Nucleotide composition analysis of tRNA from leukemia patient cell samples and human cell lines

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ABSTRACT

A technique developed for analysis of less than microgram quantities of tRNA has been applied to the study of human leukemia. Leucocytes from peripheral blood and bone marrow samples of six, untreated leukemia patients and cells of five different established human cell lines were maintained for 18 hours in media containing [^{32}P]-phosphate. Incorporation of radioactive phosphate into the cells from the patient samples was slightly less than that of the cell lines. Likewise, incorporation of [^{32}P]-phosphate into the tRNA of the patient samples (approximately 5×10^6 DPM/ μg tRNA) was also less than that incorporated into the tRNA of the cell lines. The major and minor nucleotide compositions of the unfractionated tRNA preparations from each patient sample and each cell line were determined and compared. Similarities and differences in the major and minor nucleotide compositions of the tRNA preparations are discussed with reference to types of leukemia and the importance of patient sample analysis versus analysis of cultured human cells.

INTRODUCTION

Mammalian cells undergo changes in protein synthesis which are associated with such phenomena as differentiation (1), hormonal stimulation (2), antigenic stimulation (3), regeneration (4), aging (5) and viral transformation (6). Changes in the amount and types of proteins being synthesized by cells are accompanied by changes in the cellular population of tRNA molecules (7,8). The role of tRNA in protein synthesis as translator of the genetic code into the amino acid sequences of proteins has been well documented (9). Of recent interest are those investigations that have demonstrated the involvement of tRNA in the regulation of cellular processes (10) and the replication of RNA tumor viruses (11,12). Thus comparison of tRNA molecules from normal, non-differentiating cells with that from differentiating, neoplastic or transformed cells is important to our understanding of the involvement of tRNA in these cellular phenomena. Analysis of tRNA molecules from mammalian cells (other than human) or from cells in culture is possible (13). Analysis

of tRNA molecules from human cell samples is difficult because the cell sample size is usually very small unless the cells are subsequently adapted to culture, a procedure which takes considerable time and alters cell morphology and biochemistry (14). Techniques for the analysis of tRNA from small numbers of cells (1×10^5) have already been reported by Agris *et al.* (13). The present investigation reports on the adaptation of these techniques to the analysis of tRNA from peripheal blood and bone marrow samples of human leukemia patients.

MATERIALS AND METHODS

Materials. Cell media, including a specially prepared phosphate-free minimal essential medium (13), and fetal calf serum were purchased from Grand Island Biological; [^{32}P]-phosphoric acid from New England Nuclear; human cell lines MRC-5, GM 11 and 288 were obtained from the Mammalian Genetic Mutant Cell Repository (Institute for Medical Research, Camden, New Jersey); RPMI 6410 from Associated Biomedica Systems; and the KB cell line was the gift of Dr. F. Ruddle (Yale University); DEAE-cellulose (Whatman ED-52) was purchased from Reeve Angel; ribonuclease T_1 and T_2 (Sankyo, Japan) and pancreatic ribonuclease from Calbiochem; thin layer plates (250 μ thick, microgranular cellulose) from Analtech; and No-Screen Medical X-ray film (NST-54) from Eastman Kodak.

Separation of Leucocytes from Peripheal Blood and Bone Marrow Samples. Peripheal blood and bone marrow samples taken from untreated, leukemia patients were kept in heparinized syringes left in an upright position for 2-3 hr at room temperature. Red blood cells settled, the leucocyte fraction was removed under sterile conditions and subjected to centrifugation (1000g for 10 min). The resulting white blood cell samples were resuspended in sterile saline and collected again by centrifugation before being placed in radioactive medium.

Incorporation of [^{32}P]-phosphate. An aliquot of each cell sample was used for determination of the number of viable cells by exclusion of the dye trypan blue. A minimum of 1×10^5 viable cells were added to a plastic cell culture flask containing phosphate-free minimal essential medium. The composition of the medium including the preparation of dialyzed fetal calf serum and subsequent addition of [^{32}P]-phosphoric acid has been described previously (13).

