pm SEM of four separate experiments.

Results and Discussion

The effect of various concentrations of aflatoxins on ATPase activity of mitochondria is summarised in Table I. Aflatoxin B₁, the most potent hepatocarcinogen, does not affect the ATPase activity whereas M₁ stimulates the activity to nearly three fold and this stimulation is concentration-dependent. Aflatoxin G₁ diminishes ATPase activity at lower concentrations.

Table I

Effect of aflatoxins on ATPase activity

Aflatoxin (M)	ATPase activity (umoles P/mg protein)		
	B ₁	M ₁	G ₁
Control	0.12±0.01	0.11±0.01	0.12±0.01
1 x 10 ⁻⁴	0.14±0.01	0.32±0.01	0.16±0.01
1 x 10 ⁻⁵	0.13±0.01	0.22±0.01	0.11±0.01
1 x 10 ⁻⁶	0.14±0.01	0.19±0.01	0.05±0.01
1 x 10 ⁻⁷	0.14±0.01	0.18±0.01	0.05±0.01
1 x 10 ⁻⁸	0.14±0.01	0.18±0.01	0.05±0.01

Table II summarises the effect of aflatoxins on DNP-stimulated ATPase activity. B₁ does not affect the activity but M₁ inhibits by 10 to 25%. G₁ at the same concentrations inhibits mitochondrial ATPase by 75 to 80%. No change in the ATPase activity by aflatoxin B₁ as observed in the study is in agreement with the report of CLIFFORD and REES (1967). However a two-fold stimulation of ATPase by B₁ was reported by BABABUNMI and BASSIR (1972) using *in vitro* studies. There are no reports on the effects of aflatoxins M₁ and G₁ on mitochondrial ATPase or on the uncoupler stimulated ATPase by any of these toxins.

Table II

Effect of aflatoxins on DNP stimulated ATPase activity

Aflatoxin (M)	ATPase activity (umoles P/mg protein)		
	B ₁	M ₁	G ₁
Control			
(2 x 10 ⁻⁴ DNP)	2.55±0.07	2.55±0.07	2.45±0.06
1 x 10 ⁻⁴	2.25±0.04	1.94±0.05	0.45±0.02
1 x 10 ⁻⁵	2.25±0.04	2.09±0.06	0.59±0.03
1 x 10 ⁻⁶	2.28±0.05	2.09±0.06	0.62±0.03
1 x 10 ⁻⁷	2.25±0.03	2.24±0.06	0.66±0.04
1 x 10 ⁻⁸	2.23±0.03	2.30±0.06	0.66±0.04

Results of the present study clearly show that B₁ at the concentrations used does not affect the ATPase or DNP-stimulated ATPase activity. M₁ stimulates ATPase activity but inhibits DNP-stimulated activity only slightly. G₁ inhibits mitochondrial ATPase at lower concentrations but remarkably diminishes uncoupler-stimulated ATPase activity at all concentrations studied. Thus these structurally similar toxins (difurocoumarins) exhibit marked differences in the mitochondrial energy conservation, suggesting different loci of action on the mitochondrial inner membrane.

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