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Q-FACTOR: A SERUM COMPONENT REQUIRED FOR THE APPEARANCE OF NUCLEOSIDE Q IN tRNA IN TISSUE CULTURE

Jon R. Katze

Department of Microbiology, University of Tennessee
Center for the Health Sciences
Memphis, Tennessee 38163

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SUMMARY: Animal serum contains an activity, designated Q-factor, which effects an increase in nucleoside Q-containing tRNA in tissue culture. The appearance of Q-positive $tRNA^{Asp}$ in the L-M cell line cultivated serum-free has been used as an assay to partially characterize Q-factor from fetal bovine serum and to determine that bovine amniotic fluid contains 100 fold more Q-factor than does fetal bovine serum. Q-factor is dialyzable, 500 molecular weight or less, and binds tightly to activated charcoal and dextran. Using Q-factor, evidence is presented that the Q-negative $tRNA^{Asp}$ species are precursors of the Q-positive species.

Nucleoside Q is a guanosine derivitive found in the first position of the anticodons of E. coli $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ (1). It occurs in the same tRNAs in the metazoa either as Q or a further derivitive, Q*, (2-9);however, in the present study Q will be used generically to designate Q and/or Q*. Bacterial tRNAs normally are completely modified with respect to Q (10, 11), but in eucaryotes tRNA may exhibit variable degrees of Q content depending on developmental state and/or growth conditions (3, 7, 12-14). One such growth condition, observed in tissue culture, is the concentration of an unidentified serum factor which specifically increases the Q- containing tRNA isoacceptors (3). The present communication describes an assay for and partial characterization of this serum factor.

MATERIALS AND METHODS

The L-M cell line (American Type Culture Collection) was cultured in Dulbecco's modified Eagle's medium (Gibco), supplemented with 0.5% Bacto-peptone. In a typical assay, each of four

Abbreviations: FBS, fetal bovine serum; BAF, bovine amniotic fluid.

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flasks (75 cm^2) was inoculated with 12 ml of medium containing 3-5 x 10^6 cells on day zero and fluid changed on days 1 and 4 with 12 ml of medium containing the substance to be assayed. The assay medium was supplemented with penicillin (100 units/ml) and streptomycin (100 µg/ml) and filter sterilized after addition of the test sample. Cells were harvested on day 5, followed by isolation of tRNA, acylation with labelled-aspartic acid, and chromatographic separation. Variations on this protocol yielded similar results (see below). tRNA was isolated by the method of Yang and Novelli (15), with the following modifications: the cell suspension was extracted twice with an equal volume of 88% aqueous phenol, containing 0.1%(w/v) 8-hydroxyquinoline, and the resulting aqueous phase was placed directly onto a DEAE-cellulose column. A crude aminoacyl-tRNA synthetase preparation from mouse liver was used: fresh livers (11g), suspended in 16.5 ml of buffer [0.1M Tris-HCl (pH7.5), 25 mM KCl, 20 mM 2-mercaptoethanol, 8 mM $MgCl_2$ and 0.15 M sucrose], were homogenized in a Teflon glass homogenizer, the homogenate was centrifuged (90 min 100,000 x g), the resulting supernatant was applied to a DEAE-cellulose column (30 ml), prepared and eluted as described previously (16), the active fractions were pooled, and aliquots were stored at -70 C. tRNA was aminoacylated at 37 C as described (15), with all amino acid concentrations at 10 µM. Isoaccepting $tRNA^{Asp}$ were resolved by reversed-phase chromatography using an RPC-5 column (0.9 x 20 $\pm$ 1 cm) at 37 C as described (10). Elution was performed with a linear gradient, either 300 ml 0.5 to 0.75 M NaCl or 150 ml 0.5 to 0.8 M NaCl, in 10 mM sodium acetate (pH 4.5), 10 mM $MgCl_2$, 3 mM 2-mercaptoethanol, and 1 mM EDTA (the latter gradient allows adequate resolution and reduced chromatographic time).

