J. Biosci., Vol. 3 Number 3, September 1981, pp. 215-219 © Printed in India.

Functional changes in rat liver tRNA following aflatoxin B_1 administration

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MS received 8 May 1981; revised 8 July 1981

Abstract. Administration of aflatoxin B_1 (3 mg/kg body wt) to rats leads to strong inhibition of the acceptor activity of liver tRNA as measured by charging with [^{14}C]-chorella protein hydrolysate. The maximum inhibition occurs 2 h after treatment. At increasing intervals after treatment, the inhibition appears to be gradually relieved, till control values are restored by 72 h. The charging experiment using several [^{14}C]-amino acids separately shows pronounced inhibition of acceptor activity of all tRNA species, although the degree of inhibition varies with individual species. Preliminary results seem to rule out the possibility of hypermethylation of tRNA or damage to the CCA terminus as probable causes. The resultant functional changes may be attributed to a covalent interaction of aflatoxin B_1-metabolite with tRNA.

Keywords. Aflatoxin B_1; tRNA; acceptor activity.

Introduction

Aflatoxin B_1, a toxic metabolite elaborated by the mold *Aspergillus flavus* is also a potent hepatocarcinogen (Wogan, 1973; Goldblatt, 1969). Though the exact mechanism of its carcinogenic action is not understood, it has been shown to be able to interact with cellular macromolecules (Clifford and Rees, 1969; King and Nicholson, 1969). It has been suggested that 8, 9-epoxy aflatoxin B_1 is the ultimate carcinogenic metabolite which is generated by the action of liver microsomal enzymes on aflatoxin B_1 (Swenson *et al.*, 1977), and this highly electrophilic product interacts covalently with cellular macromolecules to initiate the carcinogenic process (Swenson *et al.*, 1974; Martin and Garner, 1977). Since transfer RNA (tRNA) molecules are key components involved in the regulation of cellular growth and differentiation (Weinstein, 1970; Kerr, 1975), it is of interest to study how aflatoxin B_1 interacts with them. An earlier report from this laboratory (Aboobaker and Bhattacharya, 1977) describes how this compound can form weak complexes with rat liver tRNA *in vitro*. Evidence of some degree of covalent binding was also obtained when [^{14}C] labelled aflatoxin B_1 was allowed to react with tRNA in presence of liver microsomes (Aboobaker and Bhattacharya, unpublished). Interactions of this nature *in vivo* could lead to conformational changes and might even bring about functional perturbations in tRNA.

The present communication deals with the effect of aflatoxin B_1 treatment on the process of enzymatic aminoacylation of liver tRNA. This reaction, catalysed by aminoacyl tRNA-synthetases, is the initial step in the process of stringing together of amino acid moieties into protein chains. The amino acid acceptor activity is considered to be the primary biological function of tRNA.

Materials and methods

Aflatoxin B_1 was obtained from Calbiochem, La Jolla, California, USA. [^{14}C]- Amino acids (sp. act 60-200 mCi/mmol) and [^{14}C]-chlorella protein hydrolysate (sp. act. 27 m Ci/matom C) were from Isotope Division, BARC, Bombay. Young male Wistar rats (100-150 g) were intraperitoneally injected with aflatoxin B_1 (dissolved in dimethyl sulphoxide) at a dose of 3 mg/kg body wt. At various intervals after injection, the animals were killed and total tRNA was prepared from the liver by phenol extraction, 1M NaCl extraction and purification on DEAE-cellulose column (Yang and Novelli, 1971). The purified tRNA was stripped of endogenously-bound amino acids by incubation with Tris buffer, pH 8.0 for 1 h at 37°C. A concentrate containing all the amino-acyl-tRNA synthetases required for this study was prepared by DEAE-cellulose chromatography of the 105,000 *g* supernatant from normal rat liver (Pearson *et al.*, 1973).

