Chromatographic Studies of Tyrosyl and Phenylalanyl Transfer RNA’s of Liver and Tumor Cells

M. Hayashi, A. C. Griffin, Ronald Duff, and Fred Rapp

The Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025 [M. H., A. C. G.]. The Department of Microbiology, College of Medicine, M. S. Hershey Medical Center of the Pennsylvania State University, Hershey, Pennsylvania 17033 [R. D., F. R.]

SUMMARY

Elution profiles of tyrosyl and phenylalanyl transfer RNA’s from several normal and cancerous tissues were studied by means of the newly developed reverse-phase chromatography system, RP-5. Tyrosyl transfer RNA from normal rat, mouse, and hamster livers emerged as a single peak. All three species exhibited identical profiles. Tyrosyl transfer RNA from Escherichia coli required a considerably higher NaCl concentration for elution from the RP-5 column. Tyrosyl transfer RNA from the Novikoff ascites hepatoma emerged as two separate peaks—one identical with the normal liver and a second late-eluting peak. Tyrosyl transfer RNA from SV40-induced tumors in hamsters and also from Ehrlich ascites tumor cells exhibited a broad-based peak which eluted later than the normal and which probably consists of two or more tyrosyl transfer RNA’s.

The phenylalanyl transfer RNA elution profile from normal mouse liver consisted of two smaller peak areas, followed by a major third peak. The transfer RNA from Ehrlich tumor cells followed a similar pattern; however, the middle peak was larger than normal. In contrast, Ehrlich tumor cells with an acquired resistance to nitrogen mustard exhibited a major middle peak, while the first and third peak areas were greatly diminished.

INTRODUCTION

Chromatography elution profiles of the tRNA’s of many tissues and organs from several species have been reported from several laboratories. Consistent and reproducible patterns are beginning to emerge, and there appears to be a remarkable similarity in the tRNA’s among different tissues and organs from different species. However, specific alterations in some of these patterns have been associated with certain physiological and pathological states. From the many studies involving differentiation, embryogenesis, chemical and viral induction of cancer, etc., it would appear that the tRNA’s may have important regulatory functions (1, 6, 14, 16, 21). Several studies that compare the profiles of normal and cancerous cells have also been reported. Thus far, the Phe-, Tyr-, and aspartyl-tRNA’s appear to have been altered most in cancer cells.

Holland et al. (8) and Taylor et al. (18), using methylated albumin Kieselguhr columns, reported the appearance of a new Tyr-tRNA in tumor cells. Baliga et al. (3) and Volkers and Taylor (19), using similar columns, also observed altered elution profiles for Tyr-tRNA and certain other aminoacyl-tRNA’s in the Novikoff and Morris hepatomas. Goldman and Griffin (6) found an altered Phe-tRNA elution profile (reverse-phase chromatography) during the comparison of normal rat liver with the Novikoff ascites tumor cells. An interesting shift in the Phe-tRNA profile also occurs as the Ehrlich ascites tumor cells acquire resistance to nitrogen mustard (HN2). In view of the importance of this general approach to the comparative study of the nucleic acids of normal and cancerous cells, additional studies of the elution profiles of Phe- and Tyr-tRNA’s in Novikoff ascites cells, several SV40-transformed hamster cell lines and nitrogen mustard-sensitive and -resistant Ehrlich ascites tumor cells are presented. In these studies the recently developed reverse-phase chromatography system (RP-5) of Kelmers and Heatherly (9) and Pearson et al. (11) was used. This system has a high degree of reproducibility plus the added advantages of a greatly reduced elution time and reduced requirements for tRNA.

MATERIALS AND METHODS

The tRNA preparations were obtained from normal liver and cancer tissues by the phenol procedure of Brunngraber (5), with minor modifications (4). An aminoacyl-tRNA synthetase fraction was obtained from liver, and the charging of tRNA with labeled amino acids was carried out as described by Goldman and Griffin (6).

The recently developed reverse-phase chromatography system (RP-5) of Kelmers and Heatherly (9) was used throughout this study. The column (0.63 x 33 cm) was equilibrated with 80 ml of the following buffer solution: 0.4 or 0.5 M NaCl, 0.01 M MgCl₂, 0.002 M β-mercaptoethanol, and 0.01 M sodium acetate, pH 4.5. One-half to 2.0 mg of 14C- or 3H-labeled aminoacyl-tRNA (50,000 to 100,000 cpm) were applied to the column, followed by 10 ml of the starting buffer.
buffer. These columns were developed with the use of a linear gradient \[i.e., 50 \text{ ml each of 0.4 and 0.7 M NaCl (in some instances, 50 \text{ ml each of 0.5 and 0.7 M NaCl})}\] in the above-described buffer solution. The flow rate of these columns that operate at approximately 300 psi was 1.5 ml/min, and 1.0-ml fractions were collected. Each fraction was precipitated by the addition of excess 5\% trichloroacetic acid and filtered on Millipore filters, and the radioactivity was counted in a Packard Model 574 liquid scintillation spectrometer. During the course of this study, the counting procedure was changed, and the fractions from the column were collected directly in the scintillation vials. The vials contained 10 ml of PCS (solubilizer-phase combining system for liquid scintillation counting obtained from Amersham/Searle Corporation, Arlington Heights, Ill.), and the column samples were then counted directly. The 2 counting procedures gave identical results. One of the objectives of this study was a comparison of the elution profiles of the tRNA\text{^{Tyr}} of normal and cancerous tissues. Accordingly, in most instances, the tRNA's of the 2 tissues were charged with tyrosine that was labeled with a different isotope (3H or 14C) and cochromatographed. The entire procedure was also repeated with the labels reversed. The tyrosine-3H (specific activity, 1.0 Ci/mmole) and tyrosine-14C (specific activity, 455 mCi/mmole) were purchased from Schwarz/Mann, Orangeburg, N.Y.