Materials: FBS and dialyzed FBS (Gibco); human female serum (a gift of Dr. Audrey Roberts); human amniotic fluid, second trimester, obtained by amniocentesis (a gift of Dr. Manuel Ricardo); BAF, of unspecified fetal age (Pel-Freeze Biologicals), of specified fetal age (a gift of Irvine Scientific); fibroblast growth factor and epidermal growth factor (Collaborative Research); putrescine, spermidine and spermine (Calbiochem); the remainder of the compounds in Table 2 (Sigma Chemical Co.)

RESULTS AND DISCUSSION

Chromatographic elution profiles of tRNA from L-M cells cultivated in the presence or absence of serum (17) suggest that a serum factor is required for the appearance of Q-containing tRNA isoacceptors (3). To test this, L-M cells were cultivated serum-free and in increasing amounts of FBS (Fig. 1 and Table 1). It is evident that $tRNA^{Asp}$ peaks 1 and 3 are almost absent under serum-free conditions and increase, with a reciprocal decrease in peaks 2 and 4, as a function of the FBS content of the growth medium. Because mouse $tRNA^{Asp}$ peaks 1 and 3 contain Q, whereas

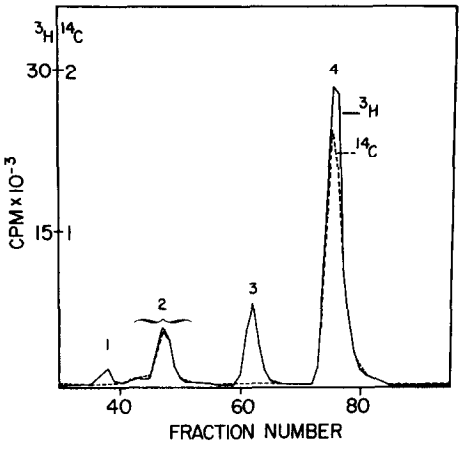

Figure 1. Representative RPC-5 cochromatographic comparison of $tRNA^{Asp}$. [^{14}C]Asp-tRNA from L-M cells cultivated serum-free and [^3H]Asp-tRNA from parallel cultures cultivated in the presence of 20% FBS. Four $tRNA^{Asp}$ peaks are designated (for simplicity and for continuity with previously published studies of mammalian $tRNA^{Asp}$, however, peaks 1, 2, and 4 are generally doublets). See Materials and Methods for procedural details.

Table 1. Effect of serum content of medium on $tRNA^{Asp}$ isoacceptor distribution.

Percent FBS in Medium	Percent of Total $tRNA^{Asp}$ in Peaks[a]				
	1	2	3	4	1.5M NaCl
0	0.7	18.4	0.7	78.9	1.2
5	0.9	17.7	3.0	78.2	0.2
10	2.5	16.9	8.4	72.0	0.1
15	3.4	15.6	13.6	66.9	0.2
20	3.6	14.3	15.6	66.3	0.2

[a] Parallel cultures of cells were cultivated in media containing 0-20% FBS, followed by tRNA isolation, aminoacylation and RPC-5 chromatography. Percent values were determined from the radioactivity present in each peak (see Fig. 1)

peaks 2 and 4 do not contain Q (3, 18), the appearance of Q-containing $tRNA^{Asp}$ in L-M cells must require a factor in FBS.

Therefore, L-M cells cultivated in the absence of serum can be used to assay this factor, hereafter designated Q-factor.

Homogeneic tRNAs arise from identical primary transcripts but differ as a result of post-transcriptional modification (12). $tRNA^{Asp}$ peaks 1 and 2 appear to represent one homogeneic set and peaks 3 and 4 another (3). This view is supported by Fig. 2, in which the data of Table 1 are plotted as the proportion of Q-positive tRNA in each homogeneic set versus the serum content of the medium. It is apparent that the two different Q-positive isoacceptors (peaks 1 and 3) increase in a coordinate fashion, approximately as a linear function of serum concentration.

