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CARCINOGENIC HYDROCARBONS INCREASE THE ACCEPTANCE OF TRANSFER RIBONUCLEIC ACID FOR METHIONINE

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Abstract—Cytosols, aminoacyl–tRNA synthetases and tRNA were isolated from postmitochondrial supernatants of rat liver preincubated with polycyclic aromatic hydrocarbons. Cytosols from subcellular preparations treated with carcinogenic hydrocarbons showed a significantly enhanced activity in charging endogenous tRNA with methionine and tRNA from the same preparations had an increased acceptance for this amino acid if incubated with aminoacyl–tRNA synthetases from normal rat liver or from *Escherichia coli*. Preparations pretreated with non-carcinogenic hydrocarbons did not differ from controls in these respects. The activity of methionyl–tRNA synthetases was not enhanced by any of the hydrocarbons tested. No stimulation of methionyl–tRNA synthesis was found if carcinogenic hydrocarbons were added directly into mixtures with cytosol or aminoacyl–tRNA synthetases and tRNA. Active intermediates into which parent carcinogenic hydrocarbons are converted by microsomal enzymes apparently modify tRNA and thus enhance its acceptance for methionine.

Evidence has been obtained in our laboratory that polycyclic aromatic hydrocarbons affect the formation of the aminoacyl–tRNA complex if added into incubation mixtures containing pH 5 enzymes from rat liver. Charging of tRNA with amino acids was stimulated by carcinogenic hydrocarbons only, while non-carcinogenic compounds, although closely chemically related, were ineffective in this respect [1]. Similar effects were also found if *N*-nitroso carcinogens were used instead of hydrocarbons [2]. However, addition of carcinogenic compounds into incubation mixtures containing partially purified aminoacyl–tRNA synthetases and tRNA had no effect on the aminoacylation of tRNA. (J.Hradec, unpublished results). This indicated that polycyclic aromatic hydrocarbons are not able to react with components of this subcellular system unless activated by an enzymic system apparently missing in the fraction of partially purified aminoacyl–tRNA synthetases.

It seems now to be generally accepted that carcinogenic polycyclic aromatic hydrocarbons such as benzo(a)pyrene are not inherently carcinogenic, but require for their carcinogenic activity a prior activation by enzymes present in microsomes [3]. During the monooxygenation of the compounds [4], reactive intermediates such as electrophilic arene oxides [5], diol epoxides [6], and free radicals [7] are formed. These reactive intermediates may be covalently bound to tissues macromolecules [8, 9]. A good correlation was found between the carcinogenic activity of some hydrocarbons and their binding to DNA [10]. These intermediates undergo further conversions to less reactive products such as phenols, quinones, dihydrodiols, and various conjugates [11, 12]. For all these metabolic conversions enzymic systems localized in microsomes are required [13].

In experiments reported in this paper different carcinogenic and non-carcinogenic hydrocarbons were preincubated with a subcellular system of rat liver containing microsomes. After this preincubation, cytosol was isolated from these systems and tested for its activity in charging endogenous tRNA with methionine. Evidence is presented that carcinogenic polycyclic aromatic hydrocarbons significantly enhance the acceptance of tRNA for this amino acids whereas non-carcinogenic compounds of the same chemical class are free of this effect.

MATERIALS AND METHODS

Animals. Wistar rats of both sexes (120–150 g) bred in this laboratory and kept on a standard pelleted diet were used.

Chemicals and radiochemicals. ATP (sodium salt) was a product of Boehringer, Mannheim, West Germany. All polycyclic aromatic hydrocarbons were purchased from Koch–Light Laboratories, Colnbrook, Bucks., U.K. For the addition into incubation mixtures, these compounds were dissolved in absolute ethanol. If required, ethyl ether was added dropwise to complete the dissolution. All solvents were redistilled before use. L-[^{35}S]methionine (180 Ci/m-mole) was a product of the Radiochemical Centre, Amersham, Bucks., U.K.

