In vitro synthesis of selenocysteinyl-tRNA$_{UCA}$ from seryl-tRNA$_{UCA}$: Involvement and characterization of the *selD* gene product

(selenocysteine biosynthesis/SelD protein/selenium donor)

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ABSTRACT The *selD* gene from *Escherichia coli*, whose product is involved in selenium metabolism, has been cloned and sequenced. *selD* codes for a protein of 347 amino acids with a calculated molecular weight of 36,687. Analysis of the *selD* gene product through expression of the gene in the phage T7 promoter/polymerase system confirmed the predicted molecular weight of the protein. Gene disruption experiments demonstrated that the SelD protein is required both for the incorporation of selenium into the modified nucleoside 5-methylaminomethyl-2-selenouridine of tRNA and for the biosynthesis of selenocysteine from an L-serine residue esterbonded to tRNA$_{UCA}^{Ser}$. tRNA$_{UCA}^{Ser}$ has been purified, aminoacylated with L-serine, and used as a substrate for the development of an *in vitro* system for selenocysteine biosynthesis. Efficient formation of selenocysteinyl-tRNA$_{UCA}^{Ser}$ was achieved by using extracts in which both the *selD* and the *selA* gene products were overproduced. The results demonstrate that selenocysteine is synthesized from L-serine bound to tRNA$_{UCA}$ and they are in accord with SelD functioning as a donor of reduced selenium.

In *Escherichia coli*, selenium is inserted in the form of selenocysteine into the 80-kDa and 110-kDa subunits of formate dehydrogenases H and N (FDH$_H$ and FDH$_N$), respectively, and into the modified nucleoside 5-methylaminomethyl-2-selenouridine (mnm^{5}Se^{2}U) (1–3). Mutants have been described that are either specifically deficient in selenoprotein synthesis (*selA*, *selB*, and *selC* genes) or unable to selenate both protein and tRNA (*selD* gene) (for review see ref. 4). The *selC* gene product has been shown to be a tRNA species (tRNA$_{UCA}$) that is aminoacylated with L-serine *in vivo* and that carries selenocysteine (5, 6). On the basis of these data it has been proposed that selenocysteine biosynthesis occurs from an L-serine residue charged to tRNA$_{UCA}$ (5). A putative role for the *selA* and *selD* gene products in this conversion process has been postulated. The *selB* gene product (SelB), on the other hand, is not essential for selenocysteinyl-tRNA formation *in vivo* (6). Recent results have shown that SelB is a guanine nucleotide-binding protein acting as a specific translation factor for selenocysteine insertion (34).

Mutants in *selD* are unique in that they are blocked in the incorporation of selenium both into FDH and into tRNA (7). They had originally been isolated as strains pleiotropically defective in FDH$_H$ and FDH$_N$ activity (8) and their additional defect in mnm^{5}Se^{2}U formation was detected only subsequently, when it was found that pleiotropic FDH mutants actually have a lesion in selenium metabolism (7). As an initial step toward identifying the biochemical function of the *selD* gene product, we have cloned and sequenced the *selD* gene from *E. coli* and devised an *in vitro* selenocysteine

biosynthesis system with the aid of extracts from cells in which the *selD* and *selA* gene products are overproduced.†

MATERIALS AND METHODS

Strains, Media, and Growth Conditions. The *E. coli* strains used in this study are listed in Table 1. Unless indicated otherwise, bacteria were grown in LB medium (13). When required, antibiotics were added to the following final concentrations: ampicillin, 50 μg/ml; chloramphenicol, 20 μg/ml; kanamycin sulfate, 50 μg/ml.

Genetic Techniques. Basic genetic techniques were as described by Miller (13). *recA* mutations were transferred by P1*kc*-mediated transduction with JC10289(pKY102) as donor (10). Recombinant DNA methods were performed according to Maniatis *et al.* (14). DNA sequencing was carried out with the method of Maxam and Gilbert (15) as modified by Gray *et al.* (16) or with the dideoxy chain-termination method (17, 18).