Q-factor activity is not retained upon ultrafiltration of FBS through membranes with selective retentions from 100,000 to 500 molecular weight (Amicon XM100, XM50, PM10, UM2 and UM05) (data not shown), indicating a molecular weight of 500 or less. Furthermore, because the activity of the UM05 (500 molecular weight nominal exclusion) ultrafiltrate does not differ significantly from that of the starting FBS, higher molecular weight activators or inhibitors of Q-factor are unlikely. Consistent with these data, in the L-M cell assay commercial dialyzed FBS exhibits less than 10% of the activity of the undialyzed product. This result is surpising in view of the report that dialysis of FBS has negligible effects on the $tRNA^{Asp}$ isoaccepting profile of the hamster cell line BHK 21/13 (19). However, different cell lines show varying requirements for Q-factor (Katze, J. R., unpublished observations), suggesting different metabolisms of or abilities to synthesize Q-factor.

Q-Factor in FBS is eliminated by treatment with activated charcoal plus dextran [10 mg/ml Norit SG1 and 1mg/ml Dextran T110, 30 min at 55 C (20)]. Heat treated FBS (2 hr at 63 C) retains 60%

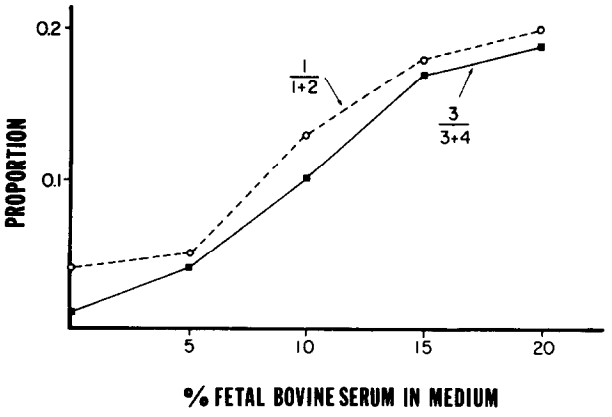

Figure 2. Proportion of Q-positive $tRNA^{Asp}$ versus % FBS medium. The data of Table 1 are plotted as the proportion of Q-positive $tRNA^{Asp}$ (peaks 1 and 3) in each homogeneic peak subset; i.e. the proportion of peak 1 in peaks 1 plus 2, and the proportion of peak 3 in peaks 3 plus 4.

of Q-factor activity, and it is not extracted by ether, ethyl acetate or benzene. In addition, the Q-factor in a UM05 ultrafiltrate of FBS is quantitatively removed by addition to and H_2O elution from columns of the following materials: Dowex 50-X8 (H^+ form), Dowex 1-X8 (Cl^- form), Sephadex G-10, Sephadex G-25, Biogel P-2 and Amberlite XAD-2. The above size, extraction characteristics and unusual binding properties eliminate most known components of animal cells as possible candidates for Q-factor. Further evidence for the singularity of Q-factor is contained in Table 2, a list of compounds devoid of Q-factor activity at the concentrations tested.

Previous reports have demonstrated Q-factor activity in several animal sera, with FBS being the most active source (3, 19). In the L-M cell assay (present study) human amniotic fluid (second trimester) is approximately equal in activity to FBS, human non-pregnant female serum is less than 20% as active as FBS, and BAF is more than 100 times more active than FBS (Fig 3). Subsequent study has shown, however, that all BAF does not exhibit this high activity, but only dark-brown colored lots (which

TABLE 2. Medium additions deviod of Q-factor activity[a]

Addition	Final Concentration
Fibroblast growth factor (bovine)	10 μg/ml
Epidermal growth factor (mouse)	100 μg/ml
Chorionic gonadotropin (human)	100 units/ml
Fetuin	200 μg/ml
Thyrotropin releasing hormone	10^{-9}M and 10^{-7}M
Glycylhistidyllysine	$5x10^{-7}$M
Spermidine	$5x10^{-7}$M
Spermine	$5x10^{-7}$M
Cadaverine	$5x10^{-7}$M
Putrescine	$5x10^{-7}$M
Estradiol (17α or 17β)	10^{-8}M
Estrone	10^{-8}M
Estrone-3-sulfate	10^{-8}M
Progesterone	10^{-8}M
Dehydroepiandrosterone sulfate	$2x10^{-7}$M

[a] Cells were cultivated, followed by tRNA isolation, aminoacylation and RPC-5 chromatography as described in Materials and Methods. In no instance was the sum of peaks 1 plus 3 (the Q-positive peaks) greater than 2% of the total $tRNA^{Asp}$.

correspond to material derived from 7-9 month fetuses). The relation of fetal age to Q-factor in FBS and human amniotic fluid remains to be determined.

