The role of queuine in the aminoacylation of mammalian aspartate transfer RNAs

Ram P. Singhal** and Vikram N. Vakharia+

**Laboratory of Cellular and Molecular Biology, Gerontology Research Center, NIA, NIH, Baltimore, MD 21224, and +Department of Chemistry, Wichita State University, Wichita, KS 67208, USA

Received 1 March 1983; Revised 26 April 1983; Accepted 24 May 1983

ABSTRACT

Can a queuine-specific tRNA function normally without replacement of G by Q in its structure? To answer this, kinetics of aspartate queuine-containing tRNA (Q-tRNA) is compared with its queuine-deficient counterpart (G-tRNA). The results indicate that Asp Q-tRNA is a more effective substrate than the Asp G-tRNA. The Asp Q-tRNA exhibits a higher reaction velocity ($V_{max} > 30\%$) and a higher reaction rate ($K_m < 55\%$) than its counterpart. The Asp tRNAs derived from human tumor lines and grown in athymic mice contain a full complement of queuine. This tumor tRNA exhibits aminoacylation kinetics similar to a normal liver tRNA. Reasons for observing the lack of a G-to-Q modification in cancer tRNAs by others are hypothesized. Two purified Asp isoacceptors from liver are compared for the aminoacylation reaction; small differences are noted in the $V_{max}$ but none in the $K_m$ values.

INTRODUCTION

The "wobble" base located in position 34 is modified in 61% of eukaryotic and 47% of prokaryotic tRNAs (1). Similarly, out of 49 mammalian tRNAs, 31 contain a modified residue in this position (unpublished results). The replacement of guanine by queuine occurs specifically at the wobble base position of asparagine, histidine, aspartate and tyrosine tRNAs. Prokaryotes contain an unmodified Q (queuosine) in each one of the four tRNAs; however, mammals contain both an unmodified Q (tRNA$^{Asn}$ and tRNA$^{His}$) as well as a modified Q (manQ in tRNA$^{Asp}$ and ga1Q in tRNA$^{Tyr}$). The G-to-Q modification in E. coli tRNAs occurs by substitution of a preformed queuine or pre-queuine (7-aminomethyl-7-deazaguanine) (2), while in mammalian tRNAs it occurs by substitution of a preformed Q (once called "Q factor") into the Q-deficient tRNA (3,4). (The "insertion" enzymes, tRNA-guanine transglycosylase, from bacteria and mammals exhibit vastly different affinities for their substrates, preQ and Q, respectively. This is one reason for the use of different substrates in the replacement of G by bacteria and mammals. For more details see review articles on Q: references 5 and 5a.)

Significant losses in the G-to-Q modification of tRNAs from several
sources have been reported, for example, in tRNAs from prenatal mice (6), one-
week old rats (7), animals raised on a Q-free diet in a germ-free environment
(8), and in tumor cells (2, 9-13). A small amount of Q-deficient tRNA is also
reported in tRNAs from other sources (7,14). The occurrence of incomplete or
total loss of the G-to-Q modification under specific conditions raises this
question: can tRNAs without Q function normally?

In several tRNAs, the nucleotides of the anticodon loop appear to be in-
volved in the recognition process (1). For example, the modification of the
2-thiouridine located in the "wobble" position of tRNA^Glu (E. coli) was
paralleled by a reduction in aminoacylation ability (15). The structural
complexity of Q and hexosylQ, and their specific location in the tRNA struc-
ture, lend support to the idea that Q can play an important role in amino-
acylation. To answer the above question and to establish role of Q in amino-
acylation, this work compares the kinetics of aminoacylation between: (a) the
two aspartate isoacceptors, (b) the aspartate Q-tRNA and its Q-deficient
counterpart (G-tRNA), and (c) the aspartate tRNA of normal liver and of tumor
origin.

