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Inhibition of Nucleoside Q Formation in Transfer Ribonucleic Acid During Methionine Starvation of Relaxed-Control *Escherichia coli*

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The elution profiles of Asp-tRNA from unstarved and starved cultures of a relaxed-control (Rel⁻) strain of *Escherichia coli* were compared by reversed-phase chromatography. Methionine starvation results in the appearance of several additional species of Asp-tRNA which are not observed with starvation for leucine or histidine. By the criterion of cyanogen bromide-effected shifts in chromatographic elution position, a large portion of the $tRNA^{Asp}$ synthesized in methionine-starved cells lacks the normal Q nucleoside. By the same criterion, virtually all of the $tRNA^{Asp}$ from unstarved, leucine-starved, and histidine-starved cells contain Q. We conclude that methionine starvation prevents the formation of the normal Q nucleoside in Rel⁻ *E. coli*.

A hypermodified derivative of guanosine, the Q nucleoside (Fig. 1), is found in the first position of the anticodons of *Escherichia coli* $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$ (8, 14a). Q or a Q-like nucleoside is also found in the same tRNA's from *Drosophila* (14a, 35) and mammalian cells (15). *E. coli* tRNA normally appears to be completely modified with respect to Q (8), whereas eukaryotic tRNA's exhibit variable, growth state-dependent degrees of Q content (15, 35).

The chromatographic properties of various isoaccepting species of tRNA are influenced by culture conditions. For example, chloramphenicol treatment of *E. coli* (11, 12, 14, 33) and amino acid starvation of relaxed control (Rel⁻) *E. coli* (1, 3, 5, 12, 13, 18, 33) result in the formation of chromatographically unique, undermodified tRNA's. The nucleotide sequences have been determined for a few such undermodified tRNA molecules (11; M. J. Fournier, personal communication) but the relation of a chromatographically novel form to the presence or absence of specific modifications remains undetermined in most instances.

In the present study, we have observed several unique species of Asp-tRNA as a result of methionine starvation. We provide evidence that at least two of these species are deficient in the normal Q nucleoside.

MATERIALS AND METHODS

Bacteria and culture conditions. *E. coli* K-12 strain RM628 (Thi⁻, His⁻, Thr⁻, Leu⁻, *metB1*, *rif-1*, Rel⁻) was constructed by conjugation between strain RM554 (Hfr Cavalli *pyrE401*, *metB1*, *rif-1*, Rel⁻) and CP79 (Thi⁻, Arg⁻, His⁻, Thr⁻, Leu⁻, Rel⁻). RM554 is a spontaneous rifampicin-resistant mutant of strain D19, which was obtained from Jon Beckwith. CP79 was obtained from Gordon Edlin. Cultures were grown to mid-logarithmic phase (2.5×10^8 cells/ml) at 37 C with vigorous aeration in minimal-salts medium (32) supplemented with 0.4% glucose and amino acids (50 μg of L-methionine, L-leucine, and L-threonine per ml and 40 μg of L-histidine per ml). For starvation, cells were collected by centrifugation, washed once in unsupplemented minimal medium, and suspended in the supplemented medium with one amino acid omitted. Incubation was continued for 4 h. One culture was grown to high density (1.6×10^9 cells/ml) in L broth (1% tryptone [Difco], 0.5% yeast extract [Difco], 0.5% NaCl, and 0.1% glucose). *E. coli* strain Q-13 (see reference 19) and *E. coli* K-12 strain W6 (4) were obtained from Alan Davis. Strain Q-13 was grown to mid-logarithmic phase in Penassay broth (Difco).

Preparation and aminoacylation of tRNA. tRNA was isolated from cells by suspension in 10 volumes of buffer A [10 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.5), 0.15 M NaCl, 10 mM 2-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, and 0.4 mg of bentonite per ml], followed by phenol extraction and diethylaminoethyl-cellulose chromatography as described previously (17). A crude preparation of aminoacyl-tRNA synthetases was prepared from *E. coli* Q-13: 2 g of cells suspended in 13 ml of buffer B (10 mM tris(hydroxymethyl)aminomethane-hydrochloride, [pH 7.5], 10 mM 2-mercaptoethanol, 5 mM $MgCl_2$, and 10% [vol/vol] glycerol) was disrupted in a French press, the lysate was centrifuged at 100,000 × *g* for 60 min, and the supernatant fluid was

