Methionine–Selenomethionine Parallels in *E. coli* Polypeptide Chain Initiation and Synthesis (36520)

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Administration of inorganic selenium to procaryotes capable of synthesizing methionine (Met) results in the appearance of selenomethionine (Se Met) into proteins (1). When selenite is administered to eucaryotes, with an absolute dietary requirement for methionine, the existing evidence is not conclusive that there is biosynthesis of Se Met or that protein-bound selenium is incorporated via peptide bonds into the polypeptide structure as seleno-analogs of the sulfur amino acids.[1]

Our recent investigations have centered on the parallels between Met and Se Met as they are activated and incorporated into polypeptides by cell-free systems. It is the purpose of these investigations to establish whether or not selenomethionine utilizes the known methionine pathway into polypeptides. Previously we have shown that in *E. coli* selenomethionine is attached to the various species of methionine tRNA (both formylatable and nonformylatable) in a reaction catalyzed by methionyl-tRNA synthetase (2).

Several investigators have recently reported the isolation of protein containing high Se/S ratios. Huber, Segal and Criddle (6) isolated β-galactosidase from *E. coli* grown in selenite and compared its chemical and biological properties with β-galactosidase from *E. coli* grown on sulfate. They showed that seleno β-galactosidase was fully active and contained 80 Se Met residues out of 150 total methionine residues in 1 mole, 135,000 g of protein. Ahluwalia (7) reported the isolation of *E. coli* alkaline phosphatase with 11% of its sulfur replaced by selenium. The seleno-enzyme had approximately 50% of the activity of the normal enzyme. Coch and Greene (8) confirmed Huber, Segel and Criddle's observations when they found that *E. coli* β-galactosidase with 70–75% of its methionine residues replaced by selenomethionine has the same catalytic activity as the unmodified enzyme.

In this paper we present evidence that selenomethionine follows methionine pathways in polypeptide chain initiation and synthesis using cell-free extracts from *E. coli*.

Methods and Procedures. The cell-free system for the study of amino acid incorporation was similar to that of Nirenberg and Matthei (3), the details being outlined in the legend on Table I. The initiation of protein synthesis in *E. coli* with either ^{3}H Met or ^{75}Se Met was carried out utilizing the method of Takeishi and Tyunosin (4), the details are shown in the legend to Table II. ^{14}C-Formyl tetrahydrofolate was prepared according to the method of Takeishi, Uketa and Nishemura (5).

Isolation of (^{14}C) formyl (^{3}H) Met-tRNA and (^{14}C) formyl (^{75}Se) Met-tRNA: The assay procedures for aminoacyl-tRNA synthetase activity [see Ref. (2)] were scaled up 10 times, one containing (^{75}Se)-selenomethionine, the other (^{3}H)-methionine. After incubation for 10 min at 37°, (^{14}C)fFH_4 was added, and further incubated for 15 min at 37°. Following incubation, the formylaminoacyl-tRNA was precipitated with 2 vol of 95% ethanol, and the redissolved precipitate was extracted with an equal volume of 88% phenol. The ether-washed aqueous layer was

[1] A future report will cover a study made by us of the incorporation of Se Met into proteins in a protein synthesizing system of eucaryote requiring dietary methionine, Fed. Proc., Fed. Amer. Soc. Exp. Biol. 31, 1972.

TABLE I. Protein Synthesis, in *E. coli* (Q-13) Cell-Free System.[a]

	Incorporation of			
	^{14}C Methionine		^{75}Se Selenomethionine	
	nmole[b]/A_{260} unit[c]	% Decrease	nmole/A_{260} unit	% Decrease
Complete system (control)	6.37	—	3.44	—
minus m RNA		26.8		11.3
Competitor 1:10[d]		32.7		5.5
1:20		39.9		18.6
1:40		34.4		23.5
minus ATP, GTP, PEP		47.3		34.0
Puromycin 4×10^{-3} *M*		4.1		19.5
4×10^{-2} *M*		40.5		11.6
S-30, boiled		24.0		34.9

[a] Protein synthesis: The incubation mixture for assay of amino acid incorporation into protein contained the following components in a final volume of 0.1 ml: 0.006 *M* mercaptoethanol, 1.5 mg/ml of soluble ribonucleic acid; *E. coli* B; (^{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; 0.344 mg F2 viral RNA; 12 A_{260} units of a 30,000*g* supernatant (S-30) fraction from *E. coli* Q-13 (3).