**MATERIALS**

Samples of human tumor lines grown in athymic mice were supplied by
Dr. Nathan Kaplan, University of California at San Diego. The cystoadeno-
carcinoma of the ovary had reached ten serial passages in mice (first passage
of 30 days and subsequent passages averaging 65 days) and the adenocarcinoma
of the lung origin had reached eight passages (first passage of 5 months and
subsequent passages averaging 53 days); these two samples were used in this
study. Samples of unfractionated tRNAs derived from L-M cells were obtained
from Dr. Jon Katze, University of Tennessee, Memphis. L-M cells of mouse
connective tissue origin (16) were grown for us in a serum free medium
supplemented with queuine (one A_260 unit per 100 ml of the growth medium) in
one case and none added in the other case (4). To remove >5-S RNAs and rRNAs,
tRNA samples were purified on Sephadex G-100 columns.

Aspartate tRNA in tRNA samples from different sources was assayed using
maximum reaction conditions (37°, 30 min). It is reported here in pmol of
aspartate incorporated in 1.0 A_260 unit of the tRNA sample: 32.6 ± 0.3,
rabbit liver tRNAs; 22.8 ± 0.5, ovary tumor tRNAs; 67.6 ± 0.3, G-tRNA from
L-M cells; 85 ± 1.2, Q-tRNA from L-M cells; 1410, tRNA^{Asp}_1 and 1450, tRNA^{Asp}_2 —
both from bovine liver. One unit of a partially-purified Asp-tRNA synthetase
preparation — containing 100 μg of proteins — catalyzed incorporation of
1 pmol of labelled aspartate in 0.25 $A_{260}$ unit of rabbit liver tRNAs at 37° in 1 min.

The conacanavalin A (ConA) Sepharose column matrix contained 10 mg of ConA per ml of the gel sediment (Pharmacia Fine Chem.). [8-3H]Guanine (8 Ci per mmol) and l-[3H]-aspartic acid (10 to 14 Ci per mmol) were obtained from Schwartz/Mann and New England Nuclear, respectively.

**METHODS**

**Aminoacylation Kinetics of Aspartate tRNAs**

The amount of aspartate tRNA present in the unfractionated tRNA sample was determined by aminoacylation of the sample (free of AA-tRNAs) with labelled aspartate using maximum incorporation conditions (17). The concentration range of the aspartate tRNA -- critical for producing aminoacylation under steady state reaction conditions, independent of the synthetase concentration -- was determined by varying the amounts of the aspartate tRNA while maintaining the synthetase in excess, and assaying for the reaction using different reaction times (data are not shown).

In a typical reaction, the unfractionated tRNA sample (free of AA-tRNA, and other RNAs), containing a known amount of the tRNA$^{Asp}$, was aminoacylated (in a reaction mixture of 200 µl containing 100 mM HEPES buffer (pH 7.6), 6 mM Mg(OAc)$_2$, 6 mM KCl, 2 mM 2-mercaptoethanol, 3 mM Na$_2$ATP, 15 µM [3H]aspartic acid, and 42 µg proteins of the crude aspartyl-tRNA synthetase). Several reaction mixtures were prepared in this manner, one for each tRNA concentration, placed in small polyethylene tubes, and maintained at 0°. One reaction mixture at a time was brought to 37° by shaking the contents for 10-15 seconds, and then (at zero time) the tRNA component was added. Aliquots (30-40 µl) were withdrawn at timed intervals and mixed with a 2 ml solution of 0.2 M NaOAc, pH 4.5 containing 2 k units of E. coli DNA, maintained in ice. An equal volume of 20% trichloroacetic acid was added and the mixture was assayed for the amount of [3H]Asp-tRNA using a fiber glass filter (GF/C, Whatman Chem. Separation, Inc.) as described elsewhere (18).