Isolations. Homogenates of rat liver prepared by the usual technique of this laboratory [14] were centrifuged at 30,000 *g* and 4° for 15 min in 10 × 85 ml rotor of the VAC 20 centrifuge (G. Janetzki, Engelsdorf/Leipzig, GDR). The upper two thirds of the supernatant were separated and used as the postmitochondrial supernatant (the S-30 fraction) for the preincubation with the compounds tested. 35 ml of this fraction were supplemented with 200 μl of ethanol

containing appropriate quantities of hydrocarbons (or the same volume of ethanol in control mixtures). Mixtures were incubated in the Dubnoff incubator at 37° for the time indicated. Reaction mixtures were chilled in ice and centrifuged at 110,000 *g* and 4° for 120 min in a 8 × 35 ml rotor of a VAC 601 ultracentrifuge (G. Janetzki, Engelsdorf/Leipzig, GDR). The upper two thirds of the supernatant were separated and dialysed against 20 mM Tris–HCl buffer, pH 7.5, containing 50 mM KCl and 7 mM 2-mercaptoethanol (buffer B). This fraction, designated as cytosol, was used for a direct testing of Met–tRNA synthesis or for further isolations of aminoacyl–tRNA synthetases and tRNA, if required. For this purpose, approx. 25 ml of cytosol were applied on to DEAE–cellulose (Whatman DE 52) columns (diameter 15 mm, length 120 mm) equilibrated with buffer B. The fraction of partially purified synthetases was eluted with 250 mM KCl in buffer B and 500 mM KCl in the same buffer was used for the elution of tRNA. The fraction of aminoacyl–tRNA synthetases was precipitated with $[NH_4]_2SO_4$ to the 70 per cent saturation, and the resulting precipitate was dissolved in buffer B and dialysed against the same buffer. This fraction was then separated into small portions required for one set of experiments, quickly frozen and kept on solid CO_2. Eluate of tRNA was dialysed against water and tRNA was extracted with phenol, precipitated with ethanol, and deacylated [15]. tRNA preparations were kept at −30°.

tRNA from normal rat liver was isolated according to Rogg *et al.* [16]. For the partial purification of aminoacyl–tRNA synthetases of *E.coli*, the procedure of Matthaei *et al.* [17] was used.

Incubations. Incubation mixtures contained in the final volume of 0.1 ml: 20 mM Tris–HCl buffer, pH 7.5, 10 mM $MgCl_2$, 7 mM 2-mercaptoethanol, 25 nM of each of 19 natural amino acids, 2.5 nM L-methionine 0.1 mCi) and cytosol, or partially purified aminoacyl–tRNA synthetases from rat liver and tRNA as indicated. Mixtures for the assay of charging of rat liver tRNA in the presence of aminoacyl–tRNA sythetases from *E.coli* were composed essentially according to Matthaei *et al.* [17]. Mixtures were incubated at 37° for 30 min. 75 μl portions of each incubation mixture were then plated on discs of Whatman GF 81 filter and filters were washed as described by Hradec *et al.* [14].

Assay of radioactivity. Radioactivity was counted in a Nuclear Chicago Mark II liquid scintillation spectrometer with and efficiency of 85 per cent for ^{35}S as determined by the channel ratio method. Protein and RNA were estimated as described earlier [14].

RESULTS

The time for which the postmitochondrial supernatant was preincubated with polycyclic aromatic hydrocarbons appeared to be of a critical importance for the effect of these compounds on Met–tRNA synthesis. Incubation of the S-30 fraction with benzo(a)pyrene for 15 min had little effect on this reaction. However, preincubation of this subcellular fraction with the hydrocarbon for 30 min had a significant effect on charging tRNA with methionine in the cytosol. A maximum enhancement was found after 60 min of preincubation while longer incubation times showed a less pronounced effect (Fig. 1). If tRNA has been isolated from

