Comparison of Transfer Ribonucleic Acids and Aminoacyl Synthetases of Liver and Ascites Tumor Cells

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SUMMARY

Aminoacyl synthetase and transfer ribonucleic acid (tRNA) fractions were obtained from normal rat liver and from Novikoff ascites tumor cells. The tRNA was precharged with tritium and 14C-labeled arginine, phenylalanine, or valine employing various combinations of the synthetase and tRNA fractions. The labeled aminoacyl tRNA's were resolved employing reversed phase chromatography. The tumor and liver cell patterns for arginyl tRNA were almost identical, while the cell types differed with respect to the phenylalanyl tRNA profile. When liver phenylalanyl tRNA was chromatographed on the reversed phase columns, three distinct peaks were obtained. In contrast, the tumor phenylalanyl tRNA did not exhibit the first peak seen in the liver profile, and the second tumor phenylalanyl tRNA peak was displaced.

INTRODUCTION

Many investigators have proposed varying roles for aminoacyl synthetases and transfer ribonucleic acids in developmental and regulatory functions of cells. Roth and Ames (13) have observed that mutants in the hisS gene of Salmonella typhimurium have a histidyl tRNA synthetase with a greatly decreased affinity for histidine. Subsequent findings from this laboratory (Silbert et al. (15)) have indicated that histidyl tRNA has a role in the repression of the histidine operon. Carbon et al. (5) have found that missense mutations affecting the A-protein of E. coli tryptophan synthetase are suppressible and that the suppression involves an altered tRNA.

There is indication already that tRNA from tumor cells may differ from certain normal tissues. Taylor et al. (16) observed differences in specific tRNA's in mouse tumors when comparisons were made with normal counterparts. Employing MAK chromatography, Baliga et al. (1) found that the profiles for tyrosyl and histidyl tRNA's from the Novikoff hepatoma differed from the normal liver controls. Of interest is the finding by Holland et al. (8) of a new species of tyrosyl tRNA in tumors produced by SV40 virus. Mach et al. (9) also have indicated that various tRNA's specific for an amino acid may differ from one plasmocytoma tumor to another. This observation in these closely related tumors would suggest a possible control function for tRNA in the biosynthesis of the different immunoglobulins elaborated by these tumors.

Employing the reversed phase chromatography procedure of Weiss and Kelmers (17), Yang and Novelli (18) demonstrated the presence of multiple isoaccepting tRNA's for 20 amino acids from a mouse plasma cell tumor. In the present study, the basic Weiss and Kelmers procedure was used to compare the aminoacyl synthetases and tRNA's of normal rat liver and of Novikoff ascites cells, a tumor of hepatic origin. The liver and tumor tRNA profiles for arginine, phenylalanine, and valine are presented in this communication.

MATERIALS AND METHODS

Preparation of Liver and Tumor Aminoacyl Synthetases and tRNA Fractions. Novikoff ascites tumor cells were collected and washed as described previously (7, 11). Six volumes of cold deionized water were added to the washed cells and the mixture homogenized in a power driven apparatus (Teflon pestle and glass tube). Ionic strength was adjusted by the addition of a concentrated solution (2.5 M sucrose, 0.25 M KCl, and 0.05 M MgCl₂—1 part to 9 of homogenized tumor cells) and the homogenate centrifuged at 15,000 X g for 30 minutes. The resulting supernatant fraction was recentrifuged at 150,000 X g (No. 50 titanium rotor, Spinco Model L2 Centri-fuge) for one hour. This supernatant fraction was adjusted to pH 5.0 by the addition of 1 N acetic acid, and the precipitate dissolved in standard buffer (0.05 M Tris-HCl; 0.25 M sucrose, 0.005 M MgCl₂; 0.025 M KCl, pH 7.6). Usually this fraction contained from 20 to 30 mg of protein per ml. Endogenous tRNA's were removed from this fraction by the addition of 0.1 gm of DEAE cellulose per ml followed by stirring for thirty minutes. After the mixture was centrifuged, the supernatant fraction containing the aminoacyl synthetase was

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3 The abbreviations used are: DEAE, diethylaminoethyl cellulose; MAK, methylated albumin kieselguhr; POPOP, 1,4-bis-(2-(5-phenyl-oxazolyl)-benzene; PPO, 2,5-diphenyloxazole; TCA, trichloroacetic acid; Tris, tris(hydroxymethyl) amino methane; tRNA, transfer ribonucleic acid.

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adjusted to 20% by volume with glycerol and 0.01 M with respect to $\beta$-mercaptoethanol. Enzymatic activity was retained for several weeks with storage at $-10^\circ C$.