The high activity of Q-factor in BAF has enabled a test of the alternative possibilities that the Q-negative $tRNA^{Asp}$ (peaks 2 and 4) are either undermodified precursors or differently modified non-precursors of Q-positive $tRNA^{Asp}$ (peaks 1 and 3). When a large excess of BAF was added to L-M cells (Table 3) nearly

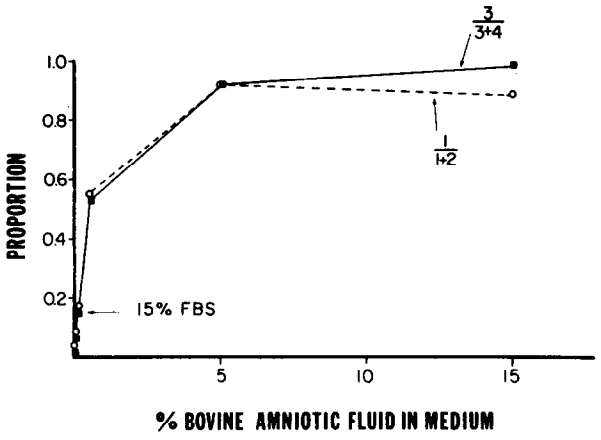

Figure 3. Proportion of Q-positive $tRNA^{Asp}$ versus % BAF in medium. tRNA was isolated, aminoacylated, chromatographed, and the resultant data analyzed as in Fig 2. These data resulted from three separate experiments with small differences in the culture protocols. For 0, 0.05, 0.1, 0.5% BAF and 15% FBS: parallel cultures were inoculated in serum-free medium day 0, fluid changed with medium additions on days 1 and 3, and harvested day 4. For 5% BAF: cells were inoculated in serum-free medium day 0, fluid changed with 5% BAF days 2 and 4, and harvested day 5. For 15% BAF: cells were inoculated in serum-free meduim day 0, fluid changed with 15% BAF on days 1 and 4 and harvested on day 6.

TABLE 3. Proportion of Q-positive $tRNA^{Asp}$ versus time in the presence of 15% BAF.

Hours in 15% BAF[a]	1/1+2[b]	3/3+4
0	0.04	0.02
4	0.25	0.24
24	0.85	0.99

[a] Parallel cultures of cells were inoculated with serum-free medium on day 0 and harvested on day 5, after being fluid changed with 15% BAF medium 4 hr and 24 hr prior to harvest. The data for 0 hr are typical control results from another experiment.

[b] After tRNA isolation, aminoacylation and RPC-5 chromatography, the resulting data were transformed into the proportion of peak 1 in peaks 1 plus 2, and the proportion of peak 3 in peaks 3 plus 4, as described in Fig. 2.

complete conversion from Q-negative to Q-positive $tRNA^{Asp}$ was obtained within 24 hours, with substantial conversion by 4 hours. In that the half-life of tRNA in tissue culture has been determined to be 36-60 hours (21), these data are consistent with peaks 2 and 4 being precursors of peaks 1 and 3.

The function of Q remains unknown, and no other biochemical or morphological phenotype has yet been linked directly with changes in the extent of the Q modification. Even in the present study, though large differences in the extent of Q-base modification have been observed, no significant alterations in cell yield, cell morphology, tRNA yield, or $tRNA^{Asp}$ acceptance could be correlated with these differences. The ultimate goal of this research is an understanding of the function of nucleoside Q. The isolation and characterization of Q-factor from BAF, currently underway, should contribute toward that goal.

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