METHIONINE-SELENOMETHIONINE PARALLELS IN RAT LIVER POLYPEPTIDE CHAIN SYNTHESIS

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1. Introduction

It has been demonstrated in our laboratory that in *E. coli*, selenomethionine (Se Met) is incorporated into polypeptides via the methionine (Met) pathway, including the initiation mechanism involving the *N*-formylated aminoacyl-tRNA [1–3]. The question may be asked whether there are methionine-selenomethionine parallels in rat liver polypeptide chain synthesis as were observed in *E. coli*. We wish to present experimental evidence which demonstrates that in rat liver as in *E. coli*, Se Met is incorporated into polypeptides via the Met pathway.

2. Materials and methods

The methods and procedures used to prepare and assay for aminoacylation of methionyl tRNA by Se Met and selenomethionyl-tRNA participation in polypeptide synthesis are described in table 1 and figs. 1 and 2 of the text and in previous communications [1, 4].

The following lists the sources of commercially obtained materials [^{75}Se] Met (1,961 mCi/mM), [^{14}C] Met (100 mCi/mM) Amersham-Searle, Des Plaines, Ill., ATP, PL Biochemicals, Inc., Milwaukee, Wisc., GTP, Miles Laboratories, Inc., Elkhart, Ind., phosphoenolpyruvate (PEP), monopotassium salt, pyruvate kinase, Sigma Chemical Co., St. Louis, Mo., rat soluble ribonucleic acid, stable amino acids, General Biochemicals, Chagrin Falls, Ohio; stable dl selenomethionine, Cyclo Chemical, Los Angeles, Calif., puromycin dihydrochloride, National Biochemicals, Cleveland, Ohio.

3. Results and discussion

We found that liver methionyl-tRNA synthetase like *E. coli* cannot distinguish between [^{14}C] Met and [^{75}Se] Met. The products of aminoacyl-tRNA synthetase activities with [^{14}C] Met and [^{75}Se] Met were simultaneously eluted from a DEAE column using a KCl gradient (fig. 1A). Gel filtration of the amino acyl-tRNA synthetases on Sephadex G-200 further demonstrated that the same amino acyl-tRNA synthetase was used for the formation of amino-acyl-tRNA compounds of [^{14}C] Met and [^{75}Se] Met (fig. 1B). Separately prepared [^{75}Se] Met-tRNA and [^{14}C] Met tRNA were mixed and were shown to co-chromatograph on a Sephadex G-25 column (fig. 2). Thus, Met and Se Met are apparently attached to the same tRNA's. In experiments using a cell-free synthesizing system [4] results show that seleno-methionyl-tRNA participated in polypeptide synthesis which is further substantiated by the fact the enzymes, RNA and energy are essential and that the analogues and puromycin inhibit the incorporation of [^{75}Se] Met and [^{14}C] Met into proteins (table 1). The ability of rat liver methionyl-tRNA synthetase to catalyze the aminoacylation of methionine tRNA

