FOUR-THIOURACIL CONTENT OF CHICKEN LIVER MITOCHONDRIAL TRANSFER RIBONUCLEIC ACID

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SUMMARY: Transfer RNA isolated from both chicken liver and mouse tumor mitochondria contains 4-thiouracil residues. The nucleoside identification is based on the absorption spectra of the tRNA and on its reaction with (^{14}C) methylamine. The concentration of the residue in chicken liver mitochondrial tRNA is similar to that found in E. coli tRNA.

INTRODUCTION: For some years it has been known that the protein synthesizing apparatus of mitochondria is similar to that found in bacteria. The resemblances include inhibitor sensitivity, similar ribosomal types, circular DNA, and the initiation of protein synthesis by a similar species of tRNA, the $tRNA^{fmet}$ (1). This particular species of tRNA points to yet another possible similarity in these two systems in that this tRNA contains a $4sU^{1}$ residue as does the tRNA isolated from many procaryotes and unlike the bulk fraction of tRNA derived from the cytoplasm of eucaryotic species (2,3). To determine if the total 4sU content of mitochondrial tRNA was similar to that of the procaryotic species, the tRNA of chicken liver mitochondria was tested for the concentration of this nucleoside both spectrophotometrically and by reaction with (^{14}C)methylamine (4). These methods showed that this mitochondrial tRNA has about the same amount of 4sU as does that obtained from E. coli.

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[1]Abbreviation used is: 4sU, four-thiouracil.

EXPERIMENTAL

Materials and Methods

Chicken livers were purchased locally from freshly killed birds. Mice (BALB/fC_3H) bearing transplantable tumors were the gift of Dr. D. H. Lavrin. Phenol was used from freshly opened bottles without prior distillation. (^{14}C)methylamine was the product of the Mallinckrodt Chemical Co. and was used at a specific activity of 1.01 Ci/M. Radioactivity was determined as previously described (5). The (^{14}C)methylamine derivatization was performed exactly as reported. Absorption spectra were recorded on the Cary 15 instrument using the 1.0 and 0.1 slide wires as required.

Mitochondrial Isolation

Liver was homogenized in 10x volume of 0.25 sucrose containing 1.0 mM EDTA and 20 mM Tris HCl buffer, pH 7.4, by three fifteen second bursts in a Waring Blendor. A nuclear and unbroken cell fraction was removed by centrifugation at 1400x g for 9 min. Mitochondria were sedimented at 12000x g for 10 min and washed twice by resuspension in a volume equivalent to 3x the original liver weight followed by centrifuging as above. Tumor tissue was homogenized in a Teflon Potter-Elvehjem type homogenizer and then fractionated in the same manner.

tRNA Isolation

The mitochondrial pellet was suspended in twice its volume of a 20 mM Tris HCl buffer, pH 7.4, containing 5 mM $MgCl_2$ and 1% sodium dodecyl sulfate. An equal volume of 88% phenol saturated with the same buffer was added and the procedure of Von Ehrenstein followed (6). Amino acids were stripped from the tRNA at pH 8.0 in 2.0 M Tris HCl by incubation for one hour at 37°. Cytoplasmic tRNA was also obtained by this procedure.

RESULTS AND DISCUSSION

A 200 g batch of chicken liver yielded 140 mg of mitochondrial protein from which 17 A_{260} units of tRNA were isolated. Absorption spectra of this tRNA and that isolated from the cytoplasm are shown in Figure 1. The shoulder seen in the 320-350 nm region is similar both in shape and in extent to that found in E. coli where it has been shown to be primarily, if not solely, due to the presence of 4sU residues (7-9). tRNA isolated from frozen mitochondria does not show absorption in this region which is consistent with the known instability of this nucleotide (10).