Cloning and Subcloning of the *selD* Gene. A cosmid bank of genomic DNA from *E. coli* MC4100 (7) was screened for complementation (19, 20) of the lesion of strain MN30; DNA from a complementing clone obtained was partially digested with *Sau*3A1 and the fragments were ligated into the *Bam*HI site of vector pACYC184 (21) to yield the complementing plasmid pMN302 (see Fig. 1) with a 3.8-kilobase (kb) *Sau*3A1 insert. The 1.2-kb *Bst*EII fragment of pMN302 was then ligated into the *Cla* I site of plasmid pBR322 (22) to yield plasmid pMN330 and into the *Sma* I site of vector pT7-6 (23) to give plasmid pMN340. For *selA* overexpression, the 1.7-kb *Sal* I–*Cla* I insert of plasmid pWL171 (7) was ligated into *Sal* I/*Cla* I-cut vector pT7-6 to result in plasmid pWL187. Formation of the SelD protein was followed by [^{35}S]-methionine incorporation (23) by strain K38 transformed with pT7-6 or pMN340.

Construction of Strains WL400 and WL60153. The chromosomal *selD* gene was inactivated by inserting a 1.2-kb *Hin*PI fragment of pACYC184 carrying the *cat* gene into the *Cla* I site of pMN330 and by subsequent homologous recombination (11, 24) to yield strain WL400 (*selD204::cat*). Strain WL60153 (Δ*selAB*, *selD204::cat*) was constructed by transducing *selD::cat* from WL400 into strain WL30153 (Δ*selAB*) (7).

Preparation of Extracts for the Enzymatic Conversion of Seryl-tRNA$_{UCA}$ to Selenocysteinyl-tRNA$_{UCA}$. Plasmids pWL187 and pMN340 were used to overexpress *selA* and *selD*, respectively, in the T7 polymerase system (23) using strain WL60153 as host. Supernatants were prepared by

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Abbreviations: FDH, formate dehydrogenase; mnm^{5}Se^{2}U, 5-methylaminomethyl-2-selenouridine.
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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M30184).

Table 1. *E. coli* strains used

Strain	Relevant genotype	Reference or derivation
MC4100	*sel⁺*	9
WL400	MC4100, *selD204::cat⁺*	This work
WL30153	MC4100, Δ(*selAB*)	7
WL60153	WL30153, *selD204::cat⁺*	This work
JC10289	Δ(*srl–recA)306::*Tn*10*	10
CES200	*recB21 recC22 sbcB15*	11
MB08	*selD165*	6–8
MN30	MB08, Δ(*srl–recA)306::*Tn*10*	This work
K38	HfrC *phoA4 pit-10 tonA22 ompF627 relA1* λ⁺	12

centrifugation at 100,000 × *g* and desalted by gel filtration through a Sephadex G-25 column. The resulting extract (termed S100/G25) was portioned, quick-frozen, and stored at −70°C under a N_2 atmosphere. Protein was determined by the method of Lowry *et al.* (25) with bovine serum albumin as a standard.

Purification and Aminoacylation of tRNA$_{UCA}$ and of Cognate Serine Isoacceptors. Sixty milligrams of bulk tRNA, prepared from *E. coli* strain FM420 carrying plasmid pMN81 (5) was applied to a BD-cellulose column (Serva) (20 × 120 mm) equilibrated with buffer 1 (50 mM NaOAc, pH 5.0) containing 0.4 M NaCl and the column was developed with 600 ml of a linear gradient of 0.4 M NaCl to 1.1 M NaCl in buffer 1. On completion of the gradient, the strongly adsorbed material was eluted with 1.5 M NaCl/50 mM NaOAc, pH 5.0/14% (vol/vol) ethanol (26). Fractions containing tRNA$_{UCA}$, as monitored by gel electrophoresis (15), were pooled, and the tRNA was precipitated by the addition of 2 volumes of ethanol and finally dissolved in 2.5 ml of buffer 2 (10 mM NaOAc, pH 4.5/10 mM $MgCl_2$/1 mM dithiothreitol/1 mM EDTA) containing 1.3 M $(NH_4)_2SO_4$.

The tRNA was applied to a Sepharose 4B column (12 × 100 mm) equilibrated with buffer 2 containing 1.3 M $(NH_4)_2SO_4$ (27). The column was developed with 300 ml of a gradient decreasing from 1.3 M $(NH_4)_2SO_4$ to 0.3 M $(NH_4)_2SO_4$ in buffer 2. Fractions containing purified tRNA$_{UCA}$ were pooled and dialyzed against three changes (2 liters each) of 10 mM NaOAc (pH 4.5), and the tRNA was collected by precipitation. The tRNA was charged with L-[^{14}C]serine (170 µCi/µmol; 1 µCi = 37 kBq) as described (5).