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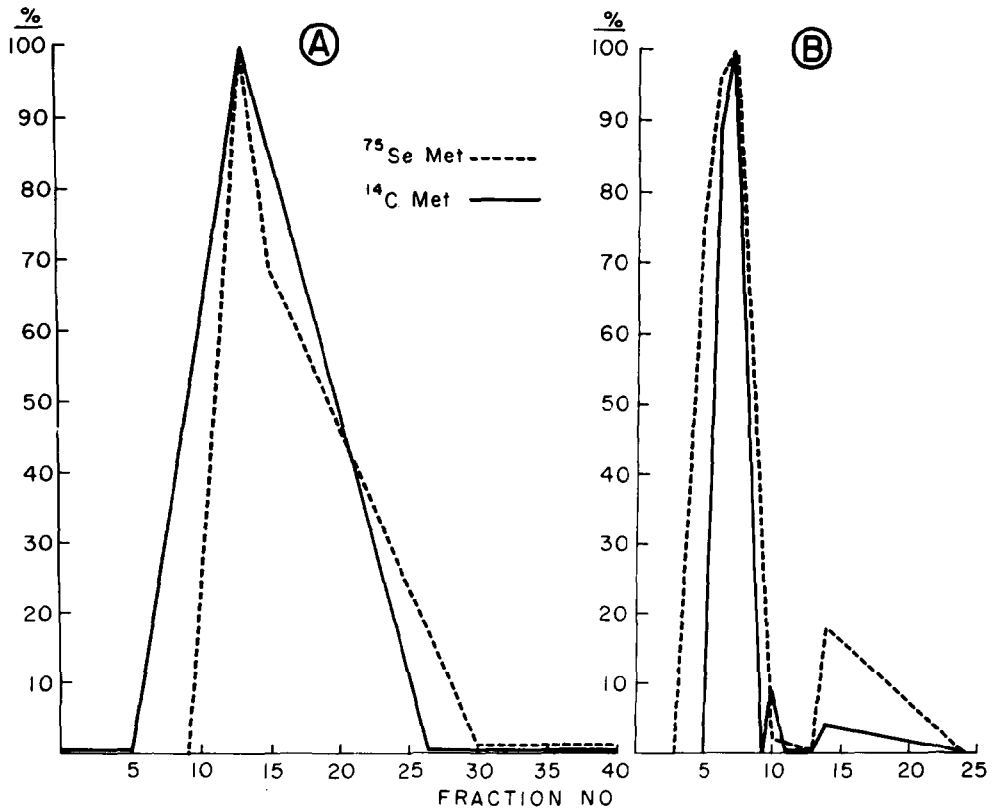

Fig. 1. Chromatography of methionyl-tRNA synthetase. (A) DEAE-cellulose: Approx. 15 g of normal rat liver was homogenized in a Dounce homogenizer in 15 ml of "synthetase" buffer (10 mM $MgCl_2$, 2 mM β-mercaptoethanol, 10 mM KCl, 0.1 M Tris, pH 7.5). The liver was extracted with 15 ml buffer and the extracts centrifuged at 10,000 rpm for 30 min. The resulting supernatant was centrifuged at 100,000 *g* for 2 hr. This supernatant was applied to a 2.5 cm × 60 cm DEAE-cellulose column (Whatman DE-23) and eluted with a 1 liter linear gradient from 0.2 to 0.7 M in KCl, buffered with 0.1 M Tris at pH 7.6 and containing 2 mM β-mercaptoethanol. Fractions were assayed separately for aminoacyl-tRNA synthetase activity with [^{14}C] methionine and [^{75}Se] selenomethionine in 100 μl reaction mixtures, containing 0.1 M Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 2 mM β-mercaptoethanol, 10 mM KCl, 2 mM ATP, 200 μg tRNA (rat liver), 40 μM of either ^{14}C-Met or ^{75}Se Met and limiting amounts of enzyme. After incubation for 10 min at 37°, 100 μl aliquots were spotted on 2.2 cm discs of Whatman 3 MM paper. The discs were washed 3 times with cold 5% trichloroacetic acid, once with 5% potassium acetate in 70% ethanol, once with 95% ethanol, once with ether and then dried under a heat lamp. Dried discs were counted for ^{75}Se (γ) (- - - -) in a Picker-Nuclear Spectroscaler III and for ^{14}C (β) (——) under 10 ml of scintillation fluid in a Packard Tri-Carb liquid scintillation counter.

(B) Sephadex G-200: Fractions 10–13 from (A) were pooled, concentrated to about 10 ml in a dialysis bag buried in polyethylene glycol (Carbowax 300) and applied to a 2.5 cm × 60 cm column of Sephadex G-200 (Pharmacia) previously equilibrated with synthetase buffer. The column was eluted with the same buffer and fractions assayed as in (A) with [^{14}C] methionine and [^{75}Se] selenomethionine.

with selenomethionine (Se Met) has been demonstrated. Using a cell-free protein synthesizing system, we observed that selenomethionyl tRNA participated in polypeptide synthesis to about the same extent as methionyl-tRNA. We conclude that in rat liver as in *E. coli*, Se Met is incorporated into polypeptide via the Met pathway.

Table 1
Protein synthesis, rat liver cell-free system.