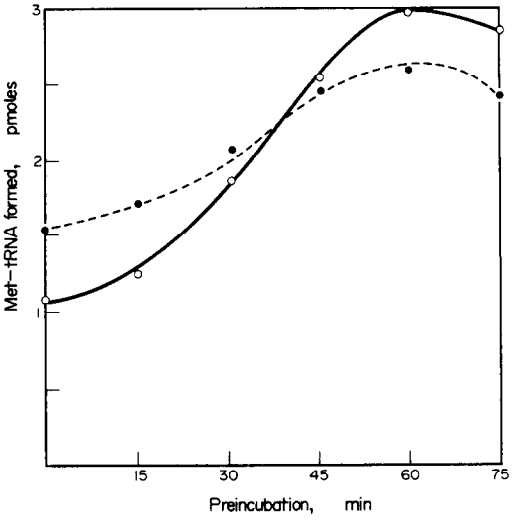

Fig. 1. Effect of different preincubation times with benzo(a)pyrene on the charging of endogenous tRNA in cytosol (●) with L-methionine and on the acceptance of isolated tRNA (○) for this amino acid. Postmitochondrial supernatants were treated with the hydrocarbon for the time indicated and cytosol, tRNA and aminoacyl–tRNA sythetases were isolated as described in Materials and Methods. Incubation mixtures contained 3.0 mg of cytosol protein, or 3 nmoles of tRNA isolated from preparations pretreated with the hydrocarbon, 150 μg of aminoacyl–tRNA synthetases from normal rat liver, and all other components as described in Materials and Methods. All values are pmoles-methionine/nmole tRNA.

subcellular preparations treated with benzo(a)pyrene for different times and tested for the acceptance of methionine in the presence of aminoacyl–tRNA synthetases from *E. coli*, a significantly increased charging of initiator tRNA with methionine was found again with a maximum after 60 min of preincubation (Fig. 1).

The stimulating effect of benzo(a)pyrene on Met–tRNA synthesis in the cytosol was strictly dose-dependent. Only additions of 10–100 nmoles of this hydrocarbon/ml of the postmitochondrial supernatant resulted in a significant enhancement of the Met–tRNA complex formation in cytosol separated from these preparations. If tRNA has been isolated from such preparations and charged with methionine in the presence of aminoacyl–tRNA synthetases from normal rat liver, an increased acceptance for this amino acid was demonstrated in mixtures containing the tRNA isolated from postmitochondrial supernatants treated with these active doses of the hydrocarbon (Fig. 2).

Principal differences were found in the charging of endogenous tRNA in cytosols preincubated with carcinogenic and non-carcinogenic hydrocarbons. In these experiments postmitochondrial supernatants were preincubated with 10 nmoles of the compound/ml of S-30 fraction for 60 min. Only cytosols pretreated with carcinogenic compounds showed an enhanced Met–tRNA synthesis in the cytosol while cytosols isolated from preparations treated with non-carcinogenic compounds charged tRNA with methionine similarly as did the control mixtures. This is in particular apparent if hydrocarbons of a similar chemical structure have been

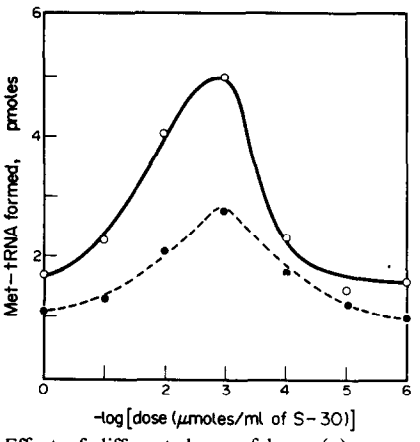

Fig. 2. Effect of different doses of benzo(a)pyrene on the charging of endogenous tRNA in cytosol with L-methionine (●) and the acceptance of isolated tRNA (○) for this amino acid. Experimental details are indicated in the legend for Fig. 1. Incubation mixtures with tRNA contained 3 pmoles of tRNA and 10 μg of aminoacyl–tRNA synthetases from *E.coli* B.

tested. Thus cytosols isolated from subcellular preparations pretreated with carcinogenic benzo(a)pyrene showed an enhanced formation of Met–tRNA complex while non-carcinogenic benzo(e)pyrene was not effective in this respect. Similar results were obtained with different dibenzopyrenes. While carcinogenic compounds of this class induced a significantly enhanced charging of endogenous tRNA with methionine in the cytosol, the related non-carcinogenic dibenzo(e,l) pyrene showed no activity in this respect. Essentially identical results were obtained with derivatives of benzo(a)anthracene. On the other hand, the addition of non-carcinogenic hydrocarbons (anthracene, pyrene or phenanthrene) into postmitochondrial supernatants had no effect on the charging of tRNA with methionine in cytosols isolated from these preparations (Table 1).