The amount of Asp-tRNA formed as a function of time was plotted, and $V_1$ for a particular tRNA$^{Asp}$ concentration was determined from the slope of the line. A Lineweaver-Burk plot ($1/V_1$ vs. 1/tRNA$^{Asp}$ concen.) and an Eadie-Hofstee plot ($V_1$ vs. $V_1$/tRNA$^{Asp}$ concen.) were obtained, and $V_{max}$ and $K_m$ values were determined by least-square fit method (with the help of two computer programs written in BASIC language for Apple II microcomputer.)
Divers Methods

Tritium-labelled guanine was incorporated into G-tRNAs, using tRNA-guanine transglycosylase from *E. coli* (2). The reaction conditions were the same as described earlier (7) except for the incubation time (12 h) needed for maximal guanine incorporation. Aspartyl-tRNAs were treated with cyanogen bromide as described earlier (15,19). The isoacceptors were resolved by chromatography on RPC-5 and BioRad Aminex A-28 columns (18,20). The liver aminoacyl-tRNA synthetase was prepared by differential centrifugation (21). A fraction rich in aspartyl-tRNA synthetase was isolated by gel filtration chromatography on Sephadex G-100 column followed by fractionation on a DEAE-cellulose column (17). tRNAs from livers and tumors were isolated using 3 to 5 phenol extractions (22); aspartate tRNAs were purified by a ConA-affinity method and gel filtration chromatography (22,23). Aspartate isoacceptors were resolved (18,20) and further purified by polyacrylamide gel electrophoresis (24) using a vertical (43 cm high x 33 cm wide) electrophoresis cell (Model 1120, BioRad Lab.).

RESULTS

1. Properties of the Aspartate Isoacceptors

A. Relative Amounts of the Aspartate Isoacceptors. The results in Table I indicate that only two major isoacceptors were present in each tRNA sample from three sources. The relative amount of tRNA*Asp*\(_1\) ranged from 7 to 13\% and that of tRNA*Asp*\(_2\) from 87 to 93\% with respect to the total aspartate tRNA (functional) present in the three different tissues.

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Relative Amount (per cent)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tRNA<em>Asp</em>(_1)</td>
</tr>
<tr>
<td>Bovine Liver</td>
<td>13.0 ± 1.8</td>
</tr>
<tr>
<td>Rabbit Liver</td>
<td>6.7 ± 0.2</td>
</tr>
<tr>
<td>Ovary Tumor(^b)</td>
<td>7.0 ± 0.2</td>
</tr>
</tbody>
</table>

\(^a\)[\(^3\)H]Aspartyl-tRNAs were resolved on RPC-5 columns and radioactivity under each peak was determined. The amount for each isoacceptor represents fraction of the total acid-insoluble radioactivity due to both species. The results are derived from at least two RNA preparations.

\(^b\)Human ovary tumor grown in athymic mice.
Figure 1. Aminoacylation of the aspartate isoacceptors (and subspecies) purified from bovine liver using an unfractonated AA-tRNA synthetase sample from the same source. (See Materials for data on various preparations). Lineweaver-Burk plots: $1/v$, pmol of the labelled aspartate incorporated per min. plotted against the reciprocal of $\mu$mol of the tRNA per liter.

B. Aminoacylation of the Isoacceptors; Bovine Isoacceptor Contains A Mixture of Active and Inactive Subspecies. Bovine liver aspartate isoacceptors were studied since this tissue contained a significant amount of tRNA$^{\text{Asp}}_1$ (see Table I). Each purified isoacceptor produced two bands — one major and one minor — on polyacrylamide gel electrophoresis. (The material retained on the ConA-affinity column gave three bands). Each band (subspecies), when submitted to two-dimensional gel electrophoresis, produced only one spot. The results indicated the presence of two subspecies (named a and b) in each aspartate isoacceptor sample. The sub species remained homogeneous on further gel electrophoresis. The rabbit liver aspartate isoacceptors, however, produced no subspecies when tested for purity.