Isolation of Radioactive tRNA. Lymphoblasts and myeloblasts which had been suspended and maintained in the radioactive medium were collected by cen-

trifugation and then extracted with a mixture of (1:1, V/V) phenol and buffer (0.05M sodium acetate, pH 5, 0.01M $MgCl_2$). Fibroblasts which attached to the culture flasks were extracted with the phenol:buffer mixture immediately after the media had been removed. The procedures for extraction of small quantities of nucleic acids and the subsequent separation of tRNA by DEAE-cellulose chromatography have been described in detail by Agris *et al.* (13).

Analysis of tRNA Nucleotide Composition. tRNA preparations were subjected to enzymic digestion to the level of mononucleotides and dinucleoside-diphosphates with the use of ribonucleases T_1 and T_2 and pancreatic ribonuclease (15). The digests were then subjected to two-dimensional thin layer chromatography (tlc) in the following solvents: (I) isobutyric acid-concentrated ammonia- H_2O (199:3:98, V/V/V): (II) isopropanol-concentrated $HCl-H_2O$ (68:17.6:14.4, V/V/V). Positions of migration of the major and minor nucleotides on the TLC plates were determined by autoradiography and compared to that of standards (15). The relative amounts of each of the nucleotides was analyzed by scraping the cellulose areas that corresponded to the exposed regions of the autoradiograph and determining the radioactivity in the cellulose by scintillation counting. Only one peripheral blood or bone marrow sample could be obtained before the patients were clinically treated. For all practical purposes this imposed a limit of one tRNA analysis for each sample. However, the nucleotide compositions of tRNA from the cell lines were determined as many as four times. The degree of error in such determinations is discussed below.

RESULTS

Incorporation of [^{32}P]-phosphate into tRNA. Peripheral blood and bone marrow samples from patients suffering from either lymphoblastic or myelogenous leukemias were obtained from local hospitals. The sample designations (H.L. I, II, etc.) and the types of leukemia as diagnosed by the hospital staff physicians are shown in Table I. Leucocytes were separated from the samples and maintained for 18 hr in medium containing [^{32}P]-phosphate as described in Materials and Methods. Commercially available human cell lines (Table I) including a white blood cell line (RPMI 6410) were also maintained for 18 hr in the radioactive medium. The amount of radioactive phosphate incorporated by the cells was determined by analysis of that remaining in the medium after the incubation period. In general 56% of the radioactive phosphate in the medium was taken up by the cells as shown in Table II. However, the cell lines incorporated slightly more phosphate (an average of 59% of the

TABLE I
Cell Types and Designations

A. Human Leukemia Peripheal Blood and Bone Marrow Samples

Type of Leukemia	Sample Type		Designation
	Peripheal Blood	Bone Marrow	
a. Lymphoblastic	+		H.L. I
	+		H.L. III
		+	H.L. V
		+	H.L. VI
b. Myelogenous	+		H.L. VIII
		+	H.L. VII

B. Human Cell Lines

Cell Type	Designation
Leukemic Myeloblast	RPMI 6410
Normal Fetal Fibroblast	MRC-5, GM 11
Normal Adult Fibroblast	GM 288
Fibroblast, carcinoma of nasopharynx	KB

phosphate in the media) than the human cell samples (53%). Lymphoblastic leukemia samples (I, III, V, VI) incorporated more phosphate (an average of 56%) than did the myelogenous leukemias (VII, VIII; 48% incorporation) and the bone marrow samples (V, VI, VII) were more active in the up-take of phosphate (55%) than the peripheal blood samples (I, III, VIII; incorporation of 51% of the phosphate).

The amounts of [^{32}P]-radioactivity found in the nucleic acid and tRNA fractions isolated from the various cell samples and cell lines are also shown in Table II. Incorporation of [^{32}P]-phosphate into total nucleic acids varied from cell type to cell type with the largest incorporations by the cell lines (18% of the radioactive phosphate incorporated by the cells versus 11% for patient cell samples). Lymphoblastic leukemias incorporated higher amounts of radioactivity into nucleic acids (an average of 13% of the radioactivity incorporated into the cells) than did the myelogenous leukemia samples (8%) and bone marrow samples (13%) more than peripheal blood samples (9%). The amounts of [^{32}P]-phosphate incorporated into tRNA by the different cell types varied in a manner similar to that incorporated into total nucleic acids (Table II). These differences in phosphate incorporation into the cells, cellular nucleic acids and tRNA are probably representative of differences in the metabolism of nucleotide pools and the biosynthesis of nucleic acids. The incorporation of [^{32}P]-radioactivity into the tRNA of human leukemia cells, although less than that of the cell lines, was considerable enough (approximately 5×10^6 DPM/ μg tRNA) for analysis of nucleotide compositions (24).

