Effect of Aflatoxin B₁ and Corticosterone on Ribosome-distributions between Free and Membrane-bound States and between Monosomes and Polysomes in Mouse Liver

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The aim of this research was to examine the inhibitory effect of aflatoxin B₁, one of the most potent hepatocarcinogen, on the translational step in mouse liver. It has been shown that polysomes were released *in vitro* from microsomal membrane prepared from rat liver by incubation with aflatoxin B₁ and that this release of ribosomes was prevented by addition of corticosterone in the incubation medium.

In this paper, the same phenomenon was proved to occur *in vivo* by an improved fractionation methods, in which ribosome-distributions can be analyzed quantitatively, not only between free and membrane-bound states but also between monosomes and polysomes. Administration of aflatoxin B₁ to mice induced reductions of membrane-bound ribosomes and polysomes, with concomitant increases of free ribosomes and monosomes in liver. Simultaneous administration of corticosterone prevented this alteration of ribosome-distributions.

From these results, it was deduced that a release of polysomes from membrane occurred primarily by administering aflatoxin, which then caused a shortening of half-life of mRNA on polysomes, resulting in an increase of the amount of monosomes.

Aflatoxin B₁, a potent carcinogen produced by *Aspergillus flavus*, has been known to inhibit both transcriptional and translational steps in liver cells.^{1,2)} Concerning with the translational step, the toxin has been reported to reduce the amount of polysomes in HeLa cells,³⁾ to release ribosomes from microsomal membrane of rodent liver *in vivo*⁴⁾ and to inhibit protein synthesis.⁵⁾ Furthermore, Rabin and his coworkers have shown that polysomes were released *in vitro* from microsomal membrane prepared from rat liver by incubation with aflatoxin B₁ and that this release of ribosomes was prevented by addition of corticosterone in the medium.⁶⁾ From this kind of experiments, they have concluded that there exists polysome-binding sites on microsomal membrane, for which aflatoxin and corticosterone compete each other to occupy; the former inhibits binding of polysomes to membrane, while the latter stimulates it.

Previously, we have reported an improved

method for the determination of ribosome-distributions, not only between free and membrane-bound states, but also between monosomes and polysomes.⁷⁾ This simple method, a discontinuous sucrose density gradient centrifugation, has been successfully applied to hypophysectomized rat liver and it has been shown that decrease of polysomes and release of polysomes from membrane occurred in hypophysectomized rat liver.⁷⁾

In the present work, changes of ribosome-distributions were determined using this method in liver cells of mouse administered with aflatoxin B₁ alone or together with corticosterone, to examine Rabin's hypothesis made by *in vitro* experiments.

MATERIALS AND METHODS

Chemicals. Aflatoxin B₁ was purchased from Makor Inc., Jerusalem and the purity was tested by thin layer chromatography. Actinomycin D was obtained from Calbiochem, California and corticosterone was from Tokyo-Kasei Pharmaceutical Co., Ltd., Tokyo.

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Animals. Male mice of C3H/He strain of 3 months old, weighing about 30 g were used throughout the experiments.

Experiments. For injection to animals, aflatoxin B₁ was dissolved in dimethylsulfoxide (DMSO), corticosterone was in 1% ethanol in 0.9% NaCl and actinomycin D in 0.9% NaCl. One tenth ml of each solution per 30 g of body weight was injected to a mouse intraperitoneally. Control animals received the solvents only.

