

Isolation and Partial Characterization of Transfer RNAs from *Astragalus bisulcatus*¹

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ABSTRACT

A procedure has been developed for the isolation of transfer RNA from the selenium accumulator plant *Astragalus bisulcatus*. This material appears free of interfering phenolic compounds, has a high guanosine to cytidine ratio, shows a major and modified nucleoside composition characteristic of plant transfer RNAs, and exhibits chromatographic and electrophoretic properties similar to transfer RNAs from other well studied bacterial and plant systems. RNAs isolated from *A. bisulcatus* seedlings incubated in the presence of ⁷⁵Se indicate some incorporation of radioactivity into the transfer RNAs, but at extremely low levels. The transfer RNAs were active in accepting amino acids, although their over-all levels of activity appeared low when compared with those from a homologous *Escherichia coli* aminoacylation reaction system.

Selenium accumulator plants, such as *Astragalus bisulcatus*, grown on seleniferous soils, absorb massive amounts of inorganic selenium, much of which is converted to water-soluble, low mol wt organic compounds (selenocystathionine, Se-methylselenocysteine, dimethyl selenide, selenohomocystine). Trace quantities of selenium appear to be required for proper growth and development of the plants (19, 22). Unlike nonaccumulator plants, where considerable amounts of selenium can be incorporated into protein in the forms of selenomethionine and selenocysteine (13), only limited amounts of selenium-containing amino acids appear to be incorporated into proteins of accumulator plants. The control mechanisms that exist in the accumulator species to limit the extent of substitution of seleno- for sulfur-containing amino acids are unknown (22). In order to examine the aminoacylation reaction as a possible control point, it is necessary to have relatively pure and biologically active tRNA. We have developed a procedure for the isolation of tRNAs from *A. bisulcatus*. These were shown to be active in accepting normal amino acids and were characterized by chemical, chromatographic, electrophoretic, and spectral methods. Several different types of nucleoside analyses were carried out to determine major and modified base compositions, and to examine the tRNAs for the possible presence of selenium-containing nucleosides—which have been isolated from *Escherichia coli* tRNAs (6, 18).

MATERIALS AND METHODS

Materials. Diethylpyrocarbonate, Trizma Base, adenosine, guanosine, and uridine were from Sigma. Sephadex G-75 and G-100

were purchased from Pharmacia Fine Chemicals and GF/C glass fiber filters from Whatman. Electrophoresis grade acrylamide and Aminex A-5 resin were from Bio-Rad. Cytidine was from Mann Research Laboratories. Snake venom phosphodiesterase (code VPH) and bacterial alkaline phosphatase (code BAPC) were from Worthington Biochemical Corp. and pancreatic RNase (type 2A) from Sigma. The antibiotic-antimycotic solution was purchased from Grand Island Biological Co., and [⁷⁵Se]selenite (97 mCi/mg) and [⁷⁵Se]selenate (40.6 mCi/mg) came from New England Nuclear. Technical data provided by New England Nuclear indicated that the selenite had a radionuclide purity of >99.9% and <1% selenate as a contaminant as determined by chromatography. The selenate had a reported radionuclide and radiochemical purity of 99.0%. TLC of both selenium forms followed by autoradiography indicated a single major radioactive component. A trace contaminant migrating as selenate appeared in the selenite sample and some contamination of the selenate sample with a selenite-like migrating component was observed. ¹⁴C-Reconstituted protein hydrolysate was from Schwarz/Mann. [³H]NaBH₄ was purchased from Amersham/Searle, code TRK.45; specific radioactivity = 2.5 Ci/mmol. The Clipper Reaper Grain Seed and Bean Cleaner was from Farrel-Ross Co., Saginaw, Mich., and the Bicon LSC Gamma Vials were purchased from Bicon Corp., Newbury, Ohio.

Phenol (J. T. Baker) and *m*-cresol (Eastman) were distilled under reduced pressure and stored at 4 C in the dark. All solutions were prepared with degassed, deionized H₂O and all other chemicals and reagents were either analytical or reagent grade.

Plant Material. Mature seed pods were harvested from *A. bisulcatus* plants growing wild near Laramie and Medicine Bow, Wyoming, in areas of high soil selenium. After air-drying, they were processed through a Wiley Mill to break the pods, and Clipper Reaper Grain Seed and Bean Cleaner to separate seeds from chaff. The seeds were stored in plastic jars at -20 C. Samples of dry seeds were homogenized in water and aliquots analyzed for selenium content (12), which ranged from 25 to 146 μg selenium per g of dry seed. Before germination, the seeds were soaked in concentrated H₂SO₄ (19) for 15 min, rinsed with ice-water, treated with 10% Clorox for 5 min, then scattered on nylon mesh trays in a dark, temperature-controlled mist chamber at 21 C for 6 to 7 days. Germination was 65 to 80%. Seeds not scarred with acid showed <10% germination. Sprouts not processed immediately were stored at -20 C.