TABLE II

Incorporation of [32 P]-phosphate into Cells and Cellular Nucleic Acids

Cell Type	Percentage of phosphate in the media incorporated by the cells ^a	Percentage of phosphate incorporated into nucleic acids ^b	Percentage of phosphate incorporated into tRNA ^b
Human Leukemias			
I	55	11	1.7
III	52	11	1.5
V	59	15	2.2
VI	56	13	1.8
VII	50	10	1.5
VIII	45	7	1.0
Cell Lines			
RPMI 6410	64	22	3.1
MRC-5	55	13	2.0
GM 11	60	19	2.1
GM 288	61	17	1.9
KB ^c	54	18	1.7

^aThe amount of phosphate incorporated by the cells was determined by analysis of the remaining radioactivity present in the cell-free culture medium after the incubation period.

^bIncorporation into nucleic acids and tRNA are shown as percentages of that incorporated by the cells.

^cResults taken from Agris *et al.* (13).

Table III

Nucleotide Compositions^a of Human Cell tRNA

Nucleotide	Leukemia Patient Samples (H.L.)						Human Cell Lines				
	I	III	V	VI	VII	VIII	MRC-5	GM11	GM288	RPMI 6410	KB
Ap	17.52 ^b	17.10	15.05	16.23	18.39	16.77	18.96	16.35	14.42	18.80	14.89
Cp	34.59	35.31	31.72	32.45	28.37	29.24	28.46	30.11	29.97	32.87	28.04
Gp	19.44	17.62	16.48	18.21	30.23	31.56	26.09	29.43	24.74	22.10	23.38
Up	17.94	16.88	21.66	19.35	16.08	18.15	16.83	17.00	19.85	17.52	16.61
riboTp	0.54	0.67	1.17	0.97	0.22	0.18	0.28	0.88	1.52	0.17	0.57
yp	2.85	2.15	0.95	1.01	1.16	1.56	2.62	2.70	2.47	1.96	2.72
Dp	1.15	1.08	2.00	1.72	0.70	1.26	1.92	0.89	1.46	1.40	1.51
^m Gp	1.66	1.32	N.D. ^c	1.48	N.D.	N.D.	1.11	N.D.	0.35	1.33	N.D.
^m Ap	0.97	0.65	N.D.	0.81	0.20	0.24	0.31	N.D.	0.22	0.33	N.D.
i ⁶ Ap	0.04	0.05	0.08	0.07	0.08	0.02	0.08	N.D.	N.D.	N.D.	0.05

^aRelative amounts of each constituent nucleotide are reported as the percentage of [32 P]-radioactivity (mole %) in each nucleotide compared to the total radioactivity in the tRNA under investigation. Other minor nucleotides found include ^mAp, ^mAp, ^mGp and an unidentified methylated Cp (24).

^bStandard deviation for the amount of each nucleotide equals 0.03 mole %.

^cN.D., Not Determined.

Nucleotide Composition of tRNA. Analysis of the nucleotide compositions of each of the tRNA samples was accomplished by two-dimensional tlc of the ribonuclease digests as described in Materials and Methods. The relative amounts of the major and minor nucleotides present in each of the tRNA samples

are shown in Table III. Although the nucleotide compositions of tRNA from the human leukemia cells could only be determined once the error in the amounts shown is probably small. Repeated determinations of the nucleotide composition of tRNA from human and mouse cell lines has shown that the analytical techniques, as reported previously (13), produce a standard deviation of ± 0.03 mole % for the relative amounts of each nucleotide (P. F. Agris and A. Nickols, personal communication and ref. 8).