In the next experiments tRNA has been isolated from postmitocondrial supernatants pretreated with different hydrocarbons and its acceptance for methionine has been tested in the presence of partially purified aminoacyl–tRNA synthetases from normal rat liver or enzymes prepared from *E. coli*. Results similar to those described for cytosols have been obtained. A significantly increased acceptance of both initiator and total $tRNA^{Met}$ was found with preparations isolated from postmitochondrial supernatants preincubated with carcinogenic hydrocarbons. Again, tRNA originating from mixtures preincubated with non-carcinogenic hydrocarbons showed a similar acceptance for this amino acid as did the control preparation (Table 1).

In further experiments the fraction of partially purified aminoacyl–tRNA synthetases was isolated from subcellular preparations pretreated with different hydrocarbons and tested for its activity in Met–tRNA synthesis with tRNA isolated from normal rat liver. No differences were found in the activity of these enzymes prepared from postmitochondrial supernatants treated with either carcinogenic or non-carcinogenic hydrocarbons. Unlike with the effect of these compounds on the acceptance of tRNA for methionine, highest doses of all hydrocarbons used resulted in a significant inhibition of Met–tRNA synthetase activity. Even doses which were most active in increasing the acceptance of tRNA for this amino acid induced a partial inhibition of Met–tRNA synthetase activity (Fig. 3).

Table 1. Effect of carcinogenic and non-carcinogenic hydrocarbons on the charging with L-methionine of endogenous tRNA in cytosols pretreated with these compounds and of added tRNA isolated from these preparations and supplemented with aminoacyl–tRNA sythetases from *E.coli*.

No.	Compound	Carcinogenicity	Reference	Charging of tRNA Endogenous	Isolated
1	Benzo(a)pyrene	+	27	2.79	5.20
2	Benzo(e)pyrene	0	27	1.51	1.80
3	Dibenzole(e,l)pyrene	0	26	1.70	1.92
4	Dibenzo(a,e)pyrene	+	26	2.27	4.27
5	Dibenzo(a,i)pyrene	+	26	2.99	5.12
6	Dibenzo(a,h)pyrene	+	26	2.74	4.95
7	Benz(a)anthracene	±	28	1.87	3.72
8	Dibenz(a,h)anthracene	+	28	2.71	5.12
9	7,12-Dimethyl-benz(a)anthracene	+	28	2.17	4.65
10	2-Aminofluorene	+	28	2.24	4.85
11	Fluoranthene	0	28	1.61	2.05
12	Dibenzo(a,e)fluoranthene	+	26	2.74	4.97
13	Anthracene	0	28	1.62	1.95
14	Chrysene	±	28	1.86	2.47
14	Pyrene	0	28	1.53	2.05
16	Phenanthrene	0	28	1.61	2.34
17	3-Methylcholanthrene	+	28	2.86	5.42

Postmitochondrial supernatants from rat liver were incubated for 60 min at 37° with 1 nmole/ml of the hydrocarbon. Cytosols from these preparations (2.5 mg of protein) were then tested for the charging of endogenous tRNA with L-methionine as described in Materials and Methods. tRNA isolated from postmitochondrial supernatants pretreated with hydrocarbons (3 nmoles) was supplemented with aminoacyl–tRNA sythetases from *E.coli* B (7 μg of protein) and tested for the charging of initiator tRNA with L-methionine as described in Materials and Methods. All values are pmoles of methionine/nmole of tRNA.