RESULTS AND DISCUSSION

It is apparent that the elution profiles for Tyr-tRNA for rat, mouse, and hamster liver are almost identical by this procedure (Chart 1, A and B). The pattern consists of a major single peak with an immediate and considerably smaller shoulder peak area. However, Tyr-tRNA obtained from E. coli eluted 30 tubes later (Chart 1B). A much steeper NaCl gradient was required to remove the microbial Tyr-tRNA from the RP-5 column [also observed by Holland et al. (8)]. In all of the chromatographic determinations, the 3H and 14C labels were reversed so we could ascertain that the profiles were not the result of contamination by or artifacts of the labeled amino acid. Comparable patterns were obtained with the labels reversed.

A comparison of the profiles of the Tyr-tRNA of normal rat liver and of Novikoff hepatoma cells revealed a small but consistent late-eluting peak (Chart 2). Comparative studies were also made of the RP-5 chromatography profiles of normal hamster liver and 3 different SV40-induced tumors (2, 13). All 3 of these tumors were transplanted into hamsters. In all of the tumors, the Tyr-tRNA elution profiles were similar and all differed from the normal hamster liver pattern (Chart 3). The major peak was shifted by several fractions. However, there were strong indications of at least 2 components that were not resolved by this chromatography system. The Tyr-tRNA from the Ehrlich ascites tumor cells also differed in elution profiles from the mouse liver pattern (Chart 4). The Ehrlich ascites tumor and SV40 tumor Tyr-tRNA patterns appear to be closely related.

The Phe-tRNA elution profile from normal mouse liver consists of 2 smaller peak areas followed by a 3rd major peak (Chart 5). Phe-tRNA from Ehrlich ascites tumor cells resembled the normal pattern; however, the middle peak was larger in the tumor than in the liver. Ehrlich ascites tumor cells with an acquired resistance to HN2 exhibited an altered Phe-tRNA pattern consisting of a major middle peak and very small initial and 3rd peak areas (Chart 5B).

The above findings with the use of the RP-5 system indicate that this is a rapid, sensitive, and highly reproducible procedure for studying the patterns of isoaccepting tRNA species. In addition, there is further indication that tumors differ from normal counterparts in several of the tRNA elution
M. Hayashi, A. C. Griffin, R. Duff, and F. Rapp

Chart 3. Elution profiles of Tyr-tRNA-\(^{14}\)C from normal hamster liver and Tyr-tRNA-\(^{3}\)H from SV40-induced tumor (cytoplasm) cochromatographed on RP-5.

Profiles. This poses the key question as to the significance of these changes in the transformation of normal to cancer cells or in the aberrant behavior of the cancer cells. The primary basis for the isoaccepting species of tRNA's [as termed by Yand and Novelli (20)] has not been completely established. The different species may correspond, at least in part, to the different codons. In addition, alterations in the base sequence (not affecting the anticodon or aminoacyl synthetase recognition site) or structural modifications such as methylation, acetylation, etc., may affect the chromatographic behavior of tRNA's specific for an amino acid.

The next logical step is to ascertain the biochemical changes responsible for the altered chromatographic behavior of specific tRNA's that have been observed during viral and chemical carcinogenesis, embryogenesis, and differentiation and also in cancer cells. It appears quite feasible to obtain sufficient quantities of the 3 isoaccepting species of tRNA\(^{phe}\), as reported above, for codon recognition studies (10) and for initial structural characterization with the use of RNase T1 digestion and DEAE-cellulose chromatography, the procedure of Randerath et al. (12), and other similar approaches. Several investigators (7, 18, 20) have already observed that the altered Tyr- and phe-tRNA's of rat hepatomas and Ehrlich ascites tumor exhibited normal codon recognition when assayed by in vitro procedures. Comparative studies of the 3rd late eluting aspartyl-tRNA peak observed in many virus-induced tumors (4) with the major 2nd peak may provide new insight into the mechanism of cancer induction. Such studies are in progress.

REFERENCES


Chromatographic Studies of Tyrosyl and Phenylalanyl Transfer RNA's of Liver and Tumor Cells

M. Hayashi, A. C. Griffin, Ronald Duff, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/33/4/902

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.