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Distribution of two selenonucleosides among the selenium-containing tRNAs from *Methanococcus vannielii*

(seleno-tRNAs/5-methylaminomethyl-2-selenouridine/methane-producing bacteria/HPLC of nucleosides)

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ABSTRACT **Naturally occurring seleno-tRNAs from *Methanococcus vannielii* account for 13–20% of the total tRNA population. Two different selenium-modified nucleosides were detected in these seleno-tRNAs. Of the total selenium incorporated, about 60% is present in 5-methylaminomethyl-2-selenouridine, and the other 40% occurs in a second selenonucleoside with spectral characteristics typical of a derivative of 2-selenouridine. The 5-methylaminomethyl-2-selenouridine was found in the seleno-tRNA species present in the early fractions of a linear salt gradient elution profile from a reversed-phase chromatographic system 5 (RPC-5) column, whereas the second selenonucleoside occurred in the tRNA species eluted late in the profile.**

Specific incorporation of selenium into biologically active macromolecules has provided some molecular basis for the nutritional role of selenium in mammals and bacteria. Of the eight selenoenzymes that have been identified to date, seven are of bacterial origin (1). Glutathione peroxidase, which occurs in mammals and birds, has not been reported in prokaryotes. The chemical form of selenium in the polypeptide chain of several of these enzymes is selenocysteine or, in two instances, selenomethionine (1, 2).

Recent studies have shown that selenium-containing amino acid tRNAs are natural components of several bacterial tRNA populations (3–5) and probably also of mammalian tRNAs (unpublished data). In each organism the incorporated selenium is distributed among several tRNA species. In the anaerobic microorganism, *Clostridium sticklandii*, the most prominent selenium-containing tRNA was identified as the major glutamate-accepting species in the bulk tRNA preparation. The presence of selenium in this $tRNA^{Glu}$ is essential for its aminoacylation activity (4). In *Escherichia coli*, a lysine isoacceptor and a glutamate isoacceptor are the two major seleno-tRNAs (5). Nucleoside analysis of bulk tRNAs from both *E. coli* and *C. sticklandii* indicated the presence of a selenonucleoside, which has been identified as 5-methylaminomethyl-2-selenouridine (mnm^5Se^2U) (6, 7). In this communication we describe the isolation and characterization of seleno-tRNAs from *Methanococcus vannielii* and the occurrence of a new selenonucleoside in addition to mnm^5Se^2U.

MATERIALS AND METHODS

Chemicals. Radioactive amino acids were purchased from Amersham and $H_2{}^{75}SeO_3$, 20 mCi/μmol (1 Ci = 3.7×10^{10} becquerels), was from New England Nuclear. Ultrogel AcA 44 was obtained from LKB; organomercurial agarose Affi-Gel 501, from Bio-Rad; μBondapak C_{18} column, from Waters Associates; nuclease P1 and ATP, from Boehringer Mannheim; and bacterial alkaline phosphatase, from Sigma. Plaskon CTFE 2300 powder and Adogen 464 (methyltrialkylammonium chloride) for packing reversed-phase chromatographic system 5 (RPC-5) column were gifts from G. D. Novelli. A reference sample of mnm^5Se^2U was prepared by a modification (6) of the procedure for synthesis of the sulfur-containing analogue (8).

Preparation of ^{75}Se-Labeled tRNAs. *M. vannielii* cells were cultured in a formate-mineral salts medium (9) supplemented with 1 μM $NiCl_2$, 2 mM cysteine, 1 mM Na_2S, and 0.5 μM $Na_2{}^{75}SeO_3$ (0.25 mCi/liter). Sonic extracts prepared from cells that had been harvested while actively fermenting were applied to a DEAE-cellulose column; after elution of most of the absorbed proteins with 250 mM potassium phosphate (pH 7.2), the acidic tRNA fraction was stripped from the column with 1 M NaCl. After precipitation with ammonium sulfate at 80% saturation and separation on an Ultrogel AcA 44 gel filtration column, the isolated tRNAs were precipitated two times with ethanol and finally were dissolved in water. Enrichment of the selenium-containing tRNAs by affinity chromatography on an organomercurial agarose gel was achieved as described (4) except that 0.2 M NaCl was included in all buffers to reduce the nonspecific interactions between tRNAs and agarose gel. RPC-5 chromatography of bulk tRNA preparations was carried out by the method of Kelmers and Heatherly (10). The amount of selenium in tRNA preparations was determined by atomic absorption spectroscopy with a Perkin–Elmer atomic absorption spectrophotometer (model 603) and also was estimated from the specific radioactivity of the incorporated ^{75}Se.