After 7 days in the cases of aflatoxin B₁ and corticosterone, and after 15 hr in the case of actinomycin D, mice were sacrificed by decapitation and livers were removed. All the mice were starved for 12 hr before sacrifice. Preparation of the post-mitochondrial fraction, treatment with sodium deoxycholate (DOC) and fractionation by the discontinuous sucrose density gradient centrifugation were carried out as described previously.⁷⁾

RESULTS

To know the effect of aflatoxin B₁ with or without corticosterone *in vivo* on ribosome-distributions in liver cells, mice were separated into three groups. One group was run as a control, receiving the solvents only. The second group was injected with aflatoxin B₁, and the third group with both aflatoxin B₁

and corticosterone. After 7 days of administration, the post-mitochondrial fraction of livers was obtained from each group of mice and submitted to the discontinuous sucrose gradient centrifugation with (Fig. 1) or without (Fig. 2) addition of 1.3% of DOC. In the absence of DOC, the distribution of ribosomes between free and membrane-bound states can be analyzed, while in the presence of DOC, the distribution of ribosomes between monosomes and polysomes can be known.⁷⁾

From the result shown in Fig. 1-B (aflatoxin treated) compared with that in Fig. 1-A (control), it can be seen that by the treatment with aflatoxin *in vivo*, the amount of membrane-bound ribosomes (fraction No. 26~31) was decreased and that of free ribosomes (fraction No. 1~25) was increased. It was also seen from Fig. 1-C (aflatoxin plus corticosterone treated) that an injection of corticosterone together with aflatoxin resulted in prevention of such a change in ribosome-distribution between free and membrane-bound states.

The distributions of total ribosomes (free plus membrane-bound ribosomes) in monosomes and polysomes in these three groups are presented in Fig. 2. In these samples,

FIG. 1. Effects of Aflatoxin and Corticosterone on Ribosome-distribution between Free and Membrane-bound States.

Mice were sacrificed after 7 days of each injection and the post-mitochondrial fractions were obtained and analyzed by a discontinuous sucrose density gradient centrifugation. A, control; B, aflatoxin B₁ (15 mg/kg body weight); C, aflatoxin and corticosterone (15 mg/kg body weight). A typical result of five independent experiments is presented.

FIG. 2. Effects of Aflatoxin and Corticosterone on Ribosome-distribution between Monosomes and Polysome.

The experimental conditions were the same as described in the legend of Fig. 1, except that the post-mitochondrial fractions were treated with 1.3% of DOC. A, control; B, aflatoxin; C, aflatoxin and corticosterone.

microsomal membrane was solubilized by treatment of the post-mitochondrial fraction with DOC. Remarkable increase of monosomes (fraction No. 21~25) and decrease of polysomes (fraction No. 1~10) are observed in aflatoxin treated mice liver as seen in Fig. 2-B, compared with the result shown in Fig. 2-A (control). This dramatic change of the distribution of ribosomes induced by aflatoxin treatment was again prevented by simultaneous injection with corticosterone as seen in Fig. 2-C. These results shown in Fig. 1 and Fig. 2 are quantitatively summarized in Table I.

It is not known at the present time what is the primary effect of aflatoxin which induces both the release of ribosomes from microsomal

membrane and the degradation of polysomes in mice liver cells. It is interesting, however, that an injection of actinomycin D, a well known inhibitor of DNA-directed RNA synthesis, for 15 hr also resulted in the same kind of changes in ribosome-distributions in liver as presented in Fig. 3; a decrease of

FIG. 3. Effect of Actinomycin D on Ribosome-distributions.

Mice were injected with actinomycin D (2.5 mg/kg body weight). After 7 hr of the injection, the post-mitochondrial fraction was prepared from livers and analyzed by a discontinuous sucrose density gradient centrifugation without (A) or with DOC treatment (B). ●—●, control; ○---○, actinomycin D treated.

TABLE I. EFFECT OF AFLATOXIN AND CORTICOSTERONE ON RIBOSOME DISTRIBUTIONS BETWEEN FREE AND MEMBRANE-BOUND STATES AND BETWEEN MONOSOMES AND POLYSOMES
Data were obtained from Fig. 1 and Fig. 2.

	Membrane-bound	Polysomes
	Free	Monosomes
Control	1.00	1.00
Aflatoxin	0.69	0.49
Aflatoxin plus Corticosterone	0.93	0.88

membrane-bound ribosomes with an increase of free ribosomes and a decrease of polysomes with an increase of monosomes.

