Aspartyl Transfer RNA Profiles in Normal and Cancer Cells

William T. Briscoe, William Taylor, A. Clark Griffin, Ronald Duff, and Fred Rapp

The Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025 [W. T. B., W. T., A. C. G.], and 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

Aspartyl-tRNA elution profiles were obtained from normal rat and hamster livers, Novikoff ascites hepatoma cells, and two different virus-induced (SV40) hamster tumors. A characteristic two-peak pattern was observed consistently in studies of the normal livers or the ascites tumor cells. However, the virus-induced tumors exhibited a third, late eluting peak in the reverse-phase chromatography system.

INTRODUCTION

Yang and Novelli (14) and Fujioka and Gallo (3) have observed an additional late eluting aspartyl-tRNA peak in myeloma cells which was not found in normal cells. A normal aspartyl-tRNA profile, however, was obtained by reverse-phase chromatography studies of normal and leukemic human lymphoblasts (4). Jackson et al. (6) reported 2 additional aspartyl-tRNA peaks in rat liver following partial hepatectomy and in livers of rats treated with growth hormone. Gallo and coworkers (3) have made the highly significant observation that the late-eluting aspartyl-tRNA peaks appear in SV40- and polyoma-transformed neoplastic cells. Using DEAE-Sephadex 3 columns, Sekiya and Oda (12) found differences in the aspartyl-, asparaginyl-, and histidyl-tRNA's of African green monkey kidney cells and cells infected with or transformed by SV40. In view of the importance of these findings, this communication will report on reverse-phase elution profiles for aspartyl-tRNA obtained from 2 virus-induced hamster tumors, a chemically induced ascites tumor of the rat, and normal hamster and rat livers.

MATERIALS AND METHODS

Animals. The rats utilized in this study were obtained from Sprague-Dawley, Inc., Madison, Wis. and the hamsters were purchased from Con Olson Co., Inc., Madison, Wis.

“Cyt” Tumor Line. This tumor material was the generous gift of Dr. S. S. Tevethia of the Baylor College of Medicine, Houston, Texas. The inoculum for this tumor was derived from fibroblastic-like cells cloned from cell cultures of the H-50 cell line, an SV40-transformed line (1).

Isotopes. H and 14C-labeled aspartic acid and serine were purchased from Schwarz/Mann, Orangeburg, N. Y.

Aminoacyl Synthetase and tRNA. Aminoacyl synthetase was prepared from normal rat and hamster livers according to Goldman et al. (5). Crude tRNA from both hamster tumors, the Novikoff ascites tumor, and normal hamster and rat livers was prepared according to the method of Brunngraber (2), except that the aqueous layer from the phenol extraction was reextracted with an equal volume of phenol (80%). Also, the ethanol precipitate was resuspended in 150 ml 0.1 M Tris-chloride buffer, pH 7.5, and 0.1 g DEAE-cellulose per ml was added and stirred for 20 min in the cold. The DEAE-cellulose was eluted with 1 M NaCl; 0.005 M disodium EDTA; 0.1 M Tris, pH 7.5; and 10-3 dithiothreitol. The tRNA in this eluate was precipitated with 2 volumes of cold ethanol. The DEAE-cellulose was reextracted with more 1.0 M NaCl buffer to harvest additional tRNA, and 0.1 M Tris supernatant fractions from the 1st DEAE-cellulose adsorption were stirred with fresh DEAE-cellulose (0.1 g/ml solution). This DEAE was washed with 0.1 M Tris, and the tRNA was eluted with 1.0 M NaCl buffer and then was precipitated with 2 volumes of cold ethanol. After standing overnight in the cold, the precipitates were centrifuged and resuspended in water with 10-3 M dithiothreitol to a concentration of 10 mg tRNA per ml.

Aminoacylation of tRNA. Charging of tRNA with amino acid was accomplished by the addition of the following to 1.0 ml synthetase: 0.3 ml ATP (0.025 M, pH 7.0); 0.3 ml 0.1 M MgCl2; 0.5 ml tRNA; and 0.3 ml aspartate-14C or 0.05 or 0.1 ml aspartate-3H. The mixture was incubated 15 min at 37°C, and the protein was removed according to the method of Goldman et al. (5). The labeled aminoacyl-tRNA was dissolved in 0.5 ml of the starting buffer of the column.