Aminoacylation. The complete reaction mixture (25 μl) contained 50 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM KCl, 4 mM ATP, 1 mM dithiothreitol, 40 μM of each unlabeled amino acid, 5 μCi of [^{3}H]glutamate, 16 μg of *C. sticklandii* aminoacyl tRNA synthetases and various preparations of tRNA. Glutamate acceptor activity was assayed by a filter paper disc method (11).

Cyanogen Bromide Treatment of tRNAs. Selenocyanate derivatives of the seleno-tRNAs were prepared and deselenized by a modification of the method described by Saneyoshi and Nishimura (12) for conversion of thiobases to thiocyanates and desthiobases. A 0.1 vol of CNBr (27 mg/ml) in 10% ethanol was added to the selenium-enriched tRNA preparation (eluate from Affi-Gel 501) in 0.1 M NH_4HCO_3 (pH 8.9). After 10 min at room temperature, 0.3 vol of 1 M potassium acetate (pH 5) was added, and the tRNAs were precipitated by the addition of 3 vol of ethanol. The precipitate was dissolved in 0.2 M potassium acetate and reprecipitated with 3 vol of ethanol.

Nucleoside Analysis. The ^{75}Se-labeled tRNA preparations were digested by treatment with nuclease P1 and bacterial alkaline phosphatase (13). The resulting ribonucleosides were separated by using a reversed-phase HPLC system as described in the legend to Fig. 3.

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Abbreviations: mnm^5Se^2U, 5-methylaminomethyl-2-selenouridine; RPC-5, reversed-phase chromatographic system 5.

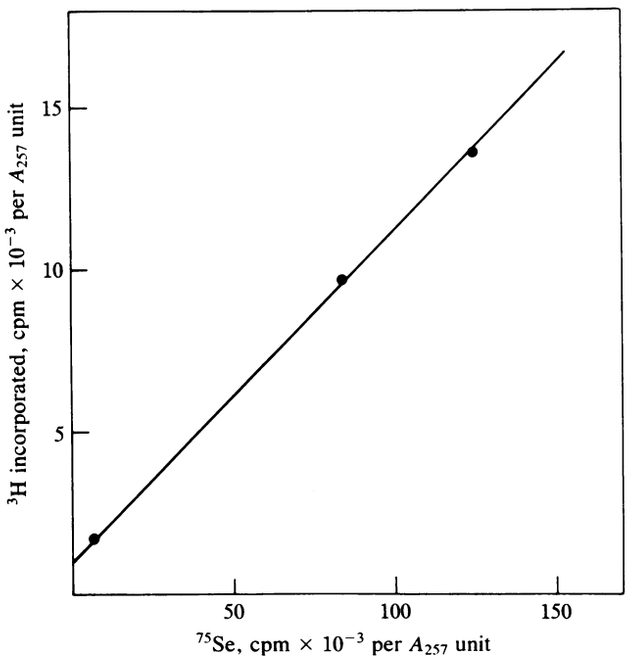

FIG. 1. Linear correlation of selenium content with [^{3}H]glutamate-accepting activity. Points on the line are tRNAs not adsorbed by Affi-Gel 501 (5.98 × 10^3 cpm ^{75}Se per A_{257} unit), unfractionated bulk tRNA (83 × 10^3 cpm ^{75}Se per A_{257} unit), and tRNAs eluted from Affi-Gel 501 by 2-mercaptoethanol (123 × 10^3 cpm ^{75}Se per A_{257} unit).