DISCUSSION

From the result shown in Fig. 1, it is clear that aflatoxin B₁ caused release of ribosomes from membrane in mouse livers *in vivo* supporting the results of Rabin's group *in vitro*.⁶⁾ Consequently, one might expect a decreased activity to synthesize serum albumin in livers of mice treated with aflatoxin B₁, since it has been proved that serum albumin is preferentially synthesized on membrane-bound ribosomes in rodent livers.⁸⁾ A preliminary experiment was carried out, in which sera from three groups of mice used in the experiments shown in Figs. 1 and 2 were analyzed by disc electrophoresis. And it was revealed that the amount of albumin in serum of aflatoxin B₁ treated mice was smaller than those of control and aflatoxin plus corticosterone treated mice. This result may indirectly support that protein synthesizing activity of membrane-bound ribosomes decreases in mouse liver treated with aflatoxin, which is prevented by simultaneous injection with corticosterone.

Protective effect of corticosterone against polysome-destructive effect of aflatoxin was also observed *in vivo* (Fig. 1-C) in agreement with the results of Rabin's group *in vitro*.⁶⁾

It has been shown by time course experiment *in vivo* using ¹⁴C-orotic acid as a precursor of RNA⁹⁾ and by inhibiting RNA synthesis with actinomycin D treatment¹⁰⁾ that mRNA on free polysomes is turning over more rapidly than that on membrane-bound polysomes. It is reasonable, then, to speculate that one of the roles of membrane in supporting protein biosynthesis in cells is to stabilize mRNA associated with membrane-bound polysomes. If it is the case, release of ribosomes with mRNA from membrane as a result of treatment with aflatoxin B₁ for 7 days as shown in Fig. 1-B should cause a decrease of polysomes and an increase of monosomes, which were observed in this experiment as shown in Fig.

2-B. The apparently similar phenomena (changes in ribosome-distributions between free and membrane-bound states and between monosomes and polysomes) could be induced when a supply of new mRNA was inhibited by treatment with actinomycin D for 15 hr.

We have concluded, then, that aflatoxin B₁ caused some structural changes of microsomal membrane when administered *in vivo* as demonstrated *in vitro* by Rabin's group, leading to release of polysomes from membrane, resulting in degradation of polysomes into monosomes during several days after the treatment.

When rats of Wistar Imamichi strain were used in stead of mice, the effects of aflatoxin B₁ on ribosome-distributions were not so clear. It may be a reflection of species-variety of the hydroxylation enzyme activity of liver microsomes by which aflatoxin B₁ is activated to induce the alteration of ribosome-distributions in liver cells, since aflatoxin must be biotransformed to have such an effect as generally proved for carcinogenic drugs.

Recently, Berdinskikh *et al.*¹¹⁾ have reported that a carcinogen, 4-dimethylaminoazobenzene, damaged binding of polysomes to microsomal membrane in rat liver as an early event in carcinogenesis. As the functional differences between free and membrane-bound polysomes have been proved to exist,^{8,12)} a damage of ribosome-membrane binding may cause a disturbance in the intracellular regulation of protein synthesis. Furthermore, this disturbance could be more serious if the stability of mRNA associated with membrane-bound polysomes decreases upon releasing from membrane.

We don't have any clear idea on the nature of interaction of membrane with aflatoxin and the biochemical mechanisms of competition between aflatoxin B₁ and corticosterone for polysome-binding sites on membrane. To clarify these problems, *in vitro* studies may be helpful as reported preliminary.^{13,14)} In these experiments, membrane fractions prepared from variously treated animals with drugs and hormones were incubated with polysomes *in*

vitro and polysomes-binding capacity of microsomal membrane was assayed.

In the light of discussions mentioned above, the results described in this paper may be one of the examples to show that ribosome-membrane interaction has an important regulatory meaning in protein biosynthesis in animal cells.

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