The assay of amino acid acceptor activity of tRNA was performed following the method of Yang and Novelli (1971). The reaction mixture (100 µl) contained 5 µmol of Tris-HCl (pH 7·5), 1 µmol of $MgCl_2$, 2 µmol of KCl, 0.4 µmol of ATP, 0.5 A_{260} unit of tRNA 0.1 µCi[^{14}C]-amino acid (or 0.5 µCi[^{14}C]-chlorella protein hydrolysate) and a saturating amount of the synthetase enzyme. The reaction mixture was incubated at 37°C for 10 min. Aliquots of 50 µl were pipetted on Whatman No. 3 MM filter paper discs which were then washed successively with cold trichloroacetic acid (containing 1% casamino acids), ethanol and ether. The dried paper discs were then counted for radioactivity in a Beckman Liquid Scintillation Spectrometer (Model LS 100). Rats injected with dimethyl sulphoxide were used for preparation of control tRNA samples. In the case of individual amino acis, the values were expressed as pmol of amino acids bound per A_{260} unit of tRNA.

Results

As we were interested in estimating the amino acid acceptance capacity of tRNA, these assays were conducted with limiting tRNA concentration in the presence of an excess amount of enzyme. Under the assay conditions, the acceptor activity was found to be linear for several amino acis upto 2.0 A_{260} of tRNA, and by 20 min the aminoacylation reaction reached a plateau.

It was observed that aflatoxin B_1 treatment led to considerable inhibition of acceptor activity of rat liver total tRNA when a mixture of amino acids derived from [^{14}C]-chlorella protein hydrolysate was used for charging (figure 1). This effect was, however, transient; this was shown by the gradual reversal of inhibition of aminoacylation when tRNA was isolated at increasing times after aflatoxin treatment. The activity returned to normal level by 72 h, reflecting renewed

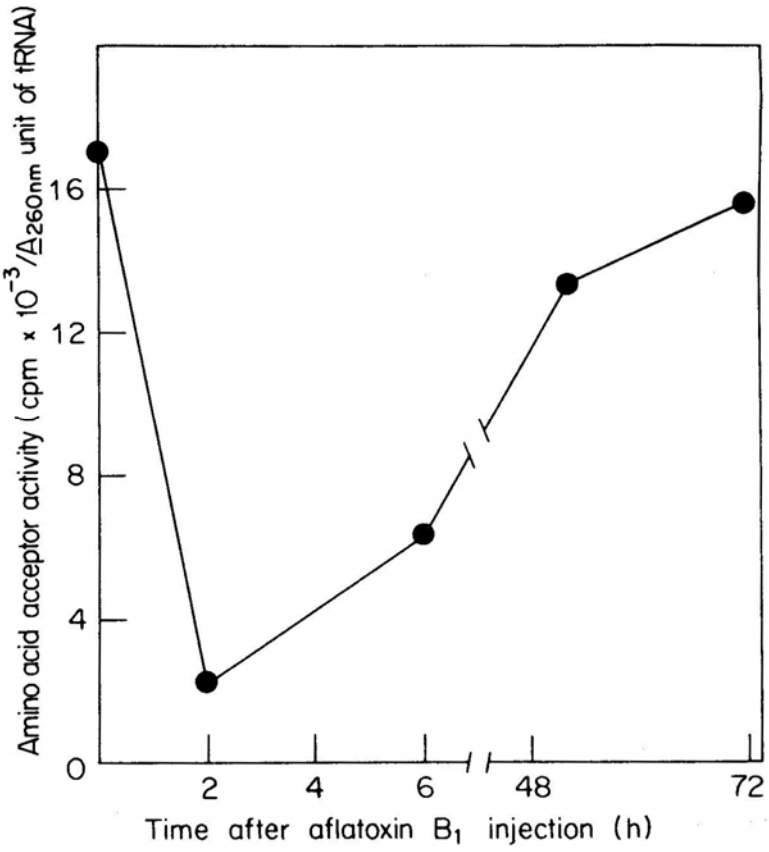

Figure 1. Acceptor activity of liver tRNA from rats at different times after administration of aflatoxin B_1 (3 mg/kg body wt.). Amino acid mixture derived from ^{14}C-chlorella protein hydrolysate was used for charging. Each point represents average value of four rats.

