RNA Sulfurtransferase Activity in Rat Liver and Chemically Induced Hepatomas

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SUMMARY

RNA sulfurtransferase activity has been detected in rat liver and in hepatomas from rats fed a diet containing 0.06% 3'-methyl-4-dimethylaminoazobenzene for 14 to 18 weeks. The reaction measured was the transfer of sulfur from cysteine to acceptor sites in Escherichia coli B transfer RNA (tRNA). Specific activities of the enzymes in liver and hepatoma supernatant fractions were similar, as were the rates and extents of sulfur transfer to tRNA. DEAE-cellulose chromatography of digests of the [35S]tRNA reaction products revealed 3 peaks associated with nucleotide material, the amounts of these peaks differing in tRNA from liver and hepatoma systems. This may suggest differences in specific sulfurtransferases in these tissues.

INTRODUCTION

Neoplastic tissues have been reported to contain higher tRNA methylase activities than their closest normal tissue counterpart (1, 3, 4, 10-12). In some cases, enzymes with different specificities were found in tumors (4, 10), and qualitative and quantitative differences in the methylated bases of tumor tRNA were reported (3, 11, 12). Methylation represents but 1 class of modifications to the tRNA molecule, and it would prove useful to gain information on the activities of other tRNA modification enzymes in normal and tumor tissues. Towards this end, we have measured RNA sulfurtransferase activity in preparations from rat liver and 3'-methyl-DAB-induced hepatomas, and we have analyzed the nucleotide content of the reaction product of these enzymes.

MATERIALS AND METHODS

Male, adult, albino Wistar rats (Hilltop Breeders, Scottsdale, Pa.) were used in these studies. Hepatomas were induced by feeding rats a diet containing 0.06% 3'-methyl-DAB as described by Sorof and Young (90), and controls were fed a diet lacking the carcinogen. After the rats had been on the carcinogenic diet for 14 to 18 weeks, the hepatomas induced were 3 to 4 times larger than liver, and involvement of all lobes was nearly complete. Livers and hepatomas were excised, minced, and homogenized in 4 volumes of 50 mM Tris-HCl, pH 7.6-5.0 M MgCl2.25 mM KC1-0.25 M sucrose. The homogenate was centrifuged at 105,000 x g for 90 min, and the clear supernatant solution was removed and dialyzed for 2 hr against 2 changes of the above buffer minus sucrose. Protein was measured by the method of Lowry et al. (8), using bovine serum albumin as standard.

RNA sulfurtransferase activity was measured by the method of Hayward and Weiss (6) as modified by Harris and Titchener (5). Assays were carried out in a volume of 0.5 ml and contained: 25 µmoles Tris-HCl, pH 8.5; 2.5 µmoles MgCl2; 2.0 µmoles ATP; 2.5 µmoles mercaptoethanol; 0.2 mg E. coli B tRNA (Schwarz/Mann, Orangeburg, N. Y.); 1.2 to 1.7 µCi [35S]cysteine (174 or 245 mCi/m mole; Amersham/Searle Corp., Arlington Heights, Ill.); and 0.1 mg of dialyzed supernatant. Incubation was at 37°, and the reaction was stopped by the addition of 1.5 ml of a 3.33 mM cysteine solution, 0.5 mg of Torula RNA (Sigma Chemical Co., St. Louis, Mo.), and 0.05 ml of a 5% cetyltrimethylammonium bromide solution. The precipitate was collected after 1 hr at 0° and dissolved in 0.25 ml of 2 M NaCl-5 mM Tris-HCl, pH 8.0, and the RNA was precipitated by the addition of 2 volumes of 95% ethanol. After 1 hr at —20°, the precipitate was collected by centrifugation at 17,300 x g for 20 min and dissolved in 0.25 ml 5 mM NaCl-0.5 mM Tris-HCl-pH 10.0. A 1 hr incubation at 37° served to hydrolyze aminoacyl-tRNA formed during the reaction. The mixture was treated with an equal volume of 88% phenol, shaken for 10 min, and centrifuged as above. RNA was precipitated from the aqueous layer by the addition of ethanol and filtered onto Millipore polyvinyl chloride filters. The filters were washed and dried for scintillation counting as previously described (5). Despite the numerous extraction and precipitation steps used in this assay, the yield of a known amount of [35S]tRNA was 81.9 ± 2.0% (n = 5).

