Marked Phenylalanyl-tRNA Activity of Herpesvirus Type 2 Infected Preparations (37287)

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Epidemiological and statistical data strongly implicate genital herpesvirus (type 2) in carcinoma of the cervix (1). Herpes-like viruses are observed with increasing frequency in cell cultures derived from healthy patients and in those tissues obtained from malignant processes (2–6). Therefore, it is of interest to compare transfer capabilities of uninfected vs viral infected systems in order subsequently to detect change in synthesis of various classes of proteins which may be peculiar to the virus and important in control mechanisms or in malignant conversions. Whether or not herpesviruses code for specific tRNAs is not known.

Subak-Sharpe and Hay (7) suggested that arginyl-tRNA might be coded by herpesviruses, but recent experimental data presented by Morris, Wagner and Roizman (8) indicated the absence of herpes simplex virus (type 1) specified arginyl- and seryl-tRNA's in infected H.Ep.2 cells as measured by extraction and hybridization techniques. To confirm these findings, Bell, Wilkie and Subak-Sharpe (9) reported also that no type-1 herpes-specified arginyl-tRNA was detected in BHK cells 7 to 9 hr postinfection. In experiments described below a comparison of tRNA obtained from herpesvirus type 2 infected H.Ep.2 cells and from uninfected controls was made. A great increase in activity of phenylalanyl-tRNA of herpesvirus type 2 infected tissue cultures was observed as compared with tRNA extracted from uninfected cells. Only minimal differences in activity were detected for 9 other amino acids including arginine.

Materials and Methods. H.Ep.2 cells, 10 large Roux bottles per preparation, were grown 4 to 5 days at 36° on glass in Eagle's medium containing 10% calf serum (10). Approximately 5×10^7 cells/bottle were harvested when cultures were terminated. Inocula of 8 to 10 virus particles/cell, as assayed on chorioallantoic membranes were used for infected systems. These cells were harvested 20 to 21 hr following incubation at 36°. Cytopathic effect was not evident at this time but viral titers were always high. Cell cultures, infected or otherwise, were harvested by scraping bottles with rubber spatulas after first removing supernatant growth fluids. Cells were collected in a solution containing 0.01 *M* Tris–HCl (pH 8.0), 0.01 *M* Mg $(C_2H_4O_2)_2$, 0.05 *M* KCl, 0.6 *M* sucrose and 1 ml/ml washed bentonite (2.5 vol, w/v). Concentrated cell preparations were homogenized by hand with all glass Toënbrock grinders. Postmitochondrial fractions, and mixed tRNA preparations were obtained following the procedure of Yang and Novelli (11). Endogenous amino acids were discharged from tRNA by incubating samples dissolved in 0.3 *M* Tris–HCl (pH 7.5), 0.01 *M* Mg $(C_2H_4O_2)_2$ and 0.001 *M* EDTA at 35° for 30 min. After ethanol precipitation tRNA was precharged with radioactive amino acids. The unit reaction mixture used was that of Yang and Novelli (11), except for 2 modifications. Approximately 30 mg tRNA for each of the 2 isotope containing preparations were employed routinely. Therefore, precharging reaction mixture constituents were increased to accommodate this tRNA quantity. Rabbit liver synthetase proved to be effective and inexpensive. Crude enzyme was prepared according to the modification of Muench and Berg (12) and the resulting solution, following French pressure cell treatment and centrifugation, was placed on DEAE-cellulose columns where enzyme fractions were eluted according to Peterson and Sober (13). Co-chromatography of aminoacyl-tRNA by the reversed-phase Freon column chromatography method of Weiss and Kelmers (14) was followed. Columns, 40 cm × 1.5 cm, were maintained at 25°, pH 4.5. Concave gradients were generated by means of a 9 chambered Phoenix gradient maker using salt solutions (800 ml) ranging from 0.25 to 1 *M* NaCl. All tube samples of tRNA were precipitated with TCA, collected on Millipore filters, washed, dried and counted directly by the liquid scintillation method (11) using a Packard Tri Carb liquid scintillation counter. The tRNAs studied were those for phenylalanine, histidine, leucine, methionine, serine, tyrosine, arginine, valine, alanine and aspartic acid. Duplicate experiments were carried out for each amino acid. For example, if a tRNA mixture was charged with ^{3}H-phenylalanine for the uninfected preparation, then ^{14}C-phenylalanine was included in the viral infected reaction mixture. In confirmation, the use of isotope was reversed in a second experiment so that ^{3}H-phenylalanine was used to precharge herpesvirus infected cell tRNA and ^{14}C-phenylalanine was added to the uninfected cellular control. This procedure provided a good