The livers of 7—8 young rats (approx. 50 gm) were homogenized in two volumes of standard buffer and centrifuged at 15,000 $X$ g for 30 minutes; the resulting supernatant fraction was subjected to 150,000 $X$ g for one hour. The aminoacyl synthetase fractions were then prepared from the material insoluble at pH 5, as described above for the tumor cells.

Transfer RNA fractions were prepared from washed tumor cells or from normal rat liver by the procedure of Brunngraber (4). An average yield of 40 mg tRNA per 100 gm of liver or packed tumor cells was obtained. A commercial source of rat liver tRNA was used for part of this investigation (General Biochemicals, Inc., Chagrin Falls, Ohio).

Aminoacyl-$^{14}C$ and $^{3}H$ tRNA's were prepared from a reaction mixture containing 7—8 mg tRNA, 50 mg DEAE cellulose-stripped synthetase protein (pH 5 fraction), 12.5 $\mu$moles ATP, 12.5 $\mu$m of amino acid-$^{14}C$ or $^{16}C$ of amino acid-$^{3}H$, and 0.25 volumes of a buffer (0.2 M Tris-HCl, 0.02 M MgCl$_2$, 0.02 M KCl, pH 7.5). The above mixture was incubated 30 minutes at $37^\circ C$ and extracted one time with an equal volume of 80% phenol at 0°C. The aqueous layer was precipitated once with 2 volumes of cold 95% ethanol for 30 minutes, and the resulting aminoacyl-tRNA was dissolved in 1.0 ml of water at pH 4.5.

Amino Acids-$^{14}C$. Uniformly labeled L-amino acids-$^{14}C$ were purchased as follows: New England Nuclear, arginine 320 mc/mole; International Chemical and Nuclear Corporation, valine 190 mc/mole.

Amino Acids-$^{3}H$. L-amino acids-$^{3}H$ were purchased from: Nuclear Chicago, arginine 380 mc/mole; New England Nuclear, phenylalanine 4.25 cm/mole; Schwarz Bioresearch, Inc., valine 340 mc/mole.

**RESULTS**

Chromatographic profiles of combinations of tritium and $^{14}C$-labeled arginyl tRNA's were obtained by the interactions of aminoacyl synthetases and tRNA's from liver and tumor cells. Similar patterns were obtained with all combinations of synthetases and tRNA's and results of a typical chromatogram are shown in Chart 1. A consistent and uniform pattern was observed in all of these determinations. The 260-mu-absorbing material emerged from the columns at fractions 54—56, and the first of the labeled arginyl tRNA appeared at approximately fraction 68. Five separate arginyl tRNA peaks were obtained in every case. In addition, the position of each of these peaks remained unchanged, as shown in Chart 1. Thus, it may be concluded that the aminoacyl synthetases and tRNA's for arginine are similar in the ascites tumor cells and in normal liver and tumor cells on the same column.

A linear gradient was employed to develop the column [1 liter each of buffer A and buffer B (0.5 M NaCl, 0.01 M MgCl$_2$, 0.01 M sodium acetate, pH 4.5)]. The column flow rate was 1 ml per minute. Initial runs were carried out in jacketed columns maintained at $27^\circ C$. However, it was observed subsequently that identical chromatograms were obtained if the columns were insulated and developed at room temperatures. Fifteen-ml fractions were collected, and the absorption at 260 m$\mu$ was ascertained. One-ml aliquots of each sample were precipitated by the addition of 10 ml of cold 5% TCA (allowed to stand for 15 minutes). The precipitates were collected by filtration on Millipore filters (type H.A., pore size 0.45 $\mu$m) and washed with an additional 10 ml of 5% TCA. Each filter was washed with 5 ml of ethanol and dried at $80^\circ C$ for 10 minutes. The filters were counted in vials containing 10 ml of scintillation fluid (4 gm of PPO, 0.2 gm of POPOP, 1 liter of sulfur-free toluene) in a Packard Tri-Carb liquid scintillation spectrometer with maximal setting for simultaneous tritium and $^{14}C$ counting.
liver cells. For comparative purposes E. coli tRNA was charged with arginine using liver aminoacyl synthetase, and liver tRNA was charged with arginine employing E. coli synthetase. The labeled arginyl tRNA's were chromatographed on the same column. As shown in Chart 2, only one major arginyl tRNA peak was obtained from the E. coli tRNA, while the microbial synthetase appeared to charge all of the arginyl tRNA species of the liver.