Enzymatic Conversion of Seryl-tRNA$_{UCA}$ to Selenocysteinyl-tRNA$_{UCA}$. The complete reaction mixture (50 µl) for conversion of seryl-tRNA$_{UCA}$ to selenocysteinyl-tRNA$_{UCA}$ contained 100 mM Hepes (pH 7.5), 10 mM Mg(OAc)$_2$, 70 pmol of L-[^{14}C]seryl-tRNA (171 µCi/µmol), 450 pmol of Na$_2$Se, 0.5 mM dithiothreitol, and S100/G25 extract to give a final protein content of 100 µg. The reaction mixture was incubated at 30°C for 30 min. After 1 volume of 0.1 M NaOAc (pH 4.6) was added to the mixture, the aminoacylated tRNA was extracted with phenol, precipitated from the aqueous phase with ethanol, and dried *in vacuo*.

Hydrolysis of the aminoacyl-tRNA ester bond was carried out by incubation for 40 min at 40°C in 150 mM NH$_4$HCO$_3$ (pH 8.8). All procedures described were conducted in an anaerobic chamber under an atmosphere of 96% N_2 and 4% H_2. After hydrolysis of the aminoacyl-tRNA ester bond, 1 µg of authentic DL-selenocysteine was added. The selenocysteine was then oxidized to the diselenide, selenocystine, by exposure to air (6). The solution was lyophilized and redissolved in water. Compounds were then analyzed by thin-layer chromatography under aerobic conditions (6). Authentic selenocystine was detected with the ninhydrin reagent.

RESULTS

Cloning, Nucleotide Sequence, and Expression of *selD*. Mutant MB08 from *E. coli* has been described as a strain that is pleiotropically defective in both FDH$_H$ and FDH$_N$ activity (8). A subsequent analysis revealed that it is deficient in the incorporation of selenium into both protein and tRNA (7). Cloning of the respective gene, *selD* (7), delivered plasmid pMN302, which carries a 3.8-kb chromosomal *Sau*3A1 fragment; its restriction map is given in Fig. 1. To narrow down the complementing region within the insert of pMN302, the plasmid was cut with *Mlu* I, the protruding ends were filled in with DNA polymerase Klenow fragment, and the linearized plasmid was religated. The mutant derivative had lost its complementation capacity, indicating that the *Mlu* I site is situated within the *selD* coding region (data not shown).

The *Mlu* I site, therefore, could be conveniently used for determining the nucleotide sequence in both directions by employing the strategy detailed in Fig. 1. The sequence (Fig. 2) contained an open reading frame coding for a putative gene product of 347 amino acids with a calculated molecular weight of 36,687.

Analysis of the sequence for restriction sites suitable for subcloning revealed that a *Bst*EII site borders the coding region at the 5' and 3' ends. To test whether the gene could be expressed and the putative gene product synthesized, the 1.2-kb *Bst*EII fragment was isolated and cloned into the expression vector pT7-6 to yield plasmid pMN340; it carries the open reading frame under the control of the phage T7 φ10 promoter (23). In an [^{35}S]methionine-labeling experiment, bacteria carrying pMN340 synthesized a protein of the size expected from the deduced amino acid sequence (Fig. 3).

The *selD* Gene Product Has a Role in Selenium Insertion into Both Protein and tRNA. The deficiency of mutant MB08 in the incorporation of selenium into selenopolypeptides and into the modified nucleotides of tRNA could be due to two independent mutations, only one of which (that responsible for incorporation into protein) is complementable by the cloned gene. To test this possibility, we disrupted the open reading frame and replaced the chromosomal gene by the plasmid-encoded, disrupted gene. The resulting strain, WL400, is an MC4100 derivative with a *cat* gene cartridge inserted in the *selD* open reading frame. It is evident that the insertional inactivation of *selD* causes the loss of the capacity to incorporate selenium into the 80-kDa and 110-kDa selenopolypeptides of FDH$_H$ and FDH$_N$, respectively, and into the modified nucleosides of tRNA (Fig. 4). Transformation of WL400 with plasmid pMN330 (*selD⁺*) restores the capacity for selenation of these macromolecules.