RESULTS AND DISCUSSION

As reported by Best (14), the phenolic extracts of *M. vannielii* have large amounts of high molecular weight nucleic acids that are difficult to separate by salt and ethanol fractionation procedures from the desired 4S material. The bulk tRNAs used in the present study were prepared from cell-free extracts by ion-exchange (DEAE-cellulose) and molecular sieve chromatography and were essentially free of larger molecular weight species. By assuming one atom of selenium per molecule of modified tRNA species, as is true for the seleno-tRNA^{Glu} from *C. sticklandii* (4), 13–20% of the tRNA population of *M. vannielii* is modified with selenium. In the numerous *C. sticklandii* cultures we have examined, 5–8% of the bulk tRNA population contains selenium; in *E. coli*, depending on growth conditions, up to 6% of the total population may contain selenium. The number of selenium-containing tRNAs in *M. vannielii*—almost one out of every five tRNA molecules—is the highest so far reported.

Because selenium-containing glutamate isoacceptors are among the major seleno-tRNAs in both *C. sticklandii* and *E. coli*, the possible presence of a seleno-tRNA^{Glu} in the *M. vannielii* preparations was examined. For these experiments tRNA samples with high and low selenium contents were prepared from ^{75}Se-labeled bulk tRNA by organomercurial agarose gel fractionation (4). A linear correlation of selenium contents and glutamate-accepting activities of these preparations was observed (Fig. 1). When the selenium-enriched preparation (123 × 10^3 cpm of ^{75}Se per A_{257} unit; see Fig. 1) was deselenized by treatment with CNBr, the glutamate-accepting activity was greatly decreased from 5.54 pmol per A_{257} unit before CNBr treatment to 0.73 after CNBr treatment. The low glutamate-accepting activity of this preparation (5.5 pmol per A_{257} unit) as compared to that of similar, enriched preparations from *C. sticklandii* (90 pmol per A_{257} unit) may be partially due to the use of the heterologous aminoacyl tRNA synthetase from *C. sticklandii* rather than a homologous synthetase derived from *M. vannielii*. From the known lability of the seleno-tRNA^{Glu} from *C. sticklandii* to CNBr treatment (7), these data indicate that a similar species is present in *M. vannielii*.