Bovine liver isoacceptors and their subspecies obtained from gel electrophoresis were aminoacylated with a homologous synthetase preparation. The results in Fig. 1 indicate Lineweaver-Burk plots of the two isoacceptors and their subspecies. $V_{\text{max}}$ and $K_m$ values derived from these results are shown in Table II. For each isoacceptor, the fast moving band of the gel electrophoresis was either completely inactive (tRNA$^{\text{Asp}}_1b$) or less active (tRNA$^{\text{Asp}}_2b$).
TABLE II: AMINOACYLATION PROPERTIES OF PURE SAMPLES OF ASPARTATE tRNAs FROM BOVINE LIVER, USING HOMOLOGOUS ENZYME

<table>
<thead>
<tr>
<th>Substrate tRNA&lt;sub&gt;asp&lt;/sub&gt; isoacceptor</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (pmol/min)</th>
<th>K&lt;sub&gt;m&lt;/sub&gt; (nM)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt;/K&lt;sub&gt;m&lt;/sub&gt; (pmol · min&lt;sup&gt;-1&lt;/sup&gt;/µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>74.5&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>7.0</td>
</tr>
<tr>
<td>1b&lt;sup&gt;a&lt;/sup&gt;</td>
<td>inactive&lt;sup&gt;e&lt;/sup&gt;</td>
<td>inactive&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;, 1b&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.56(0.55&lt;sup&gt;c&lt;/sup&gt;,0.57&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>83 (80&lt;sup&gt;c&lt;/sup&gt;,85&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>6.7</td>
</tr>
<tr>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05(1.0&lt;sup&gt;c&lt;/sup&gt;,1.1&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>82(74&lt;sup&gt;c&lt;/sup&gt;,90&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>12.8</td>
</tr>
<tr>
<td>2b&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>104&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>4.8</td>
</tr>
<tr>
<td>2&lt;sup&gt;a&lt;/sup&gt;, 2b&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.88&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>79&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>11.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isoacceptor purified by column methods (including ConA Sepharose) was further purified by gel electrophoresis.

<sup>b</sup> Isoacceptor purified by column methods only.

<sup>c</sup> Values derived from Lineweaver-Burk plot.

<sup>d</sup> Values derived from Eadie-Hofstee plot.

<sup>e</sup> The sample did not aminoacylate.

than the slow moving band. The tRNA<sub>asp</sub><sub>1a,1b</sub> preparation had about 25% of the inactive species and that of tRNA<sub>asp</sub><sub>2a,2b</sub> about 40% of the less active subspecies. Each active subspecies (tRNA<sub>asp</sub><sub>1a</sub>, tRNA<sub>asp</sub><sub>2b</sub>) was further tested for purity and for possible conversion into inactive subspecies. After recovery of the bands from the slab gel, each band was rechromatographed by two-dimensional electrophoresis. None showed a change. Electrophoresis of each Asp isoacceptor from rabbit liver produced only one band, which was active. Both isoacceptors (1<sup>a,b</sup> and 2<sup>a,b</sup>) in Table II exhibit similar reaction rates (K<sub>m</sub><sup>1a</sup> ~ 80 nM), but V<sub>max</sub> value of tRNA<sub>asp</sub><sub>1a</sub> 36% less than that of tRNA<sub>asp</sub><sub>2b</sub>. The latter isoacceptor is a better substrate -- compare V<sub>max</sub> to K<sub>m</sub> ratios.

2. Properties of Q-containing and Q-deficient Aspartate tRNAs

A. Test of the tRNA Purity

a. Presence of G-tRNA in tRNA samples by the transglycosylase reaction.

Several tRNAs from various sources were treated with labelled guanine and E. coli transglycosylase in order to determine G contents in position 34 of the tRNA structure. The results in Table III indicate the presence of G (i.e., lack of G-to-Q modification) in only the tRNA from L-M cells.
TABLE III: TEST FOR THE PRESENCE OF G IN POSITION 34