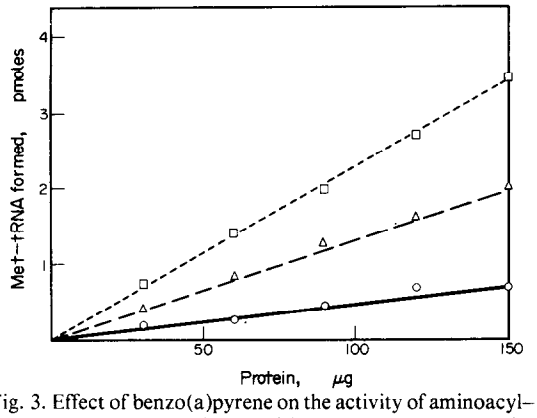

Fig. 3. Effect of benzo(a)pyrene on the activity of aminoacyl–tRNA sythetases. Posmitochondrial supernatants were preincubated for 60 min with ethanol (□), 1μmole (○) or 1 nmole (△) of the hydrocarbon/ml and aminoacyl–tRNA synthetases were isolated and incubated as described in Materials and Methods.

Addition of carcinogenic or non-carcinogenic hydrocarbons directly into incubation mixtures containing cytosol or aminoacyl–tRNA synthetases and tRNA had no effect on the charging of tRNA with methionine (data not shown).

DISCUSSION

Evidence provided in this paper is in full agreement with our previous results [1] that carcinogenic polycyclic aromatic hydrocarbons stimulated the aminoacylation of tRNA. An enzymic system not present in partially purified aminoacyl–tRNA synthetases, most probably of microsomal origin, is apparently absolutely required for this effect. This opinion is supported by the fact that an addition of the compounds directly into incubation mixtures containing purified synthetases and tRNA was not effective. It seems highly probable that the crude pH 5 enzyme fraction used in our previous experiments [1, 2] contained some microsomal enzymes. This explains why carcinogenic hydrocarbons were active if added into these systems.

Enzymes of the monooxigenase type present in microsomes [18] convert polycyclic hydrocarbons into more polar metabolites and these reactive intermediates react then with nucleic acids and proteins [19]. Evidence has been provided indicating a relation between the degree of carcinogenicity and binding to DNA under appropriate conditions of metabolism. On the other hand, no such relation was found with the binding to RNA or protein [20]. However, the administration of *N*-acetylaminofluorene and *N*-hydroxy-2-acetylaminofluorene to rats resulted in a rapid labelling of different RNA species and a preferential binding of the compounds to tRNA has been established [21]. An *in vitro* reaction of tRNA from *E. coli* with *N*-acetoxy-2-acetylaminofluorene resulted in a covalent attachment of the hydrocarbon residues to tRNA. This binding produced specific modifications in the amino acid acceptance capacity, codon recognition and ribosomal binding [22]. The same carcinogen was also demonstrated to modify the template activity of synthetic polynucleotides [23].

All these results suggest that the structure and function of tRNA may be altered by carcinogenic compounds. It seems highly probable that in our experiments tRNA has been modified by the action of active intermediates resulting from the metabolic activation of these compounds by microsomal enzymes. Such a modification of tRNA molecule may apparently result in an increased acceptance for a particular amino acid.

In our experiments aminoacyl–tRNA synthetases of bacterial origin were also used since they charge with methionine specifically only the initiator tRNA species leaving the $tRNA_M^{Met}$ uncharged [15]. Although the increased charging induced by carcinogenic hydrocarbons is apparently not specific for initiator tRNA as revealed by our experiments in which aminoacyl–tRNA synthetases from rat liver were used which charge both $tRNA^{Met}$ species, nevertheless, the stimulation of Met–$tRNA_F^{Met}$ formation by carcinogenic hydrocarbons may be highly significant. Such a modification of initiator tRNA may have important consequences in peptide initiation which is apparently the most important step in the regulation of protein synthesis at its translational level [24]. Such a modification of this tRNA species may also help to explain why *N*-nitroso carcinogens stimulate this particular step of protein synthesis [25].

It would be premature to conclude that a modification of tRNA is specific for carcinogenic compounds in general. However, our present results provide good evidence that this is true at least for polycylic aromatic hydrocarbons. Further experiments with carcinogens of different chemical classes are essential to reach a definitive conclusion in this respect.

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