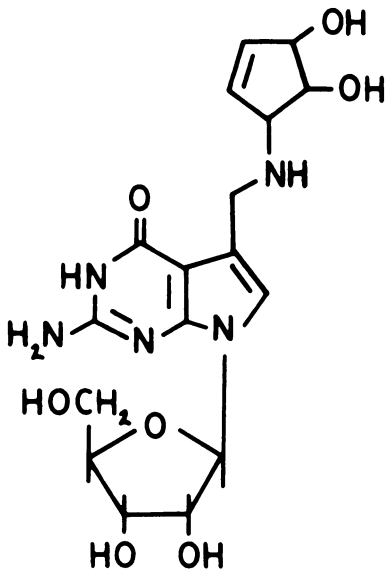

FIG. 1. *Structure of the Q nucleoside isolated from E. coli tRNA (14a).*

applied to a 7-ml column of diethylaminoethyl-cellulose equilibrated and washed with buffer B. The synthetases were eluted with buffer B containing 0.25 M KCl and frozen in small portions at −70 C until use. tRNA was aminoacylated at 37 C as described by Yang and Novelli (36), with all amino acid concentrations at 10 μM. The reaction of tRNA with cyanogen bromide (BrCN) prior to aminoacylation has been described (15).

Reversed-phase chromatography. A reversed-phase chromatography (RPC-5) column (0.9 by 20 cm) was operated at 27 C as described (17). Applied tRNA was eluted with a 300-ml linear gradient of 0.475 to 0.75 M NaCl in a buffer containing 10 mM sodium acetate (pH 4.5), 10 mM $MgCl_2$, 3 mM 2-mercaptoethanol, and 1 mM ethylenediaminetetraacetate. Each elution was followed with a wash of 1.5 M NaCl in the same buffer. One-minute (1.5 ml) fractions were collected and mixed with 10 ml of 33% Triton scintillation mixture (17). The 1.5 M NaCl fractions were diluted with 2 volumes of water, and 1.5 ml of the diluted fractions was mixed with the scintillation mixture. Radioactivity was determined in a Beckman LS-150 scintillation system, employing automatic quench compensation based on an external standard. The quench compensation reduced the appearance of ^{14}C counts per minute in the ^{3}H window to 17% of the counts per minute in the ^{14}C window. The data were corrected by hand when the ^{14}C counts per minute in the ^{3}H window exceeded 5% of the ^{3}H counts per minute. Recoveries of labeled tRNA added to each column ranged from 84 to 100%, averaging 95%.

RESULTS

Amino acid starvation. Cultures of a Rel$^-$ strain of *E. coli* K-12 (RM628) were grown to mid-logarithmic phase in supplemented minimal medium and then starved separately for each of three required amino acids (methionine, leucine, and histidine). Preparations of tRNA isolated from these cultures did not differ significantly with respect to their ability to be acylated with aspartic acid (0.12 ± 0.01 nmol of aspartic acid per absorbancy unit at 260 nm). However, when acylated with [^{14}C]aspartic acid or [^{3}H]aspartic acid and analyzed by RPC-5 cochromatography, the elution profiles of Asp-tRNA from starved and unstarved cells indicate several significant differences (Fig. 2). Methionine starvation (Fig. 2A) yields tRNA with an earlier eluting peak 2 (which is designated peak 2′) and several additional subspecies (peaks 3′, 4, 5, 9, and 10). Even though peaks 3 and 3′ (Fig. 2A) cannot be distinguished by elution position, only peak 3 is reactive with BrCN (see below). Methionine starvation of another Rel$^-$ strain, *E. coli* K-12 (W6), gave similar results (data not shown). The chromatographic profiles of Asp-tRNA from leucine-starved (Fig. 2B) and histidine-starved (Fig. 2C) cells qualitatively resemble each other and those of unstarved cells (with the exception of peak 2′), although large quantitative changes are evident.

We were bothered that three major peaks are found in the elution profile of Asp-tRNA from unstarved cells of strains RM628 and W6, because *E. coli* has been reported to exhibit only two species of $tRNA^{Asp}$ (21). Our findings could reflect the higher resolution of the RPC-5 columns used in the present study, strain differences, or growth conditions. Preparations of tRNA isolated from strain RM628 grown to late-logarithmic phase in rich medium (L broth) also contain three major species of Asp-tRNA (Fig. 2D), although each of these peaks (designated 1′, 2″, and 6′) elutes slightly later than the corresponding peak from unstarved cells grown in minimal medium. We conclude that the Rel$^-$ strains of *E. coli* used in the present study normally contain a third species of $tRNA^{Asp}$ not previously reported.