RNA Extraction. Plant tissue (10 g) was homogenized on ice with a Tekmar homogenizer (maximum speed for 15 s) using a buffered water-phenol-chloroform mixture (20 ml 60 mM Naphosphate [pH 6.0], 2.5 ml 10% SDS, 1.5 ml diethylpyrocarbonate, 0.1 ml 2-mercaptoethanol, 25 ml phenol-chloroform [1:1, v/v]). Diethylpyrocarbonate was added to the mixture immediately before use. The slurry was shaken at room temperature for 15 to 30 min, chilled in ice, and centrifuged at 7,800g for 10 min to separate the aqueous and phenolic layers. The aqueous layer was aspirated off, NaCl added to 0.2 M final concentration, and precipitated with 2 volumes of 95% ethanol at -20 C. The precipitate was collected by centrifugation (23,000g for 15 min) and the pellet

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resuspended in 2 ml 0.05 M NaCl. Sodium benzoate and cold *m*-cresol were each added to a final concentration of 20% to precipitate high mol wt rRNA, DNA, and polysaccharides (10). After stirring for 15 min at room temperature, the solution was centrifuged at 7,800g for 10 min and the pellet discarded. The supernatant was precipitated with 2 volumes of ethanol, collected by centrifugation, resuspended in 1.8 M Tris-HCl (pH 8.0), and incubated at 37 C for 90 min to deacylate the tRNAs. Under these deacylation conditions, the half-life (time required for hydrolysis of 50% of the aminoacyl ester bond) of cysteyl-, selenocysteyl-, and selenomethionyl-tRNA was 3 to 3.5 min. Methionyl-tRNA showed a $t_{1/2}$ of 5.5 min under the same conditions (ref. 23; and P. A. Young and I. I. Kaiser, unpublished data). Following chromatography on either Sephadex G-75 or G-100 columns, the tRNA peak was pooled, precipitated with ethanol, resuspended in water, and stored at -20 C.

Aminoacylation of tRNA. The reaction assay system was essentially that described earlier (8). Labeled amino acids were present in about a 10-fold excess over tRNA. Transfer RNA concentration was 13 μ g (0.3 A_{260} units) per ml of reaction mix. Aminoacyl tRNA synthetases from *A. bisulcatus* or wheat germ were used at concentrations of 1 to 2 mg protein/ml of reaction mix, while *E. coli* synthetases were at a concentration of 200 μ g protein/ml. Incubations were in a constant temperature water bath at 37 C for *E. coli* synthetases and 29 C for plant synthetases for 15 to 20 min when assaying for extent of aminoacylation. Following incubation, 100- μ l aliquots were withdrawn, pipetted onto 2.2-cm Whatman GF/C glass fibre filter discs, and washed as described (20). Dried discs were suspended in toluene scintillation fluid and counted in a Beckman LS-100 counter. Reactions were run in duplicate or triplicate and blanks containing no tRNA were processed with the samples and appropriate corrections made.

Gel Electrophoresis. Formamide was deionized by adding 2 g Bio-Rad AG11AB mixed bed ion exchange resin per 50 ml formamide. The mixture was stirred for 2 h, filtered, and the formamide stored at -20 C in the dark. Formamide-acrylamide gels (3, 14) were prepared by dissolving 15 g acrylamide and 0.4 g *N,N'*-methylene-bis-acrylamide in 99 ml of 70% formamide containing 36 mM Tris-HCl (pH 7.5), 30 mM NaH_2PO_4 , and 1 mM EDTA (11). One ml 15% $(\text{NH}_4)_2\text{S}_2\text{O}_8$ and 60 μ l tetramethylethylenediamine were added after 1-min deaeration, just before pouring into slabs (10 \times 14 cm). tRNA samples were dissolved in the electrophoresis buffer containing 25% sucrose and 0.05% bromophenol blue. About 50- μ g samples of tRNAs were applied per slot in a volume of less than 25 μ l. Following electrophoresis, the gels were fixed in 1 M acetic acid for 1 h and stained in 0.2% methylene blue containing 0.4 M sodium acetate (pH 4.8).

^{75}Se Uptake by *E. coli* B. *E. coli* were cultured with shaking at 37 C in either sulfur-deficient (<5 μ M sulfate) or complete media (1 mM sulfate) (4). Flasks containing 500 ml media were inoculated with 25 ml of an overnight growth culture (complete media) and incubated for 60 min at 37 C. After the addition of 300 μ Ci [^{75}Se]selenite (97 mCi/mg), the cultures were incubated for 3 more h with shaking. Cells were harvested by centrifugation and washed with 10 mM Tris-HCl (pH 7.4), 10 mM MgCl_2 , 60 mM NH_4Cl , and the pellets stored at -70 C. tRNAs were extracted by the phenol procedure using Tris-HCl (pH 7.5) and 100 mM NaCl with vigorous shaking at room temperature for 1 h. The suspension was centrifuged (7,800g) after chilling on ice, and the aqueous phase aspirated off to be reextracted with 1/2 volume phenol for 20 min. RNA was precipitated from the resulting aqueous phase with 2 volumes 95% ethanol at -20 C. The resulting precipitates were resuspended in 0.5 ml H_2O and applied to a Sephadex G-75 Superfine column (1 \times 90 cm) and developed with a buffer of 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 150 mM KCl. Fractions of 2 ml were collected and aliquots from each were taken and counted for ^{75}Se activity by means of Bicon LSC vials in a Beckman LSC-100 liquid scintillation counter.

^{75}Se Uptake in *A. bisulcatus*. *A. bisulcatus* seeds germinated for 6 to 8 days in a dark mist chamber were rinsed in dilute Clorox solution several seconds and then in sterile deionized H_2O containing an antibiotic-antimycotic solution at a final concentration of 100 units/ml penicillin, 100 μ g/ml streptomycin, and 0.25 μ g/ml Fungizone. Sprouts were placed in wide mouth jars with 60 ml sterile media (1- to 1.5-cm depth) consisting of either 20 μ Ci [^{75}Se]selenite (97 mCi/mg) or 50 μ Ci [^{75}Se]selenate (40.6 mCi/mg), and the above antibiotic solution at 10 times the concentration. Half of the cultures also contained 2 mM Na_2SO_4 to examine the effect of the presence of sulfur on selenium incorporation. Culture chambers were incubated in the dark at room temperature for 40 to 42 h, with mild shaking to circulate the media over the seedlings. Sprouts were then rinsed with distilled H_2O and tRNA extracted.

Nucleoside Analyses. Major nucleoside analyses of tRNAs were carried out by high pressure ion exclusion liquid chromatography on Aminex A-5 columns, following KOH hydrolysis and alkaline phosphatase treatment (9). Standard solutions were prepared (9) and calibrated using the molar extinction coefficients given by Beaven *et al.* (2).

Total nucleoside analyses of the tRNAs were accomplished using the chemical tritium-labeling method of Randerath *et al.* (15). Samples containing 6 to 15 μ g of RNA were hydrolyzed to nucleosides by a mixture of venom P-diesterase, pancreatic RNase, and bacterial alkaline phosphatase. Aliquots of the hydrolysates containing 2.5 to 4.0 nmol of nucleosides were oxidized with NaIO_4 , then reduced with [^3H]NaBH $_4$. The resulting mixture of [2',3'- ^3H]trihydroxy derivatives of the nucleosides was resolved by TLC on cellulose as described by Randerath *et al.* (17). Detection of radioactivity was accomplished by fluorography (16) for 55 to 65 h at -75 C. Radioactive spots were excised, eluted with H_2O , and aliquots counted in a Beckman liquid scintillation spectrometer at an efficiency of 32%. The identity of all radioactive spots

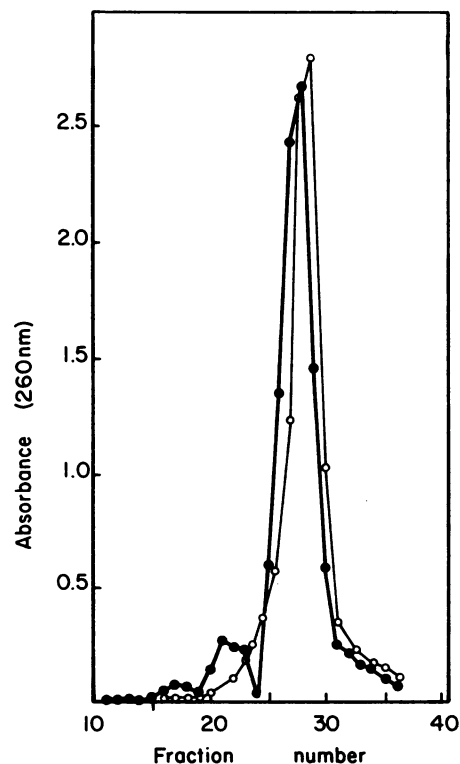


FIG. 1. Composite elution profiles of *A. bisulcatus* (●—●) and *E. coli* (○—○) tRNAs from a column (0.9 \times 150 cm) of Sephadex G-100. The column was equilibrated and run at room temperature with a buffer containing 20 mM sodium acetate (pH 5.2), 0.2 M NaCl. Fraction size was 2.3 ml.

was verified by parallel analyses of commercial nucleoside preparations except for 3-(3-amino-3-carboxypropyl)-uridine which is tentatively identified by its position relative to the map of Randerath *et al.* (17).

RESULTS AND DISCUSSION

tRNA Characterization. tRNAs isolated from sprouts of *A. bisulcatus* were shown to have chromatographic properties on Sephadex G-100 similar to *E. coli* tRNAs (Fig. 1). Aminoacylation of the plant tRNAs with a mixture of ^{14}C -labeled amino acids did not appreciably change the elution profile, and gave a single radioactive peak that eluted coincident with the UV absorbing material (data not shown). Sephadex chromatography at neutral pH in the presence of 7 M urea did expose a trailing peak of lower mol wt fragments amounting to about 20% of the total UV absorbing material (Fig. 2). This indicates that even though the extractions were carried out in the presence of diethylpyrocarbonate and the initial tissue homogenization was performed in buffer containing phenol, some nuclease activity remained. Bentonite appeared to be *less* effective than diethylpyrocarbonate in preventing nuclease activity, since preparations in its presence resulted in both a lower yield of tRNA and tRNA with a decreased amino acid acceptance activity (data not shown).

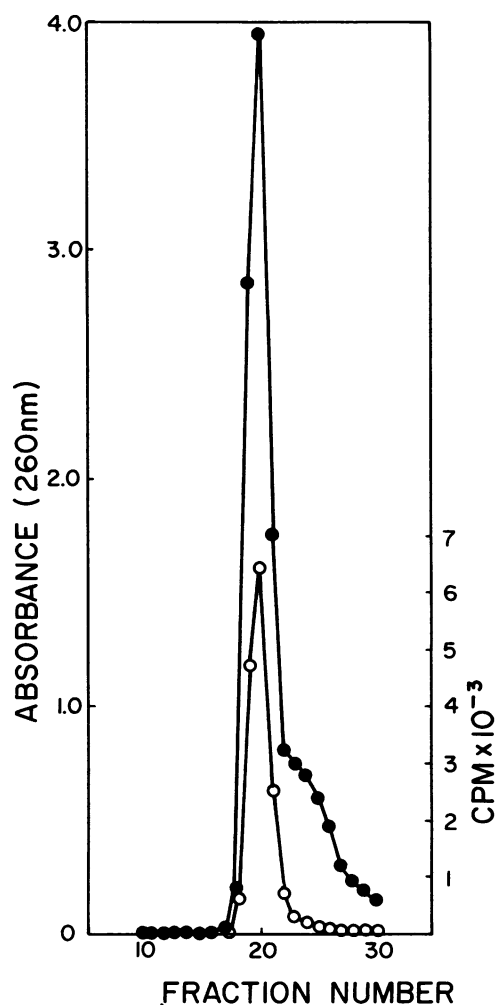


FIG. 2. Elution of a mixture of *A. bisulcatus* (●—●) and ^{32}P -labeled *E. coli* tRNAs (○—○) from a column (1 × 90 cm) of Sephadex G-75 in the presence of 7 M urea. The buffer consisted of 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 7 M urea. Fraction size was 2.3 ml. The plant tRNA was present in about a 200-fold excess.

Polyacrylamide gel electrophoresis of *A. bisulcatus* in 15% gels in the absence and presence of 70% formamide also gave patterns very similar to those found with *E. coli* and wheat germ tRNAs (Fig. 3). There is some indication of faster migrating material in the formamide-containing gels for the *A. bisulcatus* samples, again indicating that nicks may be present in the polynucleotide chains. In the absence of formamide, all tRNA samples gave one broad, diffusely stained band characteristic of a mixture of tRNAs. The reason for the slight increase in the mobility of wheat germ tRNAs in the absence of formamide is unclear. Addition of formamide to the gels results in the definition of distinct RNA staining bands. Samples from different sources revealed slightly different profiles, but all with similar mobilities.

RNA isolated from *A. bisulcatus* and passed over Sephadex (Fig. 1) gave an *A* profile characteristic of RNA, with an *A* minimum and maximum at 232 nm and 258 nm, respectively (Fig. 4). There does not appear to be a significant amount of residual contaminating phenolic compounds present in the RNA sample following Sephadex chromatography. *A. bisulcatus* tRNAs show no *A* peaks beyond 300 nm, indicating a lack of appreciable amounts of both 4-thiouridine (λ_{max} 336 nm) and 4-selenouridine (λ_{max} 360 nm). *E. coli* tRNAs show a characteristic minor *A* peak at 336 nm due to 4-thiouridine content (Fig. 4).

Nucleoside Analyses. The major nucleoside composition of *A. bisulcatus* tRNAs was determined and compared with those found for *E. coli*, wheat germ, and yeast (Table I). The *A. bisulcatus*

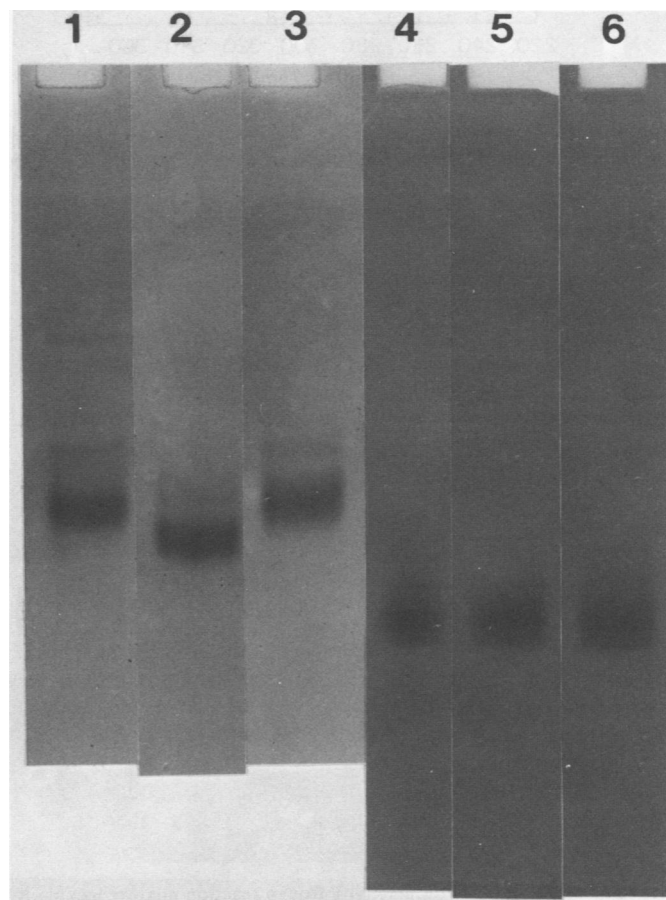


FIG. 3. Electrophoresis of tRNA samples on 15% polyacrylamide gels in the absence (samples 1-3) and presence (samples 4-6) of 70% formamide. Samples were applied to slab gels (10 × 14 cm) either 1.5 mm (samples 1-3) or 3.0 mm (samples 4-6) thick. All runs were at room temperature (tap water cooling) for 11 h at 50 mamp and 17 h at 40 mamp for the thin and thick gels, respectively. Samples: 1 and 4: *A. bisulcatus*; 2 and 5: wheat germ; 3 and 6: *E. coli* B.

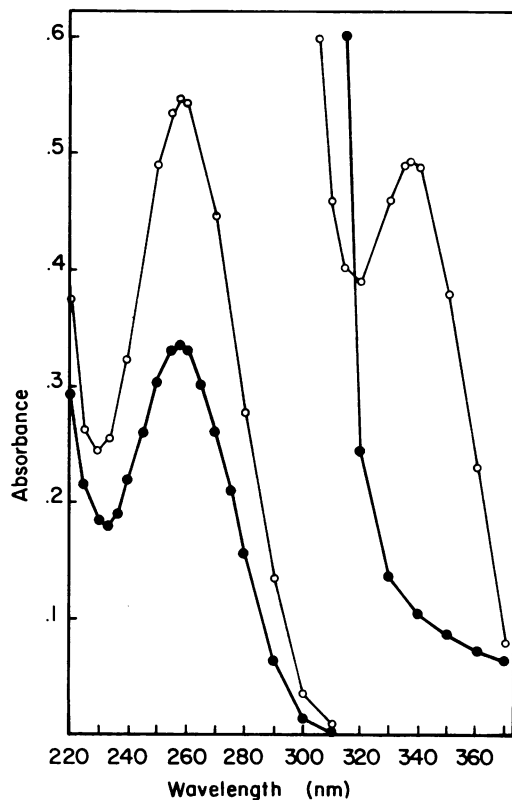


FIG. 4. UV absorbance spectra of *A. bisulcatus* (●—●) and *E. coli* tRNAs (○—○). Values above 310 nm were obtained on samples with A_{260} values of about 27 units. Silica cells of 1-cm path length were used.

tRNA samples showed high guanosine-cytidine values, characteristic of tRNAs from other systems (Table I). No anomalous minor components were observed in the *A. bisulcatus* samples.

Total nucleoside composition analyses of the *A. bisulcatus* tRNAs were accomplished by the tritium-labeling method of Randerath *et al.* (15). Figure 5 shows a representative fluorogram from a two-dimensional cellulose thin layer chromatogram of the tritium-labeled nucleoside trialcohol mixture obtained from *A. bisulcatus*. Nucleoside compositions of tRNAs from *Saccharomyces cerevisiae* and wheat germ showed values in the same range (Table II). We found no additional minor components present in the *A. bisulcatus* tRNAs, which further indicated that this selenium-accumulator plant did not contain detectable amounts of selenium-containing nucleosides. It should be noted that selenium-containing nucleosides might be unstable toward the Randerath tritium-labeling procedure and be converted to one of the observed nucleosides. (The sulfur-containing nucleoside, 4-thiouridine, is converted to uridine during this procedure; ref. 15.) The sum of modified constituents in *A. bisulcatus* tRNAs, 11.46 to 11.49 mol %, is comparable with that of tRNAs from *S. cerevisiae* and wheat germ, 11.91 to 12.89 and 12.44 to 13.06 mol %, respectively (Table II). Since these sums are similar, they also support the argument against the *A. bisulcatus* tRNA preparations containing significant amounts of other degraded nucleic acids.

Table I. Major nucleoside composition of tRNA preparations from various sources.

Sample	mol %			
	Ado	Cyt	Guo	Urd
<i>A. bisulcatus</i> 1	20.7	28.2	31.2	19.9
<i>A. bisulcatus</i> 2	20.5	27.5	31.4	20.6
<i>E. coli</i> B	19.6	30.6	33.1	16.7
<i>S. cerevisiae</i>	20.2	28.5	31.6	19.7
wheat germ	20.1	28.5	32.3	19.1

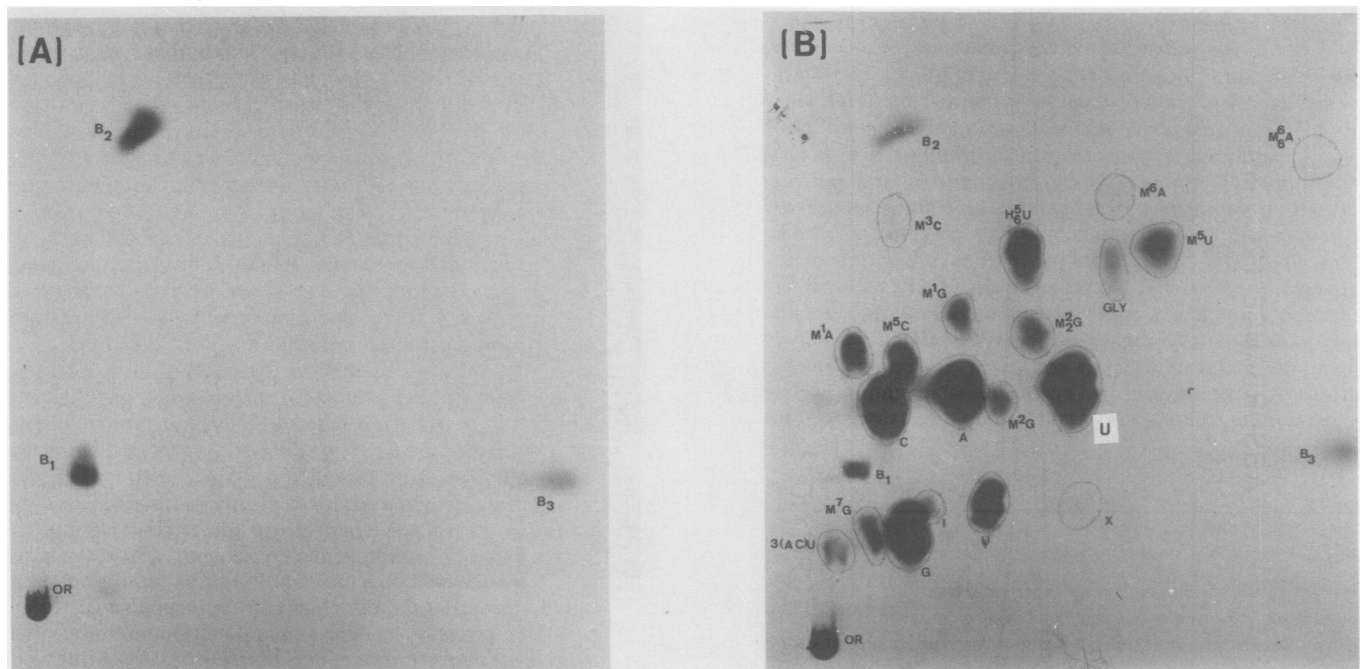


FIG. 5. A: map of ^3H radioactivity from a reaction mixture lacking RNA. B: map of ^3H radioactivity obtained from labeling 4.2 nmol of nucleosides from tRNA of *A. bisulcatus*. Fluorographic exposure of the x-ray film was for approximately 58 h at -75°C . OR (origin), B₁, B₂, and B₃ containing unidentified material present in reaction mixtures lacking RNA. The following represent [$2',3'\text{-}^3\text{H}$]trialcohol derivatives of the nucleoside; *3(AC)U: 3-(3-amino-3-carboxypropyl)uridine; **M⁷G: 7-methylguanosine; G: guanosine; I: inosine; ψ : pseudouridine; **M¹A: 1-methyladenosine; C: cytosine; M⁵C: 5-methylcytosine; A: adenosine; U: uridine; M¹G: 1-methylguanosine; M²G: 2-methylguanosine; M₂G: 2,2-dimethylguanosine; **M³C: 3-methylcytosine; H₆U: 5,6-dihydrouridine; M⁵U: 5-methyluridine; M⁶A: 6-methyladenosine; M₆A: 6,6-dimethyladenosine; x: unidentified; GLY: glycerol derived from the breakdown of alkali-labile nucleosides.

* Identified only by comparison with the map of Randerath *et al.* (17).

** Not corrected for partial loss due to alkaline degradation (7, 15).

⁷⁵Se Uptake by *A. bisulcatus*. Because we had no evidence for the presence of selenium-containing nucleosides in *A. bisulcatus* tRNAs from either their spectra, HPLC, TLC, or the tritium-labeling analysis, we attempted to label plant seedlings by incubating them in the presence of either [⁷⁵Se]selenate or [⁷⁵Se]selenite. This procedure should be more sensitive in the detection of selenium-containing nucleosides. When RNAs were isolated from these labeled seedlings incubated with [⁷⁵Se]selenite and fractionated on Sephadex G-75, the majority of the ⁷⁵Se activity coeluted with the tRNA peak (Fig. 6). Label eluting prior to the tRNA peak in Figure 6 was quite disperse and had a low specific radioactivity. It probably represents nonspecific interaction of the ⁷⁵Se with higher mol wt components. RNAs isolated from tissues incubated with [⁷⁵Se]selenate gave profiles similar to those shown in Figure 6, although the amounts of radioactivity eluting with the initial void volume peak were reduced to background levels. Addition of exogenous sulfate (final concentration 2 mM) to the culture media did not interfere with selenium incorporation into the tRNA peak. Addition of sulfur appears to increase ⁷⁵Se uptake slightly (Table III). In this system, selenate appears to be taken up

and/or used more readily by the seedlings than selenite. A preference for selenate over selenite has been noted before in uptake studies with various plant systems (1 and refs. therein). As a control, *E. coli* B were grown in the presence of [⁷⁵Se]selenite and the RNAs isolated and examined. The radioactivity was again found to be associated largely with the tRNA peak (Fig. 7). Earlier workers noted ⁷⁵Se incorporation into the tRNA fraction (18, 21). The $A_{336}/A_{260} \times 100$ - ratio, which is a measure of 4-thiouridine content in *E. coli* tRNA, varied only slightly: from 1.78 in a sample of normal tRNA to 1.75 and 1.65 in selenite and selenite plus sulfate tRNA preparations, respectively. These results indicate that significant replacement of 4-thiouridine by 4-selenouridine (6) does not occur under our conditions, which is consistent with the low levels of ⁷⁵Se incorporated. Addition of excess sulfate (final concentration 1 mM) to the growth media did not affect ⁷⁵Se uptake into the tRNAs to any appreciable extent (Fig. 7 and Table III). A high ratio of sulfur to selenium in the culture media has been used by others to suppress nonspecific substitution of selenium for sulfur in cellular constituents (5). This supports an earlier suggestion by Saelinger *et al.* (21) that selenium could be a normal trace constituent of tRNA. Additional work will be required to determine whether or not there is a similar distribution of ⁷⁵Se in tRNAs isolated from cells grown in the absence and presence of excess sulfur. The low levels of ⁷⁵Se incorporation into *A. bisulcatus* tRNA could be misleading, since the incubated seedlings may have considerable endogenous selenium that is capable of diluting the specific radioactivity of the ⁷⁵Se-salt used.

Mild enzymic hydrolysis of ⁷⁵Se-containing tRNAs to the nucleoside level followed by cellulose TLC resulted in complete separation of the four major nucleosides (data not shown; see ref.

Table II. Comparison of the nucleoside compositions of tRNAs from *A. bisulcatus*, *S. cerevisiae*, and wheat germ¹.

Nucleoside	mol %		
	<i>A. bisulcatus</i>	<i>S. cerevisiae</i>	Wheat Germ
G	27.29-27.56	23.55-27.25	27.97-27.54
A	19.25-19.21	20.11-18.99	17.96-18.31
C	26.42-25.87	28.32-26.05	26.82-26.14
U	15.56-15.92	16.02-14.83	14.79-14.97
M ¹ G	0.65- 0.59	0.79- 0.87	0.84- 0.88
M ² G	0.44- 0.97	0.71- 0.67	0.66- 0.61
M ³ G	0.62- 0.62	0.66- 0.66	0.82- 0.78
M ⁷ G	0.58- 0.56	0.35- 0.37	0.56- 0.57
M ¹ A	0.74- 0.75	0.55- 0.53	0.56- 0.88
M ⁶ A	0.10- 0.11	0.09- 0.07	0.11- 0.12
M ⁶ A	0.00- 0.00	0.02- 0.01	0.01- 0.03
M ³ C	0.05- 0.06	0.05- 0.05	0.06- 0.07
M ⁵ C	1.38- 1.35	1.19- 1.50	1.74- 2.04
3(A ₂ C)U	0.37- 0.38	N.P.- N.P.	0.71- 0.74
M ⁵ U	1.00- 0.99	1.24- 1.33	1.04- 0.97
H ⁶ U	2.27- 1.78	1.77- 1.86	1.57- 1.39
ψ	2.98- 2.98	4.10- 4.21	3.32- 3.44
I	0.19- 0.13	0.34- 0.40	0.20- 0.28
X	0.12- 0.19	0.15- 0.36	0.24- 0.26

¹Procedure is as described in Materials and Methods. Duplicate determinations are reported for each sample. See legend to Figure 5 for explanation of abbreviations. NP = not present in detectable amounts.

Table III. Incorporation of ⁷⁵Se into tRNAs of *A. bisulcatus* and *E. coli* B¹.

media	No. of tRNAs present per one ⁷⁵ Se atom
<i>A. bisulcatus</i>	
[⁷⁵ Se] selenite	8330
[⁷⁵ Se] selenite + sulfate	5560
[⁷⁵ Se] selenate	1300
[⁷⁵ Se] selenate + sulfate	1220
<i>E. coli</i> B	
[⁷⁵ Se] selenite	241
[⁷⁵ Se] selenite + sulfate	273

¹The specific activity calculation for the tRNAs is based on the original specific activity of ⁷⁵Se-containing acid as furnished by the supplier and corrected for loss due to decay. ⁷⁵Se counting efficiency in Bicon KI vials was assumed to be 100%. One absorbance unit of tRNA at 260 nm in Tris-Mg⁺⁺ buffer (1-cm path length cell) was assumed to correspond to 1609 pmoles.

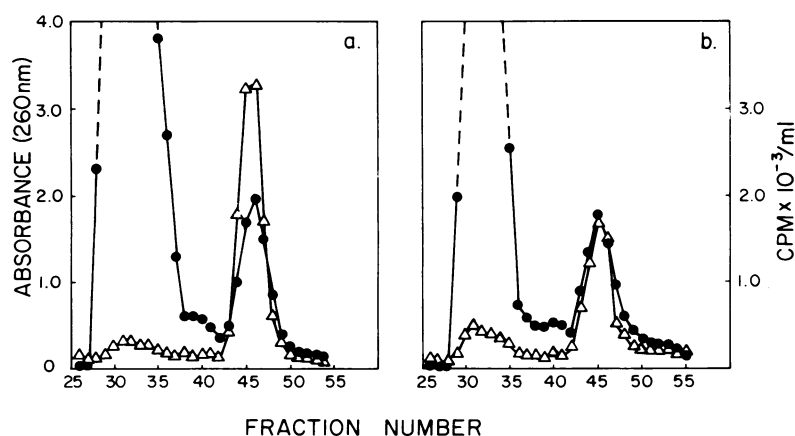


FIG. 6. Sephadex G-75 elution profile of ⁷⁵Se-labeled RNA from *A. bisulcatus* seedlings cultured in the presence of [⁷⁵Se]selenite either with (a) or without (b) sulfate added to the media. The column (1.5 × 90 cm) was equilibrated and eluted with 10 mM Tris-HCl (pH 7.4), 0.15 M KCl, and 1 mM EDTA. Fractions of 2.5 ml were collected. Absorbance (●—●); (cpm) (Δ—Δ).

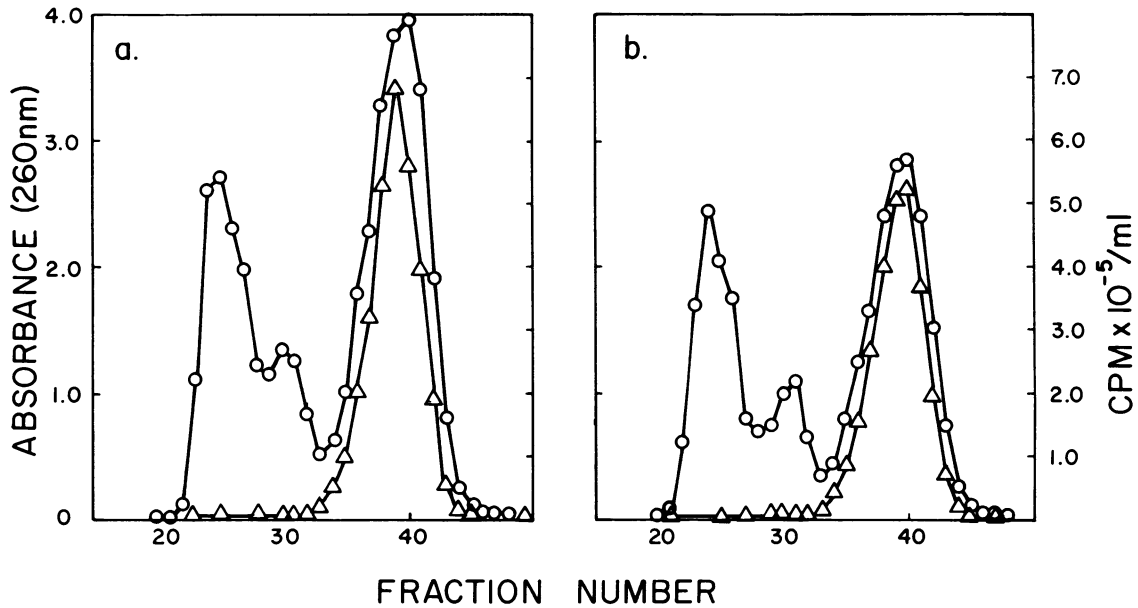


FIG. 7. Chromatography on Sephadex G-75 of ⁷⁵Se-labeled RNA isolated from *E. coli* grown in the presence of [⁷⁵Se]selenite with (a) or without (b) exogenous sulfate added. The column (1 × 90 cm) was eluted and fractions collected as described for Figure 5. Absorbance (○—○); cpm (△—△).

6). In this system, the order of separation is: adenosine, guanosine, uridine, and cytidine with cytidine migrating the fastest. Virtually all of the ⁷⁵Se label present in *A. bisulcatus* tRNA digests migrated as a single spot just ahead of the cytidine peak where selenite and selenate run. Nucleoside digests from ⁷⁵Se-labeled *E. coli* tRNAs show five distinct migrating radioactive spots in the same system. Two labeled spots migrated in the cytidine region, but neither ahead of cytidine as did the labeled component from *A. bisulcatus* tRNA. The major labeled component from *E. coli* migrated as 4-selenouridine (6). Whether the labeled component found in the nucleoside digest from *A. bisulcatus* tRNAs represents an authentic selenium-containing nucleoside is unclear from these experiments. It may represent unaltered [⁷⁵Se]selenite or [⁷⁵Se]selenate that in some way manages to remain bound to the tRNA during the isolation process. Mixing of unlabeled *A. bisulcatus* tRNAs with the above labeled selenium salts and incubation at 27 C for 2 h prior to Sephadex chromatography did not result in the tRNA peak becoming labeled (data not shown).

tRNA Aminoacylation. The ability of the isolated *A. bisulcatus* tRNAs to accept amino acids, as a measure of biological activity, was examined using a ¹⁴C-labeled mixture containing 13 amino acids. Using partially purified preparations of synthetases from *A. bisulcatus*, *E. coli*, and wheat germ, the extent of charging was always greater with the heterologous synthetases (Fig. 8). Mischarging cannot be ruled out although the presence of the majority of the amino acids in the reaction mix makes this unlikely. Use of the tRNA substrates isolated in the presence of diethylpyrocarbonate improved the extent of aminoacylation somewhat, indicating that nuclease activity during tRNA isolation might be inactivating some tRNAs. The over-all results, however, remain unchanged. Addition of CTP to the homologous *A. bisulcatus* reaction mixture to repair any tRNAs deficient in -CCA terminus did improve acceptance activity levels (up to 40% in different experiments), but still not to the extent of the heterologous systems shown in Figure 8. The extent of aminoacylation of *A. bisulcatus* tRNAs with either *E. coli* or wheat germ synthetases was only 25 to 30% of the charging levels observed with the homologous *E. coli* system. Synthetase preparations were assayed for endogenous nuclease activity by measuring perchloric acid-soluble UV absorbance using *E. coli* tRNAs as substrate. On a per mg basis, *A.*

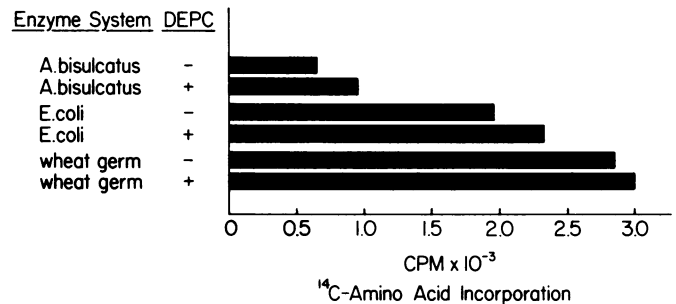


FIG. 8. Amino acid acceptor activities of *A. bisulcatus* tRNAs using homologous or heterologous aminoacyl synthetase preparations and a mixture of labeled amino acids. tRNAs isolated from the plant system in the presence of diethylpyrocarbonate (DEPC) routinely gave higher levels of acylation.

bisulcatus preparations were similar to those of *E. coli*. In the charging reactions, however, the plant enzyme was used at 5 to 10 times the concentration of the bacterial enzyme which would promote greater degradation of substrate tRNAs and result in lower aminoacylation levels. The presence of either free amino acids or an unidentified inhibitor in the *A. bisulcatus* synthetase preparations was examined for by adding the plant enzymes in various concentrations to a homologous *E. coli* aminoacylation reaction mixture. When amino acids of low specific radioactivity were used (about 5-10 mCi/mmol), little or no inhibition was found. Higher specific radioactivity amino acids (>100 mCi/mmol) were not incorporated on a per mol basis to the same extent, indicating some dilution of the label by cold amino acids. They were shown to be not limiting themselves. Most of this dilution appeared to result from amino acids released by endogenous proteolytic activity present in the *A. bisulcatus* synthetase preparation. Thus, while the tRNAs isolated from *A. bisulcatus* are clearly active in amino acid acceptance, the levels of their activity compared with *E. coli* appear low, due in part to endogenous nuclease and protease in the synthetase preparation.

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