The 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.

[b] Alkali-resistant, acid-insoluble product.

[c] Ribosomes.

[d] One mole normal substrate : 10 moles of competitor.

TABLE II. Initiation of Protein Synthesis in *E. coli* with ^{3}H Met or ^{75}Se Met.[a]

	^{75}Se (cpm)	% decrease	^{3}H (cpm)	% decrease	^{14}C (cpm)	% decrease
Cell-free system + ^{75}Se Met + ^{14}CfFH$_4$ + S-30	8939		—	—	1013	
Boiled S-30		63.4	—	—		70.4
^{3}H Met + ^{14}CfFH$_4$ + S-30	—	—	373		190	
Boiled S-30	—	—		24.6		31.9

[a] (^{14}C) formate and (^{75}Se) Se Met or (^{3}H) Met incorporated into an alkali-resistant, acid-insoluble product. Initiation of protein synthesis: In the initiation of protein synthesis in an *E. coli* cell-free system with (^{3}H) methionine, (^{75}Se)-selenomethionine and added (^{14}C) formyl tetrahydrofolate (4) (^{14}C fFH$_4$), the identical procedure outlined in Table I for protein synthesis was used, except after incubation for 10 min at 37°, the (^{14}C) fFH$_4$ was added to the mixture and incubated for 15 min. Boiled S-30 fraction from *E. coli* (Q-13) was prepared to test the dependence of initiation of protein synthesis on S-30 enzymes, manifested by formylation of methionine or selenomethionine.

chromatographed on a Sephadex G-25 column, 1 × 108 cm, eluted with 0.1 *M* sodium acetate buffer (pH 4.2). After assay for (^{14}C) and (^{75}Se), [(^{14}C) formyl (^{75}Se) Met-tRNA] or (^{14}C) and (^{3}H) [(^{14}C) formyl (^{3}H) Met-tRNA], the frontal peak of A_{260} nm was applied to a benzoylated DEAE-cellulose column. The column was eluted at 0 to 2° by a 500 ml gradient from 0.5 to 1.0 *M* NaCl containing 0.02 *M* sodium acetate at pH 6.0. Fractions were assayed for the appropriate combinations of ^{75}Se, ^{14}C and ^{3}H.

Materials and reagents. The following lists the sources of commercially obtained materials: Soluble ribonucleic acid, *E. coli* B., Schwarz Bioresearch, Inc., Orangeburg, NY; F2 virus, Miles Laboratories, Inc., Elkhart, IN; *E. coli* Q-13 mid-log (RNase II$^-$, PNPase$^-$), General Biochemicals; (^{75}Se)-Se Met, 980 Ci/mole (counted at 52% efficiency), sodium formate-(^{14}C), 36 Ci/mole (88%), and (^{3}H)-L-Met, 150 Ci/mole (57%), Amersham/Searle, Des Plaines, IL; ATP, PL Biochemicals, Inc., Milwaukee, WI; GTP, Miles Laboratories, Inc., Elkhart, IN; phosphoenolpyruvate (PEP), monopotassium salt, pyruvate kinase, Sigma Chemical Co., St. Louis, MO; stable amino acids, General Biochemicals, Chagrin Falls OH; stable seleno-DL-methionine, Cyclo Chemical, Los Angeles, CA.

Results. The properties of the cell-free system used are demonstrated in Table I. It is evident that the incorporation of either methionine or selenomethionine into polypeptides (acid-insoluble material) is dependent on an energy source, is stimulated by the addition of mRNA, and is inhibited by puromycin. These are classical characteristics of systems for the activation and incorporation of amino acids into polypeptides under the direction of mRNA.