Sulfur incorporation into nucleotide material was determined as follows. [35S]tRNA was isolated from the above reaction mixtures by phenol extraction and ethanol precipitation and was treated to remove cysteinyl-tRNA (Chart 3, legend). The [35S]tRNA was hydrolyzed in 0.3 N KOH for 18 hr at 37°, neutralized using Dowex 50-H+, and chromatographed on DEAE-cellulose according to the method of Lipsett (7). Radioactivity in each fraction was determined in Bray’s solution (2), the data being correct for quenching.
RESULTS

We have successfully adapted a RNA sulfurtransferase assay used with bacterial extracts (5, 6) to investigate the properties of the enzyme(s) in mammalian tissues. In this study we have compared sulfurtransferase activities in supernatant solutions from rat liver and 3'-methyl-DAB-induced hepatomas. The time course of the reaction is shown in Chart 1, in which no difference in either the rate or extent of sulfur incorporation into tRNA was seen for the 2 enzyme sources. In these reactions, sulfur transfer from cysteine to E. coli B tRNA was measured, and the data were corrected for incorporation in the absence of added tRNA. Sulfur transfer was proportional to added supernatant protein, the incorporation increasing in a linear fashion up to 200 µg of protein (Chart 2). Over this protein range the specific activity (pmoles [35S] per mg tRNA per mg protein) of liver extracts was 13.4 ± 4.2 (n = 5), and for hepatomas, 12.2 ± 0.8 (n = 5). The large standard errors associated with these data are due to activity differences from tissue to tissue, since replicate samples from a given tissue routinely show a standard error of less than 5%.

The sulfur incorporated into tRNA was nondialyzable, migrated with tRNA on methylated albumin-kieselguhr columns, and was completely eliminated by pancreatic RNase treatment. Since the above assay procedure utilized a step to hydroyze aminoacyl-tRNA, it is concluded that the sulfur incorporation is not due to cysteinyl-tRNA formation. The [35S]tRNA reaction product was isolated and hydrolyzed to the nucleotide level using KOH, and the nucleotide mixture was chromatographed on DEAE-cellulose. Chart 3 shows that 3 radioactive peaks were associated with nucleotide elution areas for both liver and hepatoma-labeled tRNA hydrolysates. A greater amount of nonnucleotide material was found in the liver hydrolysate (Fractions 2 to 10), but this varied from one preparation to another and probably represents residual cysteine (5). Although the total incorporation data were similar, nucleotide profiles for [35S]tRNA from 4 liver and 2 hepatoma systems showed quantitative differences between the various peaks, the major difference being in the 1st 2 peaks associated with the nucleotide region. This may indicate differences in the activity of specific sulfurtransferases in these preparations.

DISCUSSION

RNA sulfurtransferase activity was present in cell supernatants from both rat liver and 3'-methyl-DAB-induced hepatomas. The reaction involves the transfer of sulfur from cysteine to E. coli B tRNA, the heterologous substrate used in these studies. The rate and extent of the liver and hepatoma enzyme-catalyzed reactions were quite similar, in contrast with the findings for tRNA methylases, the activities of which were often elevated in tumors (1, 3, 4, 10-12). Sulfur incorporation was proportional to added supernatant solution up to 200 µg of protein, with no difference in the specific activity of the liver and hepatoma enzymes being observed. Hence, the activity of RNA sulfurtransferase in liver and fully developed hepatomas appears to be identical.

The [35S]tRNA product of the liver and hepatoma enzyme systems was isolated by phenol extraction and ethanol precipitation, hydrolyzed with KOH, and chromatographed on DEAE-cellulose by the method of Lipsett (7). Three radioactive peaks were associated with nucleotide material for both the liver and hepatoma products. Quantitative differences in the apparent thionucleotide patterns were observed for the 2 tRNA preparations, suggesting that liver and hepatoma may differ in the activity or amount of specific sulfurtransferases, although the total activities were similar.

Our results may be compared with those of Wong et al. (13), who studied the thiolation of yeast tRNA using β-mercaptopyruvate as the sulfur donor, and a partially purified sulfurtransferase preparation from rat cerebral cortex. The specific activity of the brain enzyme was higher than observed here for liver and hepatoma, and preliminary work in our laboratory substantiates this. However, sulfur incorporation from β-[35S]mercaptopyruvate was reduced to low levels by a chemical deacylation step and presumably

Chart 1. Time course of the tRNA sulfurtransferase reaction for liver and hepatoma supernatant preparations. Assays were carried out as described, with 1.72 µCi [35S]cysteine (174 mCi/mmole) and 0.1 mg of dialyzed supernatant present. Each tube was incubated for the indicated time at 37°, the reaction was stopped, and radioactivity was determined (10). Each curve represents a composite of 2 separate liver and hepatoma preparations.

Chart 2. Effect of protein level on RNA sulfurtransferase activity in liver and hepatoma preparations. The assay conditions were as described, except that various amounts of protein were added. The mixtures were incubated for 10 min at 37°, in the presence of 1.22 µCi [35S]cysteine (specific activity, 245 mCi/mmole). These data were accumulated from experiments with several different livers and hepatomas. Q, liver enzyme; A, hepatoma enzyme.
Our results suggest that not all tRNA modification enzymes are present in greater amounts in tumors, as one might predict for a tissue with rapid rates of growth, protein synthesis, and perhaps tRNA synthesis. However, these experiments used fully developed hepatomas obtained after 14 to 18 weeks of carcinogenic diet. It is possible that enzyme activities were altered in such advanced tumors. Further, the state of differentiation of the tumor should be defined, and enzyme activities should also be measured in various types of tumors. These experiments are currently in progress.

REFERENCES

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