In Vitro Conversion of L-Seryl-tRNA$_{UCA}$ to Selenocysteinyl-tRNA$_{UCA}$. An *in vitro* system was established to assess the role of *sel* gene products, in particular that of the *selD* gene, in selenocysteine biosynthesis. To this end, strain WL60153 was

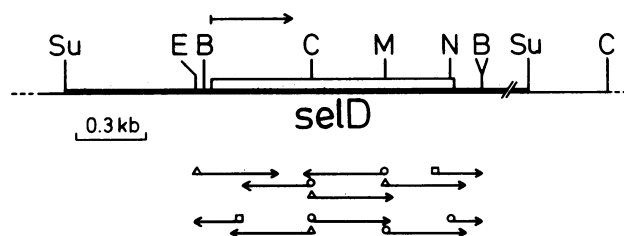FIG. 1.   Restriction map of the DNA fragment of the 3.8-kb insert of pMN302 coding for the *selD* gene. The arrow above the restriction map signifies the transcriptional orientation of the *selD* gene. The arrows below give the sequencing strategy: Δ, 5' labeling; ○, 3' labeling; □, DNA sequenced by the dideoxy chain-termination method. B, *Bst*EII; E, *Eco*RI; C, *Cla* I; M, *Mlu* I; N, *Nco* I; Su, *Sau*3A1.

Biochemistry: Leinfelder *et al.*

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-37                                          GGTTACC ATACCCGGAT TGCATTGACA GGAGATGTCC

  1 ATG AGC GAG AAC TCG ATT CGT TTG ACC CAA TAC AGC CAC GGA GCT GGT TGC GGC TGT AAA ATT TCC CCA AAA GTG TTG GAA ACC
  1 Met Ser Glu Asn Ser Ile Arg Leu Thr Gln Tyr Ser His Gly Ala Gly Cys Gly Cys Lys Ile Ser Pro Lys Val Leu Glu Thr

 85 ATC CTG CAT AGT GAG CAG GCG AAG TTT GTT GAT CCG AAT TTG CTT GTG GGT AAT GAA ACC CGC GAC GAT GCG GCG GTG TAC GAT
 29 Ile Leu His Ser Glu Gln Ala Lys Phe Val Asp Pro Asn Leu Leu Val Gly Asn Glu Thr Arg Asp Asp Ala Ala Val Tyr Asp

169 CTG GGC AAT GGC ACC AGC GTT ATC AGT ACC ACC GAC TTC TTT ATG CCG ATC GTT GAT AAT CCT TTC GAT TTT GGC CGC ATT GCG
 57 Leu Gly Asn Gly Thr Ser Val Ile Ser Thr Thr Asp Phe Phe Met Pro Ile Val Asp Asn Pro Phe Asp Phe Gly Arg Ile Ala

253 GCG ACT AAC GCC ATC AGC GAT ATC TTC GCG ATG GGG GGC AAA CCG ATT ATG GCG ATT GCG ATC CTC GGC TGG CCG ATT AAC AAA
 85 Ala Thr Asn Ala Ile Ser Asp Ile Phe Ala Met Gly Gly Lys Pro Ile Met Ala Ile Ala Ile Leu Gly Trp Pro Ile Asn Lys

337 CTT TCC CCA GAA ATT GCC CGC GAA GTG ACC GAA GGT GGA CGC TAT GCA TGT CGT CAG GCG GGT ATT GCG CTG GCT GGC GGT CAC
113 Leu Ser Pro Glu Ile Ala Arg Glu Val Thr Glu Gly Gly Arg Tyr Ala Cys Arg Gln Ala Gly Ile Ala Leu Ala Gly Gly His

421 TCC ATC GAT GCG CCG GAG CCG ATT TTT GGT CTG GCG GTA ACG GGG ATC GTA CCG ACC GAG CGG GTG AAG AAA AAC AGT ACC GCA
141 Ser Ile Asp Ala Pro Glu Pro Ile Phe Gly Leu Ala Val Thr Gly Ile Val Pro Thr Glu Arg Val Lys Lys Asn Ser Thr Ala

505 CAA GCC GGA TGC AAA CTG TTC CTG ACG AAA CCG CTG GGG ATC GGC GTT CTT ACC ACG GCT GAG AAA AAA TCA CTG TTG AAA CCA
169 Gln Ala Gly Cys Lys Leu Phe Leu Thr Lys Pro Leu Gly Ile Gly Val Leu Thr Thr Ala Glu Lys Lys Ser Leu Leu Lys Pro