There are significant similarities and differences in the major and minor nucleotide compositions of the various tRNA samples. The Gp and Cp content of the tRNA from myelogenous leukemia samples (H.L. VII, VIII) and human cell lines was found to be consistently higher (an average of 56.36 mole % versus 51.45%) than that of the lymphoblastic leukemia samples (H.L. I, III, V, VI). This difference in Gp plus Cp content seems to be caused more by differences in Gp than Cp as evidenced by the amounts of these nucleotides as reported in Table III. The riboTp content of tRNA from lymphoblastic leukemias (0.84 mole %) was greater than that of the tRNA from myelogenous leukemia cells (0.20 mole %). Transfer RNA from bone marrow cell samples (H.L. V, VI, VII) contained larger amounts of i^6 Ap (0.08 mole %) but smaller amounts Ψ p (1.04 mole %) than did tRNA from peripheal blood samples (0.04 mole % and 2.19 mole %, respectively). Comparison of the tRNA composition of cells from a bone marrow sample (H.L. VII; $Ap + Up/Gp + Cp = 0.59$) with that of a peripheal blood sample (H.L. VIII: $Ap + Up/Gp + Cp = 0.57$) from the same patient indicated that the largest differences in the nucleotide compositions resided in the amounts of the minor nucleotides. The nucleotide composition of the tRNA from the myelogenous leukemia cell line (RPMI 6410) was found to be comparable to that of the tRNA from the other commercially available cell lines which were fibroblasts (Table III).

DISCUSSION

A technique developed by Agris et al. (13) for the analysis of microgram quantities of tRNA has been shown applicable to the study of tRNA from cells of peripheal blood and bone marrow samples of leukemia patients. The nucleotide compositions of tRNA from six different patient samples have been determined. Of particular interest is the possible difference in Gp plus Cp content between cell samples from patients with lymphoblastic leukemia and those with myelogenous leukemia (Table III). If such a difference is found to occur consistently in studies of other patient samples, the analysis of the major compositions of tRNA may have clinical application (Agris, P.F., Ort-

werth, B., Henry, P., and Kardinal, C., personal communication). Analysis of tRNA from cells undergoing differentiation in culture has shown that cells in the undifferentiated state also contain tRNA with larger Gp plus Cp content than that of differentiating cells (8). Randerath, *et al.* (16) have shown that the Gp plus Cp content of tRNA from hepatomas is greater than that of tRNA from normal liver cells. Alterations in the major nucleotide composition of a cell's tRNA probably indicates a change in the relative amounts of the different tRNA species within the total tRNA population. Presumably, changes in the tRNA populations of eucaryotic cells are in response to new needs for specific species of tRNA during protein synthesis (7).

A capability to determine the minor nucleotide compositions of tRNA from patient cell samples is also important because the minor nucleotides of tRNA have been implicated in regulatory processes. In particular, Ψ p in tRNA has been shown to be involved in repressing the synthesis of enzymes for certain amino acid biosynthetic pathways (17,18,19). i^6 Ap, in those tRNA species which respond to codons beginning with Up, was found to effect, in a positive manner, the binding of tRNA to ribosomes (20). Eucaryotic tRNA species responsible for initiation of protein synthesis have been shown to lack riboTp (21). The presence of a particular minor species of tRNA^{Lys} has been shown to be correlated with a mammalian cell's ability to divide (22,23). Structural differences between this isoaccepting species of tRNA^{Lys} and one of the major species may be due to differences in modified nucleotides alone (Ortwerth, B., personal communication). The modification, *in vivo*, of Up at the first position of the anticodon in certain tRNA species was shown to effect codon-anticodon interaction (15). Analysis of the minor nucleotide compositions of tRNA from cells of leukemia patients has been possible with the technique employed in this investigation. The results shown in Table III indicate that there are significant differences in the minor nucleotide amounts present in tRNA from lymphoblastic and myelogenous leukemia samples. Interestingly, the greatest differences between the nucleotide composition of tRNA from a peripheal blood sample and a bone marrow sample from the same patient were differences in minor nucleotide amounts. This result could indicate that the tRNA samples are essentially the same but involved in different regulatory processes.

The analytical technique employed has been shown to be applicable to the small numbers of cells obtained in human samples thereby eliminating the need for culturing of cells. The adaptation of cells to culture changes cell morphology and biochemistry (14). In fact, the nucleotide compositions of the tRNA from the myelogenous leukemia cell samples (Ap + Up/Gp + Cp = 0.59 and

0.57) were found to be significantly different from that of the myelogenous leukemia cell line (RPMI 6410; Ap + Up/Gp + Cp = 0.66). The major and minor nucleotide composition of the tRNA from this cell line was comparable to that of the other cell lines possibly indicating a common, functional adaptation to cell culture conditions. Further discussion of the implications of analyzing tRNA from human leukemia cell samples will await results obtained from a greater number of patients.

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