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Guanine Incorporated$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Background incorporation of labelled G</td>
<td></td>
</tr>
<tr>
<td>tRNA, rabbit liver (control)</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>Unfractionated tRNAs, rabbit liver</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Purified Q-tRNA$^{ASP}$, rabbit liver</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>b. Maximal incorporation of labelled G</td>
<td></td>
</tr>
<tr>
<td>tRNAs from Yeast (contain no Q)</td>
<td>69.0 ± 0.7</td>
</tr>
<tr>
<td>c. Absence of Q (i.e., presence of G) in position 34</td>
<td></td>
</tr>
<tr>
<td>tRNAs from growing L-M cells $^b$</td>
<td>40.6 ± 0.4</td>
</tr>
<tr>
<td>d. Presence of Q (i.e., absence of G) in position 34</td>
<td></td>
</tr>
<tr>
<td>tRNAs from growing L-M cells with queuine $^c$</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>tRNAs from ovary tumor (adenocarcinoma)</td>
<td>3.8 ± 0.1</td>
</tr>
</tbody>
</table>

$^a$Picomoles of guanine incorporated in one A$_{260}$ unit of RNA, using E. coli tRNA-guanine transglycosylase. The values are average of five independent reactions.

$^b$tRNAs derived from the L-M cells grown in the absence of queuine.

$^c$tRNAs derived from the L-M cells grown in a serum-free medium to which queuine was added.

that was grown in culture without added queuine. Interestingly, no significant amount of guanine was incorporated into tRNAs derived from ovary tumors -- an indication of the presence of a full complement of queuine in these tRNAs. Similarly, both unfractionated tRNAs and purified aspartate tRNAs (rabbit liver) showed the full amount of Q in their structures.

b. Test of Q in tRNA samples by the lectin-affinity method. The chromatogram in Fig. 2a showed retention of the tRNA$^{ASP}$ (with manQ) only in the tRNA sample derived from L-M cells grown in culture and supplemented with queuine. However, no such tRNA$^{ASP}$ (i.e., containing manQ) was present in the tRNA sample derived from L-M cells not grown with added queuine (Fig. 2b). The results confirm the total absence of manQ in tRNA$^{ASP}$ derived from the latter tRNA sample. (The mammalian tRNA$^{ASP}$ contains mannose, linked via glucosidic linkage to queuine. The hexose of tRNA$^{ASP}$ binds specifically with ConA of the column matrix and the eluant containing $\alpha$-methylglucose competes for this binding).

B. Differences in Aminoacylation Caused by G-to-Q Modification

Aminoacylation of the tRNA$^{ASP}$ in two tRNA samples was studied using steady state reaction conditions and the rabbit liver synthetase. The tRNAs were derived from L-M cells grown in culture, with queuine (Q-tRNA$^{ASP}$) or without queuine (G-tRNA$^{ASP}$). Aminoacylation of the Q-tRNA$^{ASP}$ showed a
Figure 2. The test for the presence of manQ in tRNAs derived from L-M cells grown in culture, supplemented with or without free Q. About 3 A_{260} units of each tRNA sample was applied to a ConA-Sepharose column (0.8 x 8 cm) and desorbed with L-0-methyl-α-D-glucopyranose.

higher reaction velocity (V_{max} is 30% greater) and a higher reaction rate (K_{m} is 55% less) than its counterpart, the G-tRNA^{Asp} (Fig. 3 and Table IV). The Q-tRNA^{Asp} served as a better substrate than did the G-tRNA^{Asp} in the

Figure 3. Aminoacylation of the aspartate tRNAs derived from L-M cells grown in culture, supplemented with Q (---) or without Q (——). Upper panel, Lineweaver plot; lower panel, Eadie-Hofstee plot. (See legends to Fig. 1).
TABLE IV: KINETIC PROPERTIES: ASPARATE Q- AND G-tRNAs AS SUBSTRATES FOR THE AMINOACYLATION REACTION

<table>
<thead>
<tr>
<th>Substrate</th>
<th>V_max (pmol/min)</th>
<th>K_m (nM)</th>
<th>V_max/K_m (pmol·min⁻¹·μM⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q-tRNAs^asp</td>
<td>0.80^b</td>
<td>68^b</td>
<td>11.8^b</td>
</tr>
<tr>
<td></td>
<td>0.78^a</td>
<td>65^a</td>
<td>12^a</td>
</tr>
<tr>
<td>G-tRNA^asp</td>
<td>0.62^b</td>
<td>125^b</td>
<td>5.0^b</td>
</tr>
<tr>
<td></td>
<td>0.59^c</td>
<td>115^c</td>
<td>5.1^c</td>
</tr>
</tbody>
</table>