589 GAA CAT CAG GGA CTG GCG ACG GAA GTG ATG TGC CGG ATG AAC ATC GCA GGC GCG TCC TTT GCC AAC ATC GAA GGC GTA AAA GCG
197 Glu His Gln Gly Leu Ala Thr Glu Val Met Cys Arg Met Asn Ile Ala Gly Ala Ser Phe Ala Asn Ile Glu Gly Val Lys Ala

673 ATG ACC GAC GTT ACG GGC TTT GGT CTG CTG GGC CAC TTG AGC GAA ATG TGT CAG GGG GCT GGT GTG CAG GCA CGC GTC GAC TAT
225 Met Thr Asp Val Thr Gly Phe Gly Leu Leu Gly His Leu Ser Glu Met Cys Gln Gly Ala Gly Val Gln Ala Arg Val Asp Tyr

757 GAA GCG ATC CCG AAA CTC CCC GGT GTT GAA GAG TAC ATT AAG TTG GGC GCA GTA CCT GGC GGC ACT GAA CGT AAC TTT GCC AGC
253 Glu Ala Ile Pro Lys Leu Pro Gly Val Glu Glu Tyr Ile Lys Leu Gly Ala Val Pro Gly Gly Thr Glu Arg Asn Phe Ala Ser

841 TAC GGT CAT CTG ATG GGT GAA ATG CCG CGT GAA GTG CGC GAT CTG CTG TGT GAT CCG CAA ACT TCT GGC GGT TTG CTG CTG GCG
281 Tyr Gly His Leu Met Gly Glu Met Pro Arg Glu Val Arg Asp Leu Leu Cys Asp Pro Gln Thr Ser Gly Gly Leu Leu Leu Ala

925 GTC ATG CCG GAA GCA GAA AAT GAG GTC AAA GCT ACA GCC GCC GAG TTT GGC ATT GAA CTG ACG GCA ATT GGC GAA CTG GTG CCA
309 Val Met Pro Glu Ala Glu Asn Glu Val Lys Ala Thr Ala Ala Glu Phe Gly Ile Glu Leu Thr Ala Ile Gly Glu Leu Val Pro

1009 GCG CGC GGC GGT CGT GCC ATG GTT GAG ATT CGT TAA TTCAATGCGG TTGTTTATTG CCGAAAAACC GAGTCTGGCG CGCGCCATTG CTGATGTC
337 Ala Arg Gly Gly Arg Ala Met Val Glu Ile Arg ***

1102 CT GCCCAAACCG CACCCGAAAG GCGATGGCTT TATCGAGTGC GGTAATGGTC AGGTGGTGAC C
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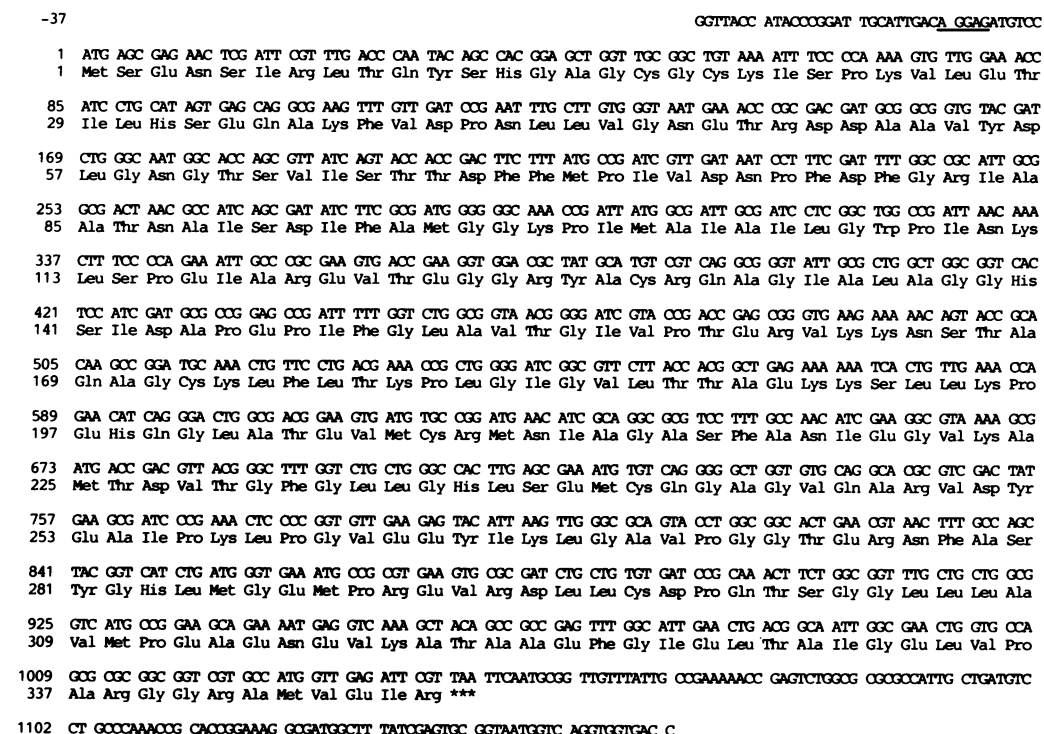
FIG. 2. Nucleotide sequence and derived amino acid sequence of the *selD* gene from *E. coli* MC4100. The sequence of the noncoding, RNA-like strand is given 5' to 3'; the adenosine of the putative ATG initiation codon is taken as +1. The putative ribosome binding sequence is underlined and the termination codon TAA is indicated by stars.