A typical RPC-5 chromatographic profile of seleno-tRNAs

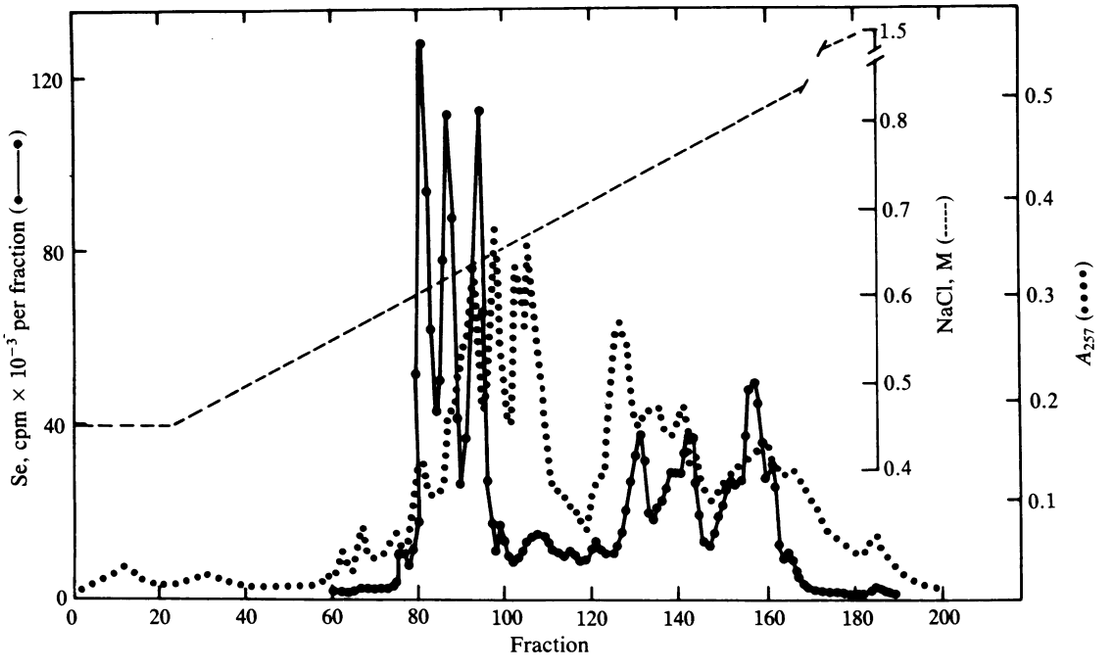

FIG. 2. RPC-5 chromatography of seleno-tRNAs from *M. vannielii*. Bulk tRNA (23.2 A_{257} units; 2.85 × 10^6 cpm) prepared from ^{75}Se-labeled *M. vannielii* cells was dissolved in 5 ml of buffer A (0.45 M NaCl/10 mM sodium acetate, pH 4.5/10 mM magnesium acetate/1 mM EDTA/1 mM dithiothreitol) and applied to a RPC-5 column (0.9 × 41 cm bed). After a 10-ml wash with buffer A, the absorbed tRNA was eluted with a linear gradient (total volume, 240 ml) of buffer A and buffer B (same as buffer A but containing 0.85 M NaCl). At the end of the gradient (fraction 160), buffer C (same as buffer A, but containing 1.5 M NaCl) was applied to the column. Fractions (1.5 ml) were collected at a flow rate of 1.0 ml/min. The column pressure was about 400–550 psi (1 psi = 6.89 kPa). Radioactivity of each fraction was measured with a Beckman Gamma 5500 counter.