Cyanogen bromide treatment. Q-containing tRNA species elute at lower NaCl concentrations from RPC-5 columns than otherwise identical, Q-negative tRNA species (15, 33), presumably because of an extra positive charge contributed by the secondary amino group in the Q nucleoside side chain (Fig. 1, reference 14a). Therefore, it was of interest to determine whether the unique species of Asp-tRNA which result from methionine starvation of strain RM628 are hypomodified with respect to Q. This was accomplished using a BrCN-depend-

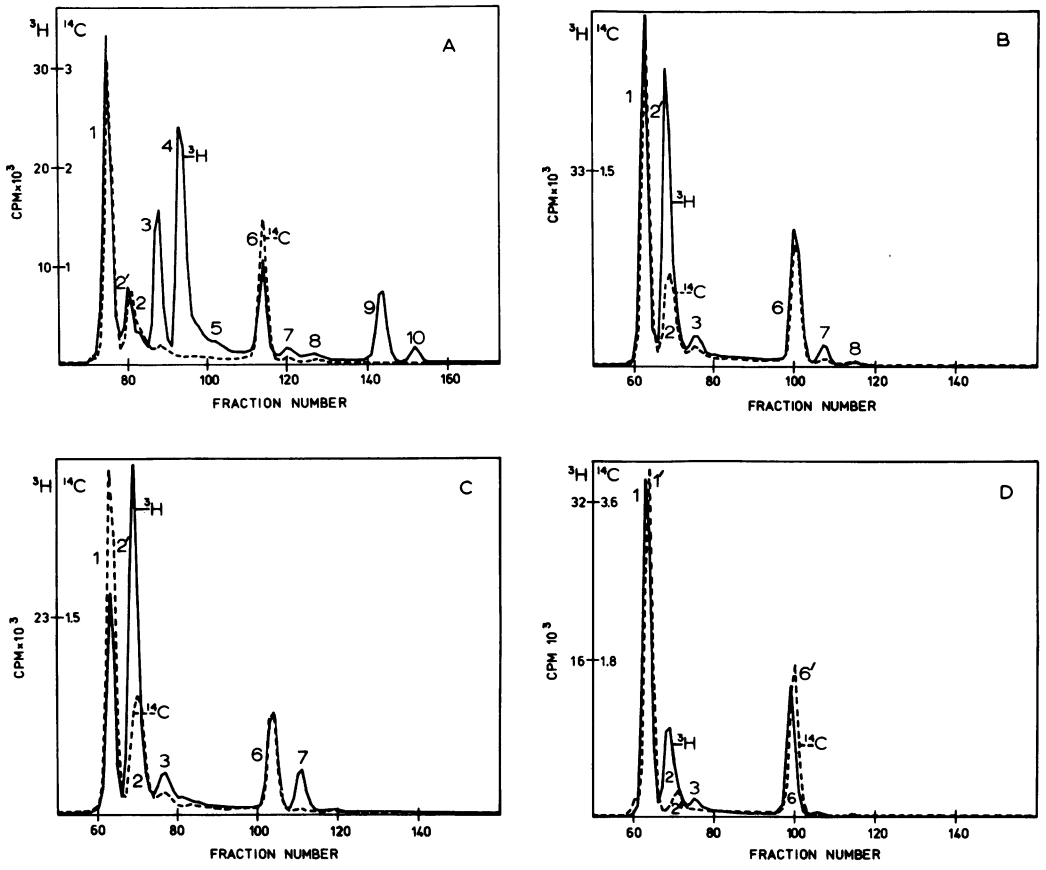

FIG. 2. *Cochromatographic comparisons of Asp-tRNA from E. coli strain RM628 grown under different conditions. The procedural details are described in Materials and Methods. (A) [^{14}C]Asp-tRNA (unstarved); [^3H]Asp-tRNA (methionine starved). (B) [^{14}C]Asp-tRNA (unstarved); [^3H]Asp-tRNA (leucine starved). (C) [^{14}C]Asp-tRNA (unstarved); [^3H]Asp-tRNA (histidine starved). (D) [^{14}C]Asp-tRNA (L broth); [^3H] Asp-tRNA (unstarved).*

ent shift to a later RPC-5 peak elution position as the criterion of Q content. BrCN is known to inactivate the amino acid acceptance activities of some *E. coli* tRNA species, but not $tRNA^{Asp}$ (25, 27), and only small losses (10 to 26%) in the specific acceptance of $tRNA^{Asp}$ were observed with BrCN treatment in the present study.