^aQ-tRNAs were derived from L-M cells, grown in a serum-free medium to which queuine was added; G-tRNAs were derived from L-M cells, grown in the absence of queuine.
^bValues derived from Lineweaver-Burk plot.
^cValues derived from Eadie-Hofstee plot.

amiacylation reaction as shown by a ratio of V_max to K_m 2.4 times higher for the former tRNA (see Table IV).

3. Properties of Normal and Tumor Aspartate tRNAs
A. Test for Q in Tumor Aspartate tRNAs
a. Test by lectin-affinity method. tRNAs derived from human ovary and lung tumors and grown in athymic mice were examined for the presence of aspartate Q-tRNAs by ConA-affinity chromatography. The results in Fig. 4

![Figure 4](image)

Figure 4 The test for the presence of manQ in the tRNAs derived from human ovary and lung tumor lines grown in athymic mice. (See Fig. 2).
Figure 5 The test for the presence of manQ in ovary tumor tRNAs using rabbit liver tRNA as a reference. Separation of aspartyl-tRNAs: 0.5 A260 unit samples were applied to RPC-5 columns (0.63 x 33 cm) before treatment (---) and after (----) treatment with CNBr.

indicate retention of some tRNA material on this column. Aminoacylation of the unfractionated tRNA, which failed to interact with the ConA (the front peak), also failed to produce Asp-tRNA. However, the retained material exhibited very high specific activity for Asp-tRNA (\( \sim 1.8 \text{ mmol aspartate per } 1.0 \text{ A}_{260} \text{ unit} \)). The results indicate the absence of G-tRNA\(^{\text{Asp}}\) and the presence of manQ-tRNA\(^{\text{Asp}}\) in the tRNA samples derived from the human tumor cell lines.

b. Test by column chromatography and reaction with CNBr. The side chain of queuine contains a secondary amine with \( pK_a \) of ca. 9. Cyanogen bromide reacts with this amine and thereby abolishes the cationic charge. In RPC-5 column chromatography, this Q-tRNA, having lost its cationic function after reaction with CNBr, elutes at a higher salt concentration. The G-tRNA, having no such cationic function (due to the missing Q), fails to react with CNBr. Therefore, the elution position of G-tRNA on an anion-exchange (e.g., RPC-5) column remains unchanged after CNBr treatment (25).

The results in Fig. 5 indicate that tRNA samples derived from both normal rabbit liver and tumor had a full complement of Q in their tRNA\(^{\text{Asp}}\) structures. (Small peaks indicated with prime numbers were apparently partial reaction products of the CNBr reaction). Chromatography of the Asp-tRNAs after the CNBr reaction caused a change in the elution position for each isoacceptor peak; therefore, neither sample contained any G-tRNAs\(^{\text{Asp}}\).

c. Test for G-tRNAs by transglycosylase reaction. The results in Table III
indicate that the tRNA sample derived from ovary tumors (and from several other tumors grown in athymic mice, results not shown) did not incorporate labelled guanine any more than the control value.

B. Comparison of Normal and Cancer Aspartate tRNAs as Substrates for the Aminoacylation Reaction

Aminoacylation of the tRNA^{Asp} of normal and ovary tumor tRNA samples was carried out using a preparation from rabbit liver rich in Asp-tRNA

### TABLE V: COMPARISON OF NORMAL AND TUMOR ASPARTATE tRNAs AS SUBSTRATES FOR THE AMINOACYLATION REACTION

<table>
<thead>
<tr>
<th>Substrate</th>
<th>v_{max} (pmol/min)</th>
<th>K (nM)</th>
<th>v_{max}K (pmol/min μM^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>rabbit liver tRNAs:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.77^a</td>
<td>75^a</td>
<td>10.3^a</td>
<td></td>
</tr>
<tr>
<td>0.76^b</td>
<td>73^b</td>
<td>10.4^b</td>
<td></td>
</tr>
<tr>
<td>ovary tumor tRNAs:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.64^a</td>
<td>75^a</td>
<td>8.5^a</td>
<td></td>
</tr>
<tr>
<td>0.62^b</td>
<td>72^b</td>
<td>8.6^b</td>
<td></td>
</tr>
</tbody>
</table>

