tRNA Sulfurtransferase: A Member of the Aminoacyl-tRNA Synthetase Complex in Rat Liver

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SUMMARY

Transfer RNA sulfurtransferase, tRNA methyltransferase, and aminoacyl-tRNA synthetase activity are associated in a complex in rat liver, which is excluded from Sephadex G-200 columns. The complex can also be isolated by subjecting cell supernatants to further centrifugation at 160,000 x g for 18 hours. The resulting pellet contains 70% of the total sulfurtransferase activity, and a 3-fold increase in specific activity is accomplished through pelleting. The data suggest that the enzymes of tRNA metabolism are organized in a large complex in rat liver.

INTRODUCTION

The protein synthetic apparatus in eukaryotic cells may exist as a complex or group of sub-complexes (1). In rat liver eighteen aminoacyl-tRNA synthetases and most of the cellular tRNA were associated in a complex with a molecular weight of 2 x 10^6 daltons or greater (2). Complexes with fewer synthetase activities have also been isolated, and were found to sediment between 18 and 20 S in sucrose density gradients (3,4). Recently, it was reported that three forms of synthetase are present in mammalian cells: free enzyme (4-9 S), enzyme complexes (18-25 S) and microsome-bound enzymes (5,6). These findings suggest that the synthetase complex has a role in transporting aminoacyl-tRNA to the ribosomes, and may somehow regulate protein synthesis. It was suggested that tRNA methyltransferase activity was also associated with the synthetase complex (7). In an attempt to learn whether other tRNA modifying enzymes are present we have measured and detected tRNA sulfurtransferase activity in this fraction. We find that most of the sulfurtransferase activity in rat liver supernatants is present in a complex cosedimenting with aminoacyl-tRNA synthetases and tRNA methyltransferase.

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MATERIALS AND METHODS

Male albino rats of the Wistar strain (200-300 g) were obtained from Hilltop Breeders, Scottdale, PA and were maintained on a standard Purina rat chow diet until needed. Escherichia coli B tRNA was purchased from Schwarz Mann, Orangeburg, NY and Sephadex G-200 was a product of Pharmacia Inc., Piscataway, NJ. [^{35}S]-L-cystine (13.2 Ci/mmole), [$^{14}CH_3$]S-adenosyl-L-methionine (40 mc/mole) and a mixture of 15 [^{14}C]-L-amino acids (u) were purchased from New England Nuclear Corp., Boston, MA. All other reagents were of the highest quality commercially available.

PREPARATION OF CELL SUPERNATANTS

The livers were excised and chilled immediately in 50 mM Tris-HCl buffer, pH 7.6, also containing 50 mM $MgCl_2$, 25 mM KCl, 1.0 mM 2-mercaptoethanol and 0.25 M sucrose (buffer A). The tissue was minced and then homogenized by 10 up-and-down strokes in a loose fitting, followed by 3 up-and-down strokes in a tight-fitting Dounce homogenizer. The homogenate was centrifuged at 105,000 x g for 90 minutes using a Beckman L3-50 centrifuge. The lipid layer was removed by aspiration and the entire supernatant was centrifuged an additional 18 hrs at 160,000 x g (49,000 rpm in a Beckman 50 Ti rotor). The resulting supernatant was carefully discarded and the loose pellet was suspended in buffer A without sucrose. A glass-teflon homogenizer was used for this step, after which insoluble material was removed by centrifugation at 2,000 x g for 10 min. Protein was determined by the method of Lowry et. al. (8) using bovine serum albumin (fraction V) as standard.

ENZYMATIC ASSAYS

Sulfurtransferase activity was measured by the method of Hayward and Weiss (9), as modified and previously reported (10). Aminoacyl-tRNA synthetase activity was determined by the method of Harris et. al. (11), and methylation of tRNA was measured using the procedure of Turner and Handcock (12). The components of each assay mixture are given in the legend to Figure 1.

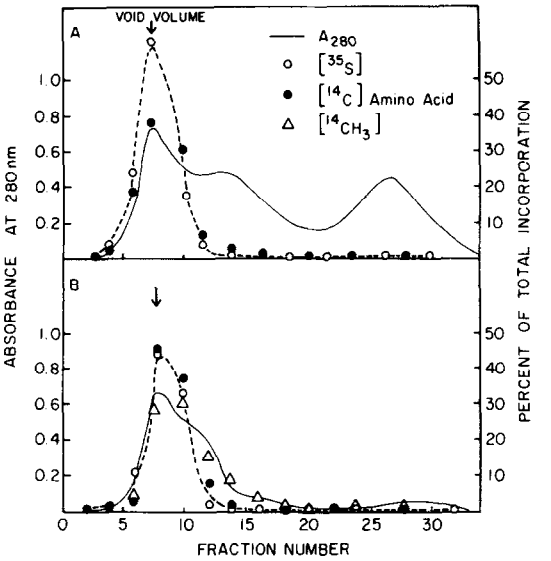

Figure 1

Sephadex G-200 chromatography of subcellular fractions prepared from rat liver. Five ml of the 105,000 x g supernatant (Panel A) and 2 ml of the 160,000 x g pellet suspension was chromatographed on a 2.5 x 20 cm column of Sephadex G-200, previously equilibrated at 4° with buffer A without sucrose. Elution was carried out at a flow rate of 0.5 ml per min with the same buffer. The eluate was continuously monitored at 280 nm using an ISCO UA-5 ultraviolet analyzer. Each fraction was assayed at 37° as described above, and the enzyme activities are plotted as percentages of the total measured activity. The data are corrected for incorporation in the absence of added protein.