Under the conditions used here (pH 8.9, 10 min, room temperature), the reaction of BrCN with tRNA is limited to Q, 3-(3-amino-3-carboxyl-*n*-propyl) uridine, and thionucleosides (26, 32, 34). By examining the effect of BrCN on the RPC-5 elution properties of Asp-tRNA, we may detect specifically the interaction of BrCN with Q. This follows from several observations: (i) BrCN treatment eliminates a positive charge from Q (27, 32, 34), probably as a result of interaction of BrCN with the secondary amine of the Q side chain (14a, 32). The loss of charge is believed to account for the BrCN effected later elution of Q-containing tRNAs from RPC-5 columns (15, 35). (ii) *E. coli* $tRNA^{Asp}$ does not contain 3-(3-amino-3-carboxyl-*n*-propyl)uridine (9). (iii) The reaction of BrCN with thionucleosides does not result in alteration of charge (26, 27, 32) and therefore should not effect later elution of tRNA's from RPC-5 columns. Moreover, the synthesis of 4-thiouridine, the only thionucleoside in *E. coli* $tRNA^{Asp}$ (9), should be inhibited by any interruption in protein synthesis (see below) and cannot account for the specific effects of methionine starvation presented in Fig. 2 and 3 (see below).

The changes in chromatographic properties effected by BrCN treatment of tRNA from unstarved and methionine-starved cells are shown in Fig. 3 and, to facilitate comparison,

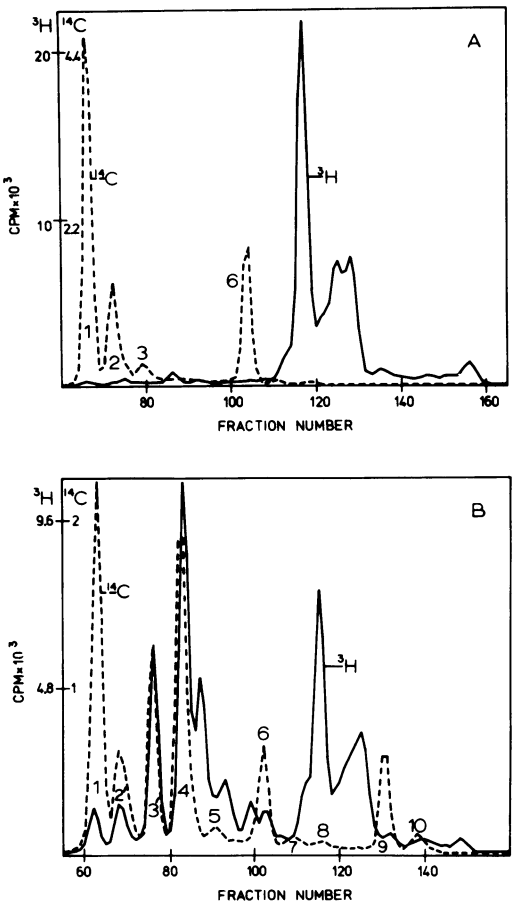

FIG. 3. *Effect of BrCN on the elution profiles of Asp-tRNA from E. coli strain RM628 grown under different conditions. The procedural details are described in Materials and Methods. (A) [^{14}C]Asp-tRNA (unstarved); [^3H]Asp-tRNA (unstarved, treated with BrCN). (B) [^{14}C]Asp-tRNA (methionine starved); [^3H]Asp-tRNA (methionine starved, treated with BrCN).*

these data are normalized and expressed quantitatively in Table 1. It is apparent that virtually all of the Asp-tRNA from unstarved cells is shifted to later elution by BrCN (Fig. 3A, Table 1). However, a more complex result is obtained with tRNA from methionine-starved cells (Fig. 3B, Table 1). After BrCN treatment, peaks 1, 2', 6, and 9 (and perhaps 10) are diminished, the majority of peaks 3' and 4 are unaltered, and at least three peaks appear which were not observed with BrCN-treated tRNA from unstarved cells. The residual Asp-tRNA eluting in the positions of peaks 1, 2', 3', and 4 after BrCN treatment suggests that a large portion of $tRNA^{Asp}$ from the methionine-starved cells either lacks, or is aberrantly modified with respect to, the Q nucleoside. Results similar to those shown in Fig. 3B were also obtained with tRNA from methionine-starved strain W6 of *E. coli* K-12 (data not shown). BrCN-treated tRNA from leucine- and histidine-starved cells gave results (data not shown) very similar to BrCN-treated tRNA from unstarved cells (Fig. 3A). Therefore, we conclude that methionine starvation, but not leucine or histidine starvation, of Rel$^-$ *E. coli* interferes with the formation of the normal Q nucleoside.