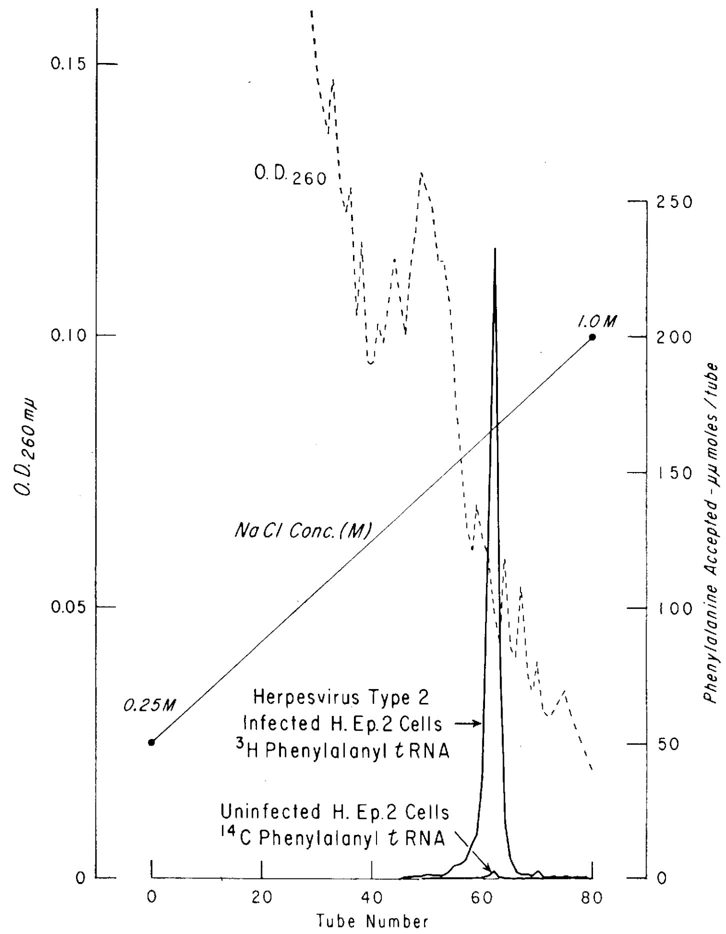

FIG. 1. Chromatography of phenylalanyl-tRNA of herpervirus type 2 infected and uninfected H.Ep.2 cells. Preparations of tRNA were precharged with ^{3}H- or ^{14}C-phenylalanine as described in the Methods section. The OD$_{260}$ plot represents the total tRNA elution profile. tRNAs were eluted from 40 × 1.5 cm reversed phase Freon columns with 800 ml of a 0.25 to 1.0 *M* NaCl gradient. Approximately 30 mg tRNA/preparation were added to the column. Columns were maintained at 25°, pH 4.5. Samples, 10 ml/tube, were precipitated with TCA, collected on Millipore filters and counted.

check as to isotope purity and established the reliability of the charging procedure.

Results and Discussion. Profiles displayed (Fig. 1) depict OD_{260} readings as tRNAs are eluted from a column. Phenylalanyl-tRNA of type 2 herpesvirus infected cells presents a large sharp peak, while that of the host system is minimal. Data diagramed in Fig. 1 and combined in Table I records pmoles of phenylalanine accepted per tRNA peak. For peak 2, the herpesvirus type 2 infected system shows a 110-fold greater activity than that of the uninfected control. In addition to the above data, similar experiments performed with herpesvirus type 1 infected cells indicate that the major phenylalanyl-tRNA activity peak is only double that of the uninfected H.Ep.2 cell control.

According to our method, 2 major peaks are detected for leucine (Table I). Leucyl-tRNA of cells infected with herpesvirus type 1 or 2 have the same low values. Uninfected H.Ep.2 cellular leucyl-tRNA peaks show acceptance of 7 to 8 times more amino acid than leucyl-tRNA peaks of virus infected systems. Several peaks are observed for methionyl-tRNAs of uninfected and type 1 herpesvirus infected preparations. The amount of methionine accepted by tRNA obtained from herpes 1 infected preparations is 4 times higher (52 pmoles) than that for uninfected cellular tRNA of peak I (13 pmoles) and approximately 3 times greater (29 vs 11 pmoles, 9 vs 3 pmoles, respectively) in other small peaks. These differences may or may not be significant. Type 2 herpesvirus studies with methionine have not been performed. Amino acid acceptance values for uninfected vs type 2 herpesvirus infected tRNA of the remaining amino acids listed are the same for both infected and control preparations.

Why phenylalanyl-tRNA of the type 2 herpesvirus system shows such tremendous activity is not known. It remains to be seen if phenylalanine is an important constituent of a structural protein peculiar to the virus. Another possibility is that the demand for phenylalanine may be required in the synthesis of certain enzymatic proteins such as thymidine kinase, etc. The problem will be simplified when sequencing experiments on tRNA obtained from type 2 herpesvirus infected preparations progress further. We still do not know if phenylalanyl-tRNA is coded by the virus or if preformed host molecules are used exclusively. It may be that modifications such as methylation or other conversions are promoted as a consequence of viral invasion.

Summary. By means of co-chromatographic techniques using ^3H- and ^{14}C-labeled amino acids in appropriate reaction mixtures, it is possible to detect and compare tRNAs of

TABLE I. A Comparison of Activities by Selected Aminoacyl-tRNA of Herpesvirus Infected and Uninfected H. Ep. 2 Cells.[a]

Amino acid accepted (pmoles/tRNA peak)

		Peaks				
Amino acid	Source of tRNA	1	2	3	4	5
Phenylalanine	Cells, uninfected	0	0	0	0	0
	Herpesvirus type 2 infected	5	553	3	5	5
	Herpesvirus type 1 infected	0	9	0	0	0
Leucine	Cells uninfected	31	14			
	Herpesvirus type 2 infected	4	2			
	Herpesvirus type 1 infected	4	2			

[a] Procedures are the same as those of Fig. 1. Calculations are based on the amount of radioactivity (^3H, ^{14}C) incorporated into aminoacyl-tRNA complexes.

herpesvirus type 2 infected with uninfected H.Ep.2 cellular controls. Individual tRNAs are separated by reversed-phase Freon column chromatography. In terms of picomoles of amino acid accepted per phenylalanyl-tRNA peak, herpes virus type 2 infected preparations are 110 times more active than uninfected controls. In comparative experiments for 9 other amino acids tested there are either no differences or only 3- to 8-fold increases in activity by one system or the other. Possibilities as to the demand for phenylalanine by viral infected preparations are suggested but whether or not a different tRNA is coded by the virus is not known.

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