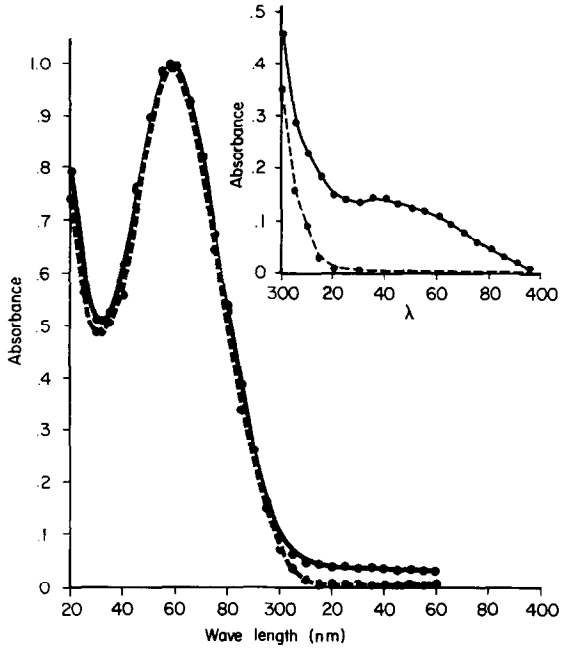

Fig. 1. Absorption spectra of mitochondrial and cytoplasmic tRNA. Mitochondrial tRNA, solid line; cytoplasmic tRNA, dotted line. tRNA dissolved in H_2O, final pH 6.6 for each.

A further demonstration of the presence of 4sU was given by the reaction of the cytoplasmic and mitochondrial tRNA with (^{14}C)methylamine. In this reaction, the 4sU residue is con-

verted into an N^4-methylcytosine residue (4). The results of this experiment with the mitochondrial tRNA are shown in Figure 2. As shown, a net uptake of 0.6 mole methylamine per mole tRNA was observed. The cytoplasmic tRNA showed a slightly positive reaction with the difference between the periodate control and the experimental assay being 0.2 mole per mole.

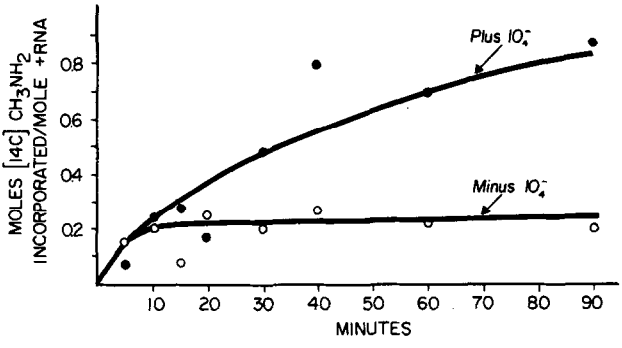

Fig. 2. Kinetics of incorporation of ^{14}C from methylamine into mitochondrial tRNA. Assay is for Cl_3CCOOH insoluble radioactivity (4).

These findings for chicken liver mitochondrial tRNA have been extended in a brief study of the mitochondrial tRNA from a transplantable mouse tumor. From 35 tumors (77 g), 320 mg of mitochondrial protein were obtained and this yielded 11 A_{260} units of tRNA. The absorption spectra had a shoulder in the 320-350 nm region and also reacted with (^{14}C)methylamine to the extent of 0.5 mole (^{14}C)methylamine per mole tRNA. The control without added periodate was 0.2 mole per mole.

The mitochondrial preparations used in these studies were not sterile although precautions were taken to limit bacterial contamination. Therefore the question arises whether the absorption spectra and methylamine reacting material was of bacterial rather than mitochondrial origin. It has been shown

that two 4sU residues are present per three tRNA molecules (8). If the methylamine reaction does represent a reasonable measure of the 4sU content, then about 90 per cent of the tRNA in the chicken liver mitochondria, and about 45 per cent of that from the tumor mitochondria would be from bacterial tRNA in the absence of mitochondrial 4sU residues. This should be reflected in the yield of tRNA from the mitochondria. Chicken liver mitochondria yielded 5.8 μg tRNA/mg mitochondrial protein and for the tumor mitochondria the yield was 1.7 μg tRNA/mg. While comparisons between species and tissues are not compelling, 4.1 μg tRNA/mg mitochondrial protein was found in a study of rat liver mitochondria in which only a very small amount of bacterial contamination was demonstrated (11). This argument does not rule out the presence of bacterial tRNA in these studies but would seem to suggest that this tRNA was not the sole source of 4sU.

The question of the existence in mitochondrial tRNA of the other thiolated nucleotides is left open by this study since only the presence of 4sU was tested. If such thiolated nucleotides are to be found in mitochondrial tRNA, then they, together with 4sU, could provide the physical basis for the observed chromatographic and enzymological differences observed in comparisons of these and the cytoplasmic tRNA's (12-15).

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