	Incorporation of [^{14}C] Methionine		[^{75}Se] Selenomethionine	
	nmole* per A_{260} unit**	% of control	nmole* per A_{260} unit**	% of control
Complete system (control)	18.3	100	16.8	100
Minus tRNA		93.8		79.7
Competitor 1:10		48.4		24.0
Minus ATP, GTP, PEP		25.0		28.0
Puromycin 4×10^{-2} M		71.8		72.0
S-30, boiled		11.1		20.5

Protein synthesis: The incubation mixture for assay of amino acid incorporation into protein contained the following components in a final vol of 0.1 ml: 0.1 M cacodylic acid buffer (pH 7.8); 0.01 M magnesium acetate; 0.1 M KCl, 0.006 M mercaptoethanol, 1.5 mg/ml of rat soluble ribonucleic acid; [^{14}C] Met or [^{75}Se] Met (4×10^{-4} M), 1.02×10^{-4} M of each of 19 unlabeled amino acids, methionine not included; 1 μmole ATP, 0.25 μmole GTP; 5 μmole phosphoenolpyruvate; 0.1 mg pyruvate kinase; 12 to 20 A_{260} units of a 30,000 *g* supernatant (S-30) fraction from rat liver. Incubation mixture was heated at 37° for 15 min, 0.1 ml 0.3 N NaOH was added, the mixture was further incubated for 30 min and neutralized with 0.5 N HCl. 100 μl aliquots were spotted on 2.2 cm discs of Whatman 3 MM paper. The discs were washed 3 times with cold 5% trichloroacetic acid, once with 5% potassium acetate in 70% ethanol, once with 95% ethanol, once with ether and then dried under a heat lamp. Dried discs were counted for ^{75}Se in a Picker Nuclear Spectroscaler III and for ^{14}C under 10 ml of scintillation fluid in a Packard Tri-Carb liquid scintillation counter. Competitive inhibition between methionine and selenomethionine during protein synthesis: Reaction mixtures were as indicated above with the exception that the concentration of non-radioactive selenomethionine or methionine was added as indicated in the presence of the labeled analogue.

*Alkali-resistant, acid insoluble product.
**Ribosomes.

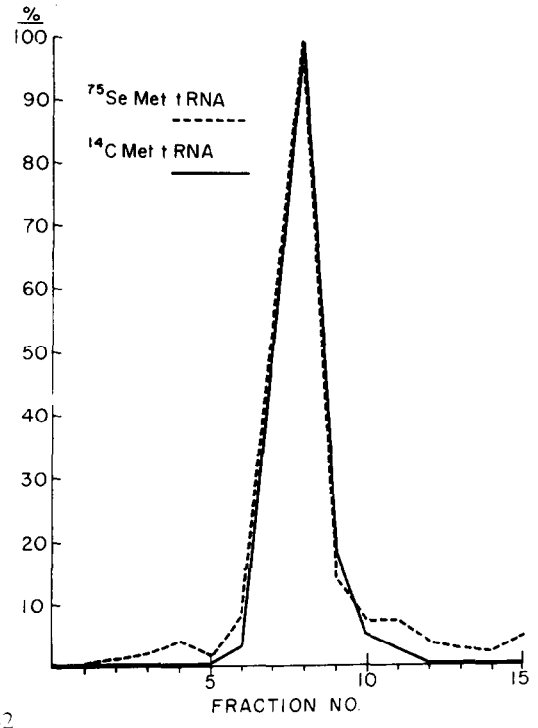

Fig. 2. Co-chromatography of [^{75}Se] selenomethionyl and [^{14}C] methionyl tRNA. Two reaction mixtures (fig. 1A) with synthetase from fig. 1B were scaled up to 1 ml (2 mg tRNA), one containing [^{75}Se] selenomethionine, the other [^{14}C] methionine. Following incubation, the mixtures were extracted with an equal volume of 88% phenol, and precipitated with 2 vol of 95% ethanol. The precipitates were redissolved in 0.02 M sodium acetate at pH 6.0, mixed and chromatographed on Sephadex G-25 using the same buffer. Fractions were analyzed as before (fig. 1) for ^{75}Se and ^{14}C.

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