Tumor and liver tRNA's were charged with \(^{3}\text{H}\)- or \(^{14}\text{C}\)-labeled phenylalanine utilizing synthetases from each tissue. The elution profiles that were obtained revealed some possible differences between the tumor and liver tissues. It may be observed in Chart 3 that three distinct peaks were present when liver tRNA was charged with phenylalanine-\(^{14}\text{C}\) in the presence of liver synthetase preparations. Chromatography on the same column of tumor tRNA charged with phenylalanine-\(^{3}\text{H}\) (in the presence of tumor synthetase) revealed an absence of the first and a displacement of the second phenylalanyl tRNA peaks. However, a normal elution profile for phenylalanyl tRNA was obtained from the combination of tumor synthetase and liver tRNA (Chart 4). When the tumor tRNA was charged with phenylalanine in the presence of liver synthetase, the elution profile indicated a low initial peak and a displaced second peak (Chart 4). It would appear that there are differences between tumor and liver in the tRNA's that react with phenylalanine.
Finally, a comparison of valyl tRNA's from tumor and liver cells was made employing reversed phase chromatography. As shown in Chart 5, some displacement was observed between the tumor and liver valyl tRNA peaks. Some difficulty was encountered in the complete resolution of these valine peaks. Further studies employing different gradients are in progress to improve the separation of the valine tRNA profile.

**DISCUSSION**

Several individual acceptor tRNA's for arginine, phenylalanine, and valine were found in normal liver and in tumor cells by the reversed phase chromatography procedure. These observations are in agreement with those of Yang and Novelli (9) who reported multiple “isoaccepting” tRNA's in mouse plasma cell tumors. The elution profiles for the arginyl tRNA's of the liver and tumor cells were almost identical. In contrast the phenylalanine-accepting tRNA's in tumor differed from those in normal liver. The initial phenylalanyl tRNA peak present in liver was not observed in the eluates from the tumor cell preparations. In addition, the second phenylalanyl tRNA peak of the tumor and liver cells differed with respect to their rate of emergence from the columns.

Other investigators have reported differences in specific tRNA's in tumors (1, 9, 16). Of related interest are the findings that chemical carcinogens react with transfer and other RNA's (6, 12) and the appearance of different types of tRNA's in tumors produced by SV40 virus (6). Whether these chromatographically different tRNA's are the result of alterations in the sequence of nucleotides or of other changes within the molecule remains to be established. It would appear that extensive methylation may alter the chromatographic behavior of tRNA's. Borek (3) has reported that the methylation capacity of extracts from several animal tumors exceeds the methylating capacity of extracts of appropriate control tissues. This group found also that the rates and extent of methylation were greater in human mammary carcinomas and carcinomas of the colon than in normal adjacent tissues. McFarlane and Shaw (10) observed a several-fold elevation in tRNA methylase capacity in tumors produced by adenovirus-12, and Silber et al. (14) noted a three-fold increase in methylase capacity five days after murine leukemia virus infection.

The possibility exists that the tumor and liver may differ with respect to enzymes that affect the tRNA's, i.e., ribonuclease, phosphodiesterase, phosphatases, etc. Yang and Novelli (18) indicated that the multiple isoaoccting tRNA peaks may be due to aggregation, degradation, or differential removal of the pCpCpA terminal. However, they excluded these changes as factors in the production of artifactual species of tRNA. In the present study, similar procedures were utilized in order to minimize the possibility that the different aminoacyl tRNA's were artifactual. Since the comparisons of liver and tumor aminoacyl tRNA's were carried out under the same conditions of isolation, chromatography on the same columns, etc., it is believed that the differences that were observed do represent real changes in the tRNA's. Uncharged tRNA's of arginine, phenylalanine, and valine exhibited different elution profiles than the charged species. This was observed also by Yang and Novelli (18) for the tRNA's of lysine and leucine.

The tRNA's used in this study were prepared from total liver and tumor cells. It is possible that mitochondrial tRNA may have contributed to the elution patterns that were observed (2). Preliminary attempts to extract tRNA's from liver and tumor cell mitochondria gave some indication that the concentration of tRNA's present in these organelles would not have influenced the findings as reported. However, further studies of the compartmental distribution of aminoacyl synthetases and tRNA's in mammalian cells should be carried out.

With the firm establishment of differences in the chromatographic behavior of tRNA's from tumors and normal counterparts, it should be possible to investigate the chemical or physical changes responsible for these differences. Extension of this approach to other normal, precancerous, and cancerous tissues may provide some new insight as to the role of aminoacyl synthetases and tRNA's in carcinogenesis and in the behavior of malignant tumor cells.

**REFERENCES**

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