^aValues derived from Lineweaver-Burk plot.
^bValues derived from Eadie-Hofstee plot.
DISCUSSION

Use of the Q-containing and Q-deficient tRNAs\textsuperscript{Asp} as Substrates in the Aminoaerylalion Reaction

Activities of the aspartate tRNAs in Q-tRNA and G-tRNA samples from the L-M cells were assayed, using a partially purified Asp-tRNA synthetase preparation from rabbit liver. The G-tRNA sample was free of any Q-tRNA\textsuperscript{Asp}, and similarly the Q-tRNA sample was free of G-tRNA. This was determined by the transglycosylase reaction (Table III), and the ConA-affinity method (Fig. 2).

The results indicate that important differences exist in both the reaction velocity and the reaction rate (Table V). For aspartate incorporation, the reaction rate of the G-tRNA is reduced to about half that of the Q-tRNA and the reaction velocity is reduced to 3/4th that of the Q-tRNA. The ratio of $V_{\text{max}}$ to $K_m$ -- often used as an indication of substrate efficiency for the enzyme -- is about 5 for the G-tRNA\textsuperscript{Asp}, but increases to a value of 12 for the Q-tRNA\textsuperscript{Asp}. This kinetic work was repeated four times using identical reaction conditions. The values from the repeat experiments were in close agreement (> 97%) with those reported in Fig. 3 and Table IV. (See materials for characteristics of tRNA and synthetase samples).

The results support the notion that the Q-deficient (undermodified) tRNA is a poor substrate and exhibits a diminished activity in the aminoaerylalion reaction. G-to-Q modification appears to influence the binding of the substrate with the synthetase and also, the reaction rate (i.e., complex dissociation into the synthetase and the product or subproducts). Both complex formation and complex dissociation apparently depend upon the nature of the substrate. The formation of a relatively stable complex between G-tRNA\textsuperscript{Asp} and the synthetase can tie up the enzyme. Activity of the synthetase can therefore depend upon the cellular level of the G-tRNA. Thus, the presence of large amounts of G-tRNAs in one-week old animals reported earlier (26) should correlate with higher cellular concentrations of the tRNA\textsuperscript{Asp} needed for sustained Asp-tRNA synthesis (17).

Earlier work with the rat liver tRNA\textsuperscript{Asp} showed the $V_{\text{max}}$ value of the aminoaerylalion of a purified Q-tRNA\textsubscript{1,2} sample to be 16 times that of a G-tRNA\textsuperscript{Asp}, which contained other tRNAs besides G-tRNA\textsuperscript{Asp}. The $K_m$ values...
for the two tRNA samples were the same (17,26). The values of this study may be incorrect since an unfractionated G-tRNA sample was compared with a pure Q-tRNA^{Asp} sample. (The purity of the tRNA^{Asp} in the two samples was not comparable).

Contrary to these results, studies involving usage of the histidine G and Q isoacceptors in globin synthesis showed no affect of Q in the translation process (27,28). Another study (29) of an E. coli mutant with Q-deficient tRNAs, reports similar V_{max} values and only 12% higher reaction rate of the Q-tRNA^{Tyr} than the G-tRNA^{Tyr} (K_{m} values: 0.47 and 0.60 μM, respectively).

The importance of Q, however, was clearly shown in the case of Dictostelium discoideum (30), where G-tRNA^{Asn} was specifically present during developmental transition of vegetative amobae caused by nutrient starvation. G-tRNA^{Asn} was completely deacylated in vivo, while Q-tRNA^{Asn} was aminoacylated (also see 25).