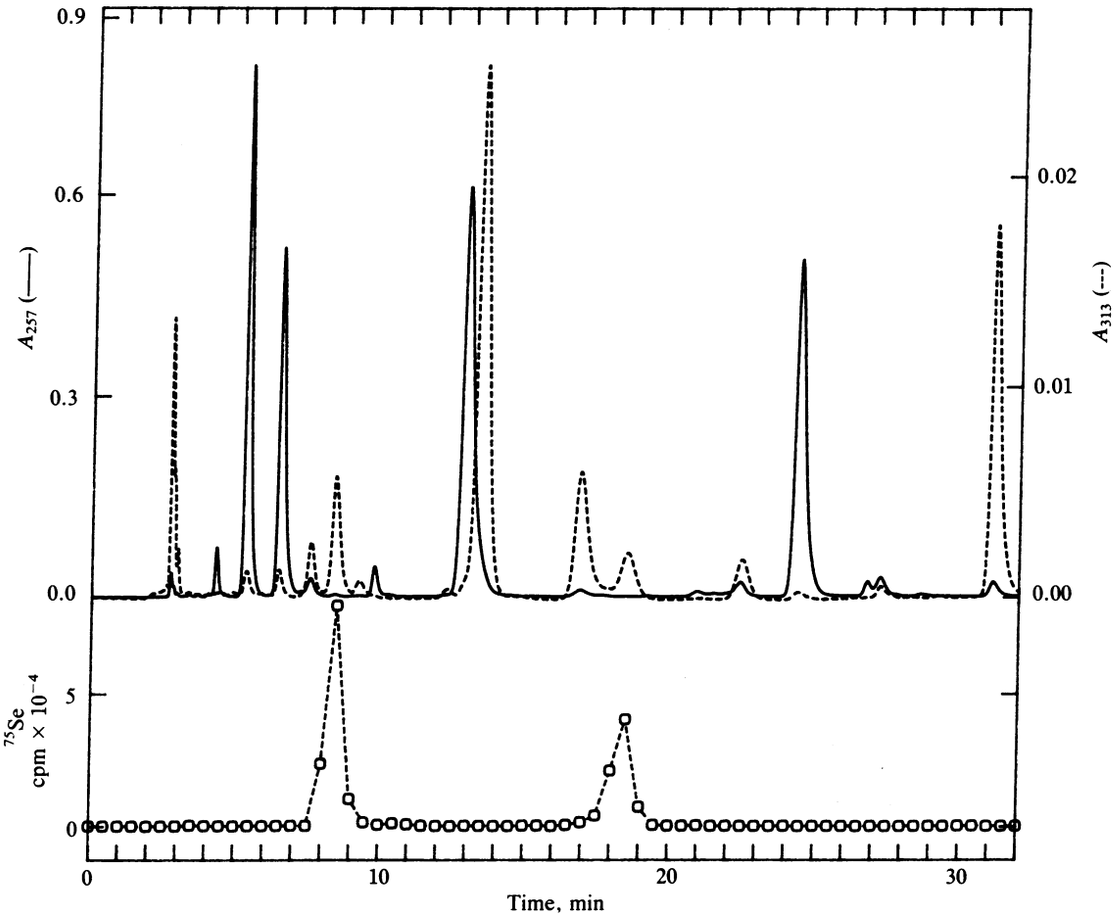

FIG. 3. HPLC analysis of nucleosides from *M. vannielii* tRNA. ^{75}Se-Labeled tRNA (1.9 A_{257} units; 223,000 cpm) was hydrolyzed enzymically as described and chromatographed on a Waters μBondapak C_{18} column at room temperature and 1 ml/min flow rate. The mobile phase at sample injection (0 min) was 97% 10 mM ammonium acetate, pH 5.4/3% methanol. The column was eluted with increasing methanol gradients as follows: from 0 to 12.5 min, 3–5%; from 12.5 to 27.5 min, 5–20%; and finally from 27.5 to 30 min, 20–100%. The effluent from the column was monitored at 257 (——) and 313 (-----) nm, and the ^{75}Se in 0.5-min (0.5 ml) fractions (□) was determined as described in the legend to Fig. 2. In this separation system, the major ribonucleosides (the four major peaks of 257 nm absorbance) were eluted in the order cytidine, uridine, guanosine, and adenosine. As previously reported for *M. vannielii* tRNA (14), no 5-methyluridine or 7-methylguanosine was detected. The prominent 313-nm absorbance peak at 13 min immediately after guanosine is due to 4-thiouridine. The 313-nm absorbance peak at 17 min is due to oxidized dithiothreitol present in the digestion mixture.

isolated from actively growing *M. vannielii* cells is shown in Fig. 2. This pattern is similar to those observed for *C. sticklandii* (4) and *E. coli* (5) tRNAs. As would be predicted, glutamate-accepting activity was detected in the fractions eluted early in the profile with 0.5–0.6 M NaCl.

Recent studies with *E. coli* have shown that a selenium-containing nucleoside, 5-methylaminomethyl-2-selenouridine, accounts for most of the ^{75}Se incorporated into tRNAs of this organism (6). When bulk ^{75}Se-labeled tRNA from *M. vannielii* was enzymically hydrolyzed to nucleosides and analyzed by reversed-phase HPLC, two selenium-modified nucleosides were detected (Fig. 3). Together these accounted for 85% of the ^{75}Se applied to the column. The ^{75}Se-modified nucleoside that was eluted early in the profile at 8 min contained 58% of the recovered ^{75}Se. This was identified as mnm^{5}Se^{2}U by its UV spectrum (Fig. 4) and by cochromatography on the HPLC column with authentic mnm^{5}Se^{2}U (data not shown). The other ^{75}Se-modified nucleoside that was eluted later at 18 min (Fig. 3) contained 36% of the recovered ^{75}Se. The UV spectrum of this compound at pH 5 (λ_{max}, 310 nm) is similar to but distinct from that of mnm^{5}Se^{2}U (λ_{max}, 313 nm) (Fig. 4). The pH-dependent UV spectral shifts exhibited by the unknown nucleoside (at pH 1, λ_{max} was 312 nm; at pH 9, λ_{max} was 290 nm) resemble those of mnm^{5}Se^{2}U and 2-selenouridine (15), suggesting that this new selenonucleoside also may be a derivative of 2-selenouridine. It was distinguished from 5-methyl-2-selenouridine by direct chromatographic comparison and from 5-hydroxymethyl-2-selenouridine by the failure of the deselenized nucleoside to cochromatograph with 5-hydroxymethyluridine. However, from the chromatographic properties of the new selenonucleoside, it might be the selenium analog of 5-methylcarboxymethyl-2-thiouridine, which has been identified in certain eukaroytic tRNAs (16).