The samples (0.5 ml 3H-labeled and 0.5 ml 14C-labeled aminoacyl-tRNA's) were chromatographed by means of the reverse-phase system (RP-2) (13). The columns were developed with a linear gradient (1 liter each of 0.25 and 0.5 M NaCl solutions containing 10 mM MgCl2, 10 mM sodium acetate, pH 4.5; 10-3 M β-mercaptoethanol). The elution rate was 1 ml/min, and 10-ml fractions were collected. Portions of each fraction were precipitated with 5% trichloroacetic acid, collected on membrane filters, and the radioactivity was counted in a liquid scintillation counter.
RESULTS

The typical or normal aspartyl-tRNA profile resulting from reverse-phase chromatography is apparent in the profile for hamster liver in Chart 1. This pattern, made up of a small peak area followed by a single major peak, was consistently observed in normal rat and hamster liver and in the Novikoff ascites tumor cells, a tumor originally induced by administration of one of the azo carcinogens (8). The initial peak showed some degree of splitting, suggesting the possibility of heterogeneity in this region. However, the reverse-phase system, as used in this study, did not provide any major resolution of this leading peak area. Of more significance is the high degree of reproducibility of the initial and of the major peak areas in a large number of runs involving many different liver and ascites tumor preparations and several different columns.

In contrast to the above normal profiles, a 3rd aspartyl-tRNA peak appeared in all instances when tRNA from SV40-induced tumors was analyzed on the reverse-phase chromatography system (Chart 1). It should be noted that the initial peak and the major aspartyl-tRNA peaks were almost identical to those obtained in the normal liver and ascites tumor chromatograms. The 3rd aspartyl-tRNA peak was of the same general magnitude as the initial peak. Another SV40 tumor [PARA (defective SV40) adenovirus type 7] with the T-antigen present in the cytoplasm (10, 11) also exhibited a 3rd aspartyl-tRNA peak. In all of the chromatographic determinations, the $^3$H and $^{14}$C labels were reversed in order to ascertain that the profiles were not the result of contamination by or artifacts of the labeled amino acids. Comparable patterns were obtained with the labels reversed.

DISCUSSION

From the findings of this laboratory and of other investigators, it appears that at least several virus-induced tumors differ from normal tissues and from other tumors with respect to the appearance of a 3rd (late eluting) aspartyl-tRNA peak in the reverse-phase chromatography system. Several key questions arise from these preliminary findings: (a) Are all virus-induced tumors characterized by this 3rd aspartyl-tRNA peak? (b) What is the mechanism(s) that may be involved in the appearance of this 3rd peak? (c) How does this 3rd peak differ structurally from the other 2 aspartyl-tRNA peaks? (d) Does this offer any possibility of ascertaining viral involvement in human tumors?

With respect to the 1st of the above questions, it can only
be stated that, from the limited number of studies reported to date, all of the virus-induced tumors are characterized by a 3rd aspartyl-tRNA peak. Obviously, the question cannot be answered until a spectrum of tumors has been studied. There are some conflicting reports as to the presence or absence of a 3rd aspartyl-tRNA peak in some noncancerous tissues. Thus far, however, we have not detected the 3rd peak in any normal tissues that have been surveyed, nor has it been found in a chemically induced tumor of hepatic origin (8).

Several explanations may be offered as to the origin of this 3rd aspartyl-tRNA peak. It could represent a tRNA coded by the virus genome, a modification of preexisting tRNA, or a fetal or embryonic tRNA resulting from derepression of the host cell genome. Utilizing the hydridization of aminoacyl-tRNA-3H with SV40 DNA, Sekiya and Oda (12) reported that none of the altered tRNA's in their studies were encoded in the SV40 genome. If the 3rd aspartyl tRNA peak proves to be a constant feature of virus-induced tumor cells, it would appear worthwhile to isolate the aspartyl-tRNA's for some initial structural characterization. Analysis of the profiles on DEAE-cellulose columns of RNase T1 digests of the specific tRNA's or use of the procedure of Randerath et al. (9) for base composition should reveal whether the 3rd aspartyl-tRNA is the result of an altered nucleotide sequence or of an enzymatic modification of one or more of the bases (methylation, formation of pseudouracil, etc.).

With respect to the last of the questions posed above, it will be essential to study the profiles, by reverse-phase chromatography and perhaps by other systems, of a number of normal tissues or organs from humans and also a variety of tumors and other abnormal tissues of human origin. Finally, a large 3rd aspartyl-tRNA peak was found in the 1st of the human mammary tumors studied in this laboratory (Chart 3).

**REFERENCES**

Aspartyl Transfer RNA Profiles in Normal and Cancer Cells


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/32/8/1753

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.