DISCUSSION

Chloramphenicol treatment of *E. coli* results in the formation of tRNA's undermodified with respect to 4-thiouridine (33), dihydrouridine (11, 14, 33), the 2-methylthio moiety of 2-methylthio-N^6-(Δ-isopentenyl)adenosine (11), pseudouridine (in the anticodon region, but not in the TψC loop) (11), and possibly 2-methyladenosine, 1-methylguanine, and uridine 5-oxyacetic acid (33). Starvation of Rel$^-$ *E. coli* for

TABLE 1. *Effect of BrCN on the relative proportions of the isoaccepting species of $tRNA^{Asp}$ from unstarved and methionine-starved E. coli.*

Peak no.	Radioactivity[a] (%)			
	Unstarved		Methionine starved	
	−BrCN	+BrCN	−BrCN	+BrCN
1	50.7	0.8	26.3	2.9
2	19.3	1.3		
2'			9.8	3.6
3	5.4			
3'			13.6	10.2
4		2.0	26.7	22.3
5			2.7	?
6	22.4		8.2	? (<3.1)
7	1.1		1.7	?
8	0.9		1.5	?
9			7.7	? (<1.7)
10			1.7	?
Other[b]		95.2		60.5
1.5 M NaCl	0.2	0.6	0.1	0.4

[a] Values were determined from the data represented in Fig. 2 by dividing the radioactivity present in each peak by the total radioactivity recovered from each chromatographic run. The value for a peak which is obscured by a new BrCN-dependent peak is represented by ?. See Materials and Methods for procedural details.

[b] The materials in new peaks resulting from BrCN treatment are summed together and designated Other.

amino acids also yields tRNA which is undermodified, apparently with respect to the same bases affected by chloramphenicol treatment (5, 18; M. J. Fournier, personal communication). These hypomodified tRNA's formed during inhibition of protein synthesis appear to be precursors of normal, fully modified tRNA's which accumulate during inhibition of protein synthesis while tRNA synthesis continues (11, 12, 18, 33). It has been suggested that this incomplete modification could result from instability of the modifying enzymes involved (5, 13, 14, 34). Superimposed upon this general effect of protein synthesis inhibition on tRNA modification, amino acid starvation of Rel⁻ *E. coli* also can inhibit the formation of specific modifications which utilize amino acids as precursors; e.g., methionine starvation blocks the formation of all methyl groups (see reference 33) and the 3-amino-3-carboxypropyl group (24, 28); cysteine starvation prevents the formation of thiobases (10, 12).

The means by which the Q nucleoside is synthesized is unknown, but the present results suggest that it is a methionine-dependent mechanism. The role of methionine could be direct, i.e., serving as a precursor for part of the Q structure. It is known that the methyl group of methionine is not incorporated into Q nucleoside in *E. coli* (6), but it remains to be determined whether other moieties of the methionine carbon skeleton can be incorporated [as has been found with 3-(3-amino-3-carboxy-*n*-propyl)uridine of *E. coli* (24, 28) and the Y nucleoside of yeast (22)]. The role of methionine also could be indirect, i.e., it could be required for other modifications which must precede the formation of Q. An obligately ordered modification pathway has been suggested for certain tRNA post-transcriptional modifications which appear to occur in sequence (13, 23, 29; M. J. Fournier, personal communication). Studies to determine the precise role of methionine in Q biosynthesis are in progress.

The differences observed here between Asp-tRNA from cells grown to low density in minimal medium and to high density in enriched medium (Fig. 2D) are consistent with previous reports that the degree of tRNA post-transcriptional modification is variable, even under "normal" growth conditions (2, 9, 20, 30). Indeed, it is very likely that all of our findings result from differential inhibition of one or more post-transcriptional modifications of tRNA. It is also possible that some of our data could result from differential transcription of separate $tRNA^{Asp}$ genes, although there is no precedence for such a phenomenon in *E. coli*.

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