constructed, which carries a deletion of the *selAB* genes plus an insertionally inactivated *selD* gene. It was transformed with either plasmid pWL187 (*selA*⁺) or pMN340 (*selD*⁺); after induction of expression of *selA* and *selD*, crude extracts were prepared. tRNA$_{UCA}$, purified by the procedure given in Fig. 5, charged with L-[^{14}C]serine was used as substrate. The seryl-tRNA$_{UCA}$, as well as serine-inserting seryl-tRNA (as control), were incubated with extracts from strain WL60135 in which either the *selA* or *selD* gene had been overexpressed. The extracts were used either singly or in combination, and in the

presence or absence of sodium selenide. The products of the reaction were subjected to thin-layer chromatography and visualized by autoradiography (Fig. 6). L-Serine was converted to selenocysteine only when the reaction mixture
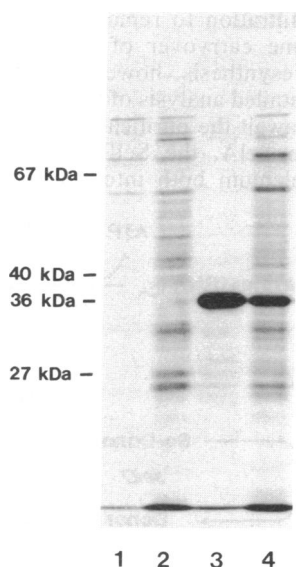

FIG. 3. Expression of the *selD* gene product from plasmid pMN340 in the T7 system, monitored by [^{35}S]methionine labeling and NaDodSO₄/PAGE (23). Lanes: 1 and 2, vector pT7-6; 3 and 4, pMN340 (*selD*⁺). Cells were induced in the presence (lanes 1 and 3) or absence (lanes 2 and 4) of rifampicin.
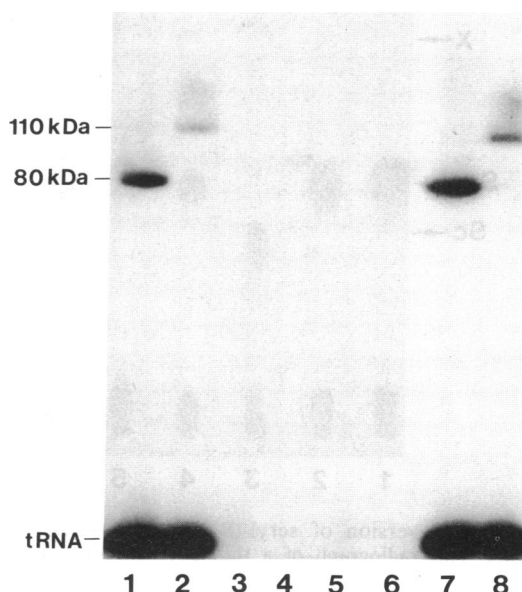

FIG. 4. ^{75}Se incorporation by wild-type *E. coli* MC4100 and by the *selD*::*cat* mutant WL400. An autoradiograph of a NaDodSO₄/ 10% polyacrylamide gel is shown in which denatured lysates of labeled cells (1) were electrophoretically fractionated. The migration positions of the 80- and 110-kDa selenopolypeptides of FDH$_H$ and FDH$_N$, respectively, and of selenated