turnover of tRNA species. Further experiments were conducted to determine whether different species of tRNA were affected to similar or different degrees with respect to their individual amino acid acceptor activity. Consequently, liver tRNA was isolated from rats 2 h after aflatoxin B1 administration, and the individual acceptor activity was measured using 14 different [^{14}C]-amino acids. These results are shown in table 1. In each case, the activity was considerably lower than the corresponding control value. The degree of inhibition varied from 34% to 91 %. The most significantly inhibited tRNA species were proline (91% inhibition), aspartic acid (84%) and leucine (80%). Valine, tyrosine, threonine, serine, arginine and alanine-specific tRNA species were inhibited to the extent of about 70%, while tRNAs accepting glutamic acid, glycine, isoleucine, lysine, and phenyl-alanine were affected marginally (3540% inhibition).

Table 1. Amino acid acceptor activity of liver tRNA following aflatoxin B_1 administration.

Amino acid	Acceptor activity (pmol/A_{260} unit tRNA)		Ratio
	Control (DMSO)	AFB_1—Treated	Control
Alanine	12.3	4.0	0.32
Arginine	42.0	16.1	0.38
Aspartic acid	24.0	4.0	0.16
Glutamic acid	36.0	16.4	0.45
Glycine	32.0	19.0	0.59
Isoleucine	8.8	4.2	0.48
Leucine	15.0	3.0	0.20
Lysine	12.0	6.4	0.53
Phenylalanine	15.8	10.4	0.66
Proline	27.2	2.4	0.09
Serine	23.0	6.7	0.29
Threonine	12.5	3.3	0.26
Tyrosine	4.5	1.5	0.33
Valine	35.1	10.5	0.30

Rats killed 2 h after administration of AFB_1. Each figure represents average of four animals.
DMSO—Dimethyl sulphoxide
AFB_1-Aflatoxin B_1

Discussion

Among the several biochemical effects produced by aflatoxin B_1, is the strong inhibition it exerts on the biosynthesis of RNA and protein (Wogan, 1973). Since aminoacylation of tRNA is among the early steps in the formation of protein chains, interference at this step may well be expected to lead to inhibition of protein synthesis. It is not known at present whether this effect is relevant to carcinogenesis; this might as well be some manifestation of the acute toxic effect of aflatoxin B1.

It is not clear what mechanism is involved in the inhibition of acceptor activity of tRNA. Changes in the post-transcriptional modification of tRNA may alter its functional property. Hypermethylated tRNA, for example, have decreased amino acid acceptor activity (Bagewadikar and Bhattacharya, 1977). Whether aflatoxin B_1 treatment could bring about alteration in *in vivo* methylation of tRNA was tested by measuring the incorporation of label from injected [$^{14}CH_3$]-methionine into tRNA. Results, however, showed that aflatoxin B_1 treatment had no effect on this process (data not shown). Another possibility is that the carcinogen administration might lead to impairment of the terminal CCA group of tRNA which is absolutely essential for its functional integrity. Transfer RNA with damaged CCA terminus would exert an inhibitory effect on the amino-acylation of normal tRNA if added into the reaction mixture (Herrington and Hawtrey, 1970). However, since, in preliminary studies, it was observed that tRNA prepared from livers of aflatoxin B_1-treated rats failed to produce inhibition of aminoacylation of normal tRNA,

destruction of the CCA terminus can be ruled out. This is also confirmed by the observation that inhibition of the acceptor activity cannot be reversed when CTP is added to the aminoacylation reaction system (data not shown).

Certain lines of evidence point to the possibility that the critical target for carcinogens is extragenic (Farber, 1973; Pitot and Heidelberger, 1963; Weinstein, 1970). One such target may well be tRNA. In recent years, several carcinogens have been shown to react with these macromolecular components (Blobstein *et al.*, 1975; Pietropaolo and Weinstein, 1975; Davoud and Griffin, 1976). The findings reported here also seem to be significant from this standpoint. Functional changes have been detected in tRNA molecules after their covalent reaction with certain carcinogenic metabolites (Fink *et al.*, 1970; Fujimura *et al.*, 1972; Bagewadikar and Bhattacharya, 1977). A similar *in vivo* situation may be involved in the case of aflatoxin metabolites. Further work along this line is in progress.

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