If methionine and selenomethionine utilize the same enzymic pathways during polypeptide formation then an excess of one of these compounds should inhibit the incorporation of the other. In fact, this was found as is shown in Table I, thus demonstrating the equivalence of methionine and its seleno-analog.

In procaryotes, *N*-formylmethionyl-tRNA (Fmet-tRNA$_F^{met}$) serves as the initiator of protein synthesis. To further define the analogy between methionine and selenomethionine, we have examined *E. coli* extracts for the ability to form (FSe Met-tRNA$_F^{met}$). We found that both (^{14}C) formate and (^{75}Se) selenomethionine are incorporated into an acid-insoluble product when precursors are incubated with *E. coli* enzymes and tRNA. To prove this more conclusively, we isolated the putative compound first by ethanol precipitation then by phenol extraction and chromatographed it to see if the (^{14}C) formate and (^{75}Se) selenomethionine labels moved to-

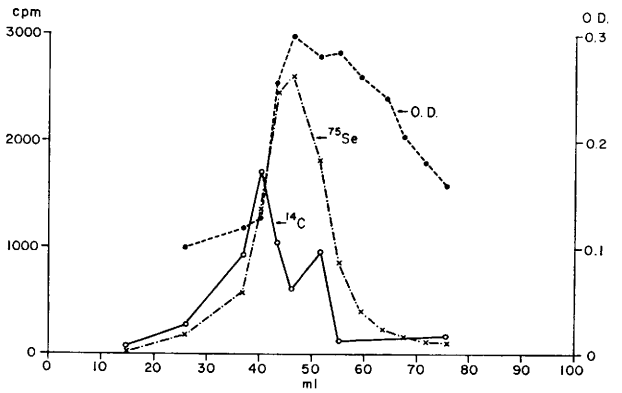

FIG. 1. Chromatography of *N*-(^{14}C)formyl(^{75}Se)-selenomethionyl-tRNA^{Fmet} on benzoylated DEAE-cellulose. The preparation of the tRNA is described in the text. The tRNA was isolated from the mixture by phenol extraction and ethanol precipitation. The precipitate was dissolved in 0.1 *M* sodium acetate at pH 4.2 and chromatographed on Sephadex G-25 using the same buffer. The frontal peak of A_{260} nm was applied to a 1 × 100 cm column of benzoylated DEAE-cellulose. The column was eluted at 0 to 2° by a 500 ml gradient from 0.5 to 1.0 *M* NaCl containing 0.02 *M* sodium acetate at pH 6.0. Fractions were analyzed for ^{75}Se and ^{14}C by a combination of γ and β counting techniques.

gether in a manner consistent with their attachment to tRNA. In Fig. 1, the labeled products from Sephadex G-25 are seen to have similar mobilities during ion-exchange chromatography on benzoylated DEAE-cellulose. The formate/selenomethionine ratio on a molar bases approached unity with each purification step.

To complete the analogy between methionine and selenomethionine, we returned to the polypeptide synthesizing system to determine if FSeMet-tRNA$_F^{met}$ could serve in the initiation of protein synthesis by transferring the FSeMet moiety to protein. This was shown to take place as indicated in Table II, where incorporation of (^{14}C) formate and (^{75}Se)-selenomethionine into an alkali-resistant, acid-insoluble product is demonstrated.

Discussion. Our results demonstrate that selenomethionine participates in all the known reactions of methionine during polypeptide chain initiation and synthesis in *E. coli*. The relationship between this conclusion and the status of selenium as an essential trace element is not clear at this time. It may be that Se Met like Met may serve as an initiator of protein synthesis.

Summary. Using a cell-free synthesizing system and natural messenger (f_2 viral RNA), we observed that selenomethionyl-tRNA participated in polypeptide synthesis to about the same extent as methionyl-tRNA. Studies on the role of Se Met in the initiation of protein synthesis indicated that *N*-formyl Se Met-tRNA can function as an initiator of protein synthesis as it has been established for *N*-formyl Met-tRNA. It is concluded that in *E. coli* Se Met is incorporated into polypeptides via the Met pathway, including the initiation mechanism involving the *N*-formylated aminoacyl-tRNA.

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