Sulfurtransferase assays contained per 0.5 ml: 25 μmoles, Tris-HCl, pH 8.0; 2.5 μmoles $MgCl_2$; 2.0 μmoles ATP at pH 7.0; 2.0 μmoles 2-mercaptoethanol; 0.4 mg E. coli B tRNA; 1 μCi [^{35}S]cysteine(13.2 Ci/mmole) and 0.1 ml of each fraction.

Aminoacyl-tRNA synthetase assays contained the following components in a final volume of 0.15 ml: Tris-HCl, pH 7.5, 10 μmoles; magnesium acetate, 2.5 μmoles; ATP, adjusted to pH 7.0, 0.2 μmole; KCl, 1.0 μmole; 2-mercaptoethanol, 0.5 μmole; [^{14}C]amino acid mixture (15 amino acids), 0.5 μCi; E. coli B tRNA, 0.05 mg; and 0.1 ml of each column fraction.

Methylase assay mixtures contained the following components in a total volume of 0.15 ml: Tris-HCl, pH 8.0, 5 μmoles; $MgCl_2$, 0.5 μmole; 2-mercaptoethanol, 0.5 μmole; E. coli B tRNA, 0.05 mg; [$^{14}CH_3$]^{2}S-adenosyl-L-methionine, 0.1 μCi; and 0.1 ml of each enzyme fraction.

The amount of radioactivity incorporated into tRNA was measured using a Beckman Model 9000 scintillation spectrometer.

RESULTS AND DISCUSSION

It was previously reported that tRNA sulfurtransferase activity is present in 105,000 x g supernatants from rat liver and hepatomas (11,13) as well

as in partially-purified preparations from rat brain (14). We noted considerable variability in enzyme activity in rat liver supernatants from one preparation to the next. This may be related to the current finding that most, if not all, sulfurtransferase activity can be sedimented at 160,000 x g after 18 hr of centrifugation. Hence, assay of the upper two-thirds of a rat liver 105,000 x g supernatant results in a substantial loss of total activity, since considerable activity was found in a fraction immediately above the microsomal pellet. Figure 1A shows that all of the sulfurtransferase activity in a 105,000 x g supernatant elutes with the void volume on a Sephadex G-200 column. The data also show that this activity co-elutes with a mixture of aminoacyl-tRNA synthetases. Since most of these latter enzymes have molecular weights of 50-150,000 daltons it has been concluded that they exist in a supramolecular complex (1-6). It now appears from these data that tRNA sulfurtransferase may be a part of this complex. The complex can be sedimented from 105,000 x g supernatants by further centrifugation at 160,000 x g for 18 hrs. If the resulting pellet is suspended and chromatographed on Sephadex G-200 all sulfurtransferase activity is associated with the void volume, as demonstrated in Figure 1B. Both aminoacyl-tRNA synthetase and tRNA methyltransferase activity are associated with the excluded complex. No measurable enzymatic activities are detected in any other fractions. These findings indicate that little, if any, breakdown of the complex occurs on centrifugation or resuspension of the resultant 160,000 x g pellet. Rechromatography of fraction 8 on Sephadex G-200 further demonstrated the relative stability of the complex as all protein eluted with the void volume in a single sharp peak (data not shown). However, storage of the complex at 0-4° for one week resulted in a loss of 30-60% of the aminoacyl-tRNA synthetase and sulfurtransferase activities, even in the presence of 50% glycerol. Since it was previously noted that freezing and thawing disrupted the synthetase complex (2) we have not attempted storage under these conditions.

The data of Table I demonstrate that nearly 70% of the sulfurtransferase

Table I

tRNA Sulfurtransferase Activity in Various Fractions from Rat Liver

Enzyme	Specific Activity[a] (Units/mg)	Total Protein (mg)	Total Units	% Yield
105,000 x g[b] Supernatant	16.9	356	12,042	100
160,000 x g Supernatant	5.2	154	1,586	13.1
160,000 x g[c] Pellet	37.2	113	8,399	69.7

[a]Specific activity is expressed as units per mg protein. One unit of enzymatic activity equals that amount of enzyme which catalyzes the incorporation of 1 pmole of sulfur into tRNA after 10 min of incubation. The assay conditions are those given in the legend to Figure 1, except that each assay contained 5 nmoles [^{35}S] L-cysteine (200 mCi/mmole) and 0.1 mg of enzyme protein.

[b]The 105,000 x g supernatant was allowed to stand at 0° for 18 hrs before assay.

[c]The 160,000 x g pellet was washed twice using 1.5 ml of Buffer A (see Methods), and suspended in 2 ml of the same buffer using a glass-teflon homogenizer.

activity sediments in the pellet fraction with about 30% of the protein, thus accounting for the observed increase in specific activity. Other experiments show that the remainder of the protein and sulfurtransferase activity is found in the pellet washes. No inhibition was seen when the various enzyme fractions were mixed in equal proportions, indicating that the increase in specific activity for the pellet fraction is not the result of the removal of some inhibitory substance. We observed only a slight loss of enzymatic activity for the 105,000 x g fraction on overnight storage at 0°, while freezing caused a 50% loss of sulfurtransferase activity. As with the complex isolated by Sephadex G-200 chromatography, after one week at 0-4° a 50-60% loss of activity was observed for all pellet enzyme activities.

The data reported here give further support to the concept that mammalian protein synthesis and RNA processing are carried out by large complexes present in the cytoplasm. With regard to tRNA processing, one can envision the acceptance of a precursor tRNA by the enzyme complex, possibly at the nuclear membrane. The latter steps of tRNA maturation (thiolation, methylation, etc.) may be carried out and the mature tRNA acylated and delivered to the ribosome for protein synthesis. At no time is the newly synthesized tRNA molecule free in the cytoplasm. Regulation of protein synthesis through the action of this synthetase-modification enzyme complex seems a possibility and is being investigated in this laboratory.

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