These results support our observation that the Q component of the tRNA in some cases can influence synthesis of the aminoacyl-tRNAs. Although at present no clear evidence exists for specific interaction of the Q with the codons or with the amino acid residues of the synthetase, the presence of a bulky side chain with a cationic group, and a hexose residue in the manQ structure, offer such possibility. Our results suggest the Q of the tRNA^{Asp} can function as a controlling factor for the Asp-tRNA synthesis. However, it must be noted that the Q-deficient tRNA exists only in special instances, for example, in some tumor cells, or when cells grown in a serum-free medium or the animal is fed on a Q-deficient diet. Since Q is present widely in plants (31,32), and can by synthesized in bacteria (33), occurrence of Q-deficient tRNAs in animals appear to be unlikely.

**Tumor tRNAs. G-to-Q Modification, Reaction Kinetics**

Several workers have reported that the cancer tRNAs contain insufficient amounts of Q (2,10-13,34). We have examined this possibility in human tumors transplanted and grown in athymic mice. The tRNAs from these well-characterized tumors were tested for the presence of Q by three different methods: (a) tRNA-guanine transglycosylase reaction (Table III), (b) specific interaction of ConA with the mannose moiety of the manQ in the tRNA^{Asp} (Fig. 4), and (c) treatment of the Asp-tRNAs with CNBr followed by chromatography on RPC-5 columns (Fig. 5). Each method indicated the presence of a full complement of Q in the tumor tRNA structure. The tRNAs derived from human ovary and rabbit liver here show identical
reaction rates and only a small loss (18%) in the reaction velocity of the tumor tRNA\textsuperscript{Asp}. This loss in the reaction velocity can arise from the use of a heterologous enzyme used with the tumor tRNA (Table V).

The cancerous cells growing in the host with a common vascular system should have access to the "free Q" and also perhaps to transglycosylase. Therefore, tumors grown in mice can possibly produce a full complement of Q in their tRNAs. Insufficient G-to-Q modification in cancer tRNAs reported by others can arise from several possibilities: (a) presence of a potent inhibitor of the transglycosylase; (b) tumor transglycosylase exhibiting more affinity for the G than for the Q; (c) insufficient amount of Q available to the tumors; and (d) the Q can be required to derepress the genome for tumor enzyme synthesis (hence, a lack of Q in the cells would produce no or little synthesis of the tumor enzyme and a failure of G-to-Q modification in the tumor tRNAs). Although any one of the above possibilities can effectively explain the observed loss of Q in specific instances, supporting experimental evidence is lacking at present.

Comparison of Aspartate Isoacceptors

The ratio of the amount of the minor to that of the major aspartate isoacceptor varies in two animal species, and also in the normal liver and the tumor cells (Table I). Each bovine liver isoacceptor exhibits an inactive (tRNA\textsuperscript{Asp}\textsubscript{1b}) or a less active (tRNA\textsuperscript{Asp}\textsubscript{2b}) subspecies. The inactive forms, which are not the product of gel electrophoresis, are either produced during the purification or are present as such in vivo. It is inactive (or less active) tRNA\textsuperscript{Asp} instead of something else since it is purified by an affinity method specific for this tRNA (35).

Marked differences appear to exist in the reaction velocities, but none in the reaction rates of the two active isoacceptors (bovine liver tRNA, see Fig. 1 and Table II). Although the complete sequence of the tRNA\textsuperscript{Asp}\textsubscript{1} is not known, our preliminary results indicate that it differs substantially from the tRNA\textsuperscript{Asp}\textsubscript{2} structure (36).

Acknowledgements

The authors thank Dr. Jon Katze, Department of Microbiology and Immunology, College of Medicine, University of Tennessee for supplying tRNA samples from L-M cells, Dr. Nathan O. Kaplan, Chemistry Department, University of California at San Diego for providing samples of the tumors grown in nude mice, and Dr. Susumu Nishimura, National Cancer Center Research Institute, Tokyo, for a sample of E. coli transglycosylase. The research
was supported by a grant from Wichita State University.

REFERENCES