In view of the finding that two different selenonucleosides occur in the tRNAs of *M. vannielii*, the distribution of these selenonucleosides among the different tRNA species was investigated. The three prominent ^{75}Se-containing tRNA fractions, 81, 87, and 94, that were eluted early from the RPC-5 column (Fig. 2) were recovered separately, digested to nucleosides as described in *Materials and Methods* and analyzed as described in the legend to Fig. 3. The ^{75}Se in each of these samples was recovered (90–95%) in a single ^{75}Se-labeled selenonucleoside that exhibited chromatographic properties identical to those of mnm^{5}Se^{2}U. In contrast, similar analysis of the ^{75}Se-labeled tRNAs that were eluted later from the RPC-5 column showed that 70–80% of the ^{75}Se in the pooled fractions 106–170 (Fig. 2) was present in the unidentified selenonucleoside. Only 20–30% of the ^{75}Se in these tRNA species was recovered in mnm^{5}Se^{2}U. If

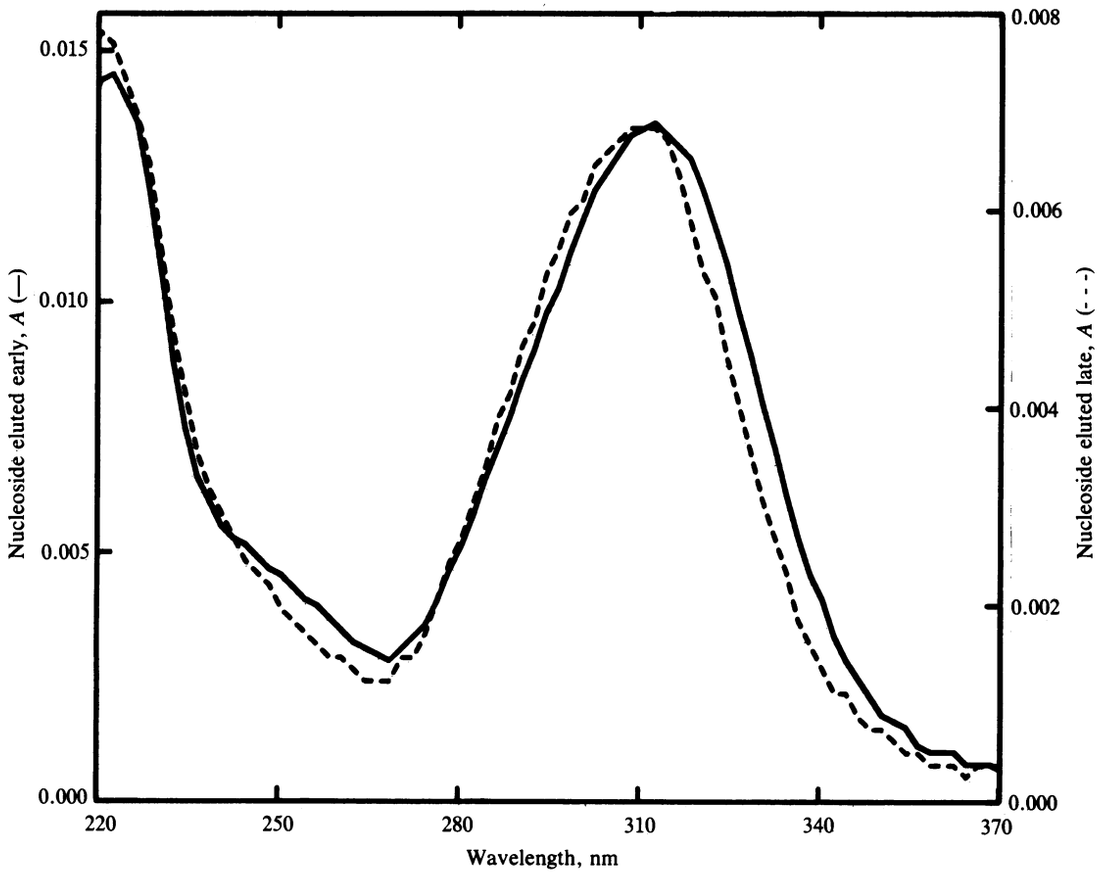

FIG. 4. UV spectra of the selenonucleosides from *M. vannielii*. The selenonucleoside fractions eluted early and late from several chromatographic separations (including that of Fig. 3) were each pooled, the volume of both pools was reduced to 50 μl by drying under a stream of argon, and each pool was subjected to HPLC analysis as described in Fig. 3. UV spectra at the apices of the selenonucleoside peaks were obtained by using a Hewlett–Packard 1040A detector. Spectra recorded immediately before or after the peaks served as references.

the elution position of the new selenonucleoside from the HPLC column is indeed a reflection of its greater hydrophobicity, this might account for its preferential distribution in the tRNA species that were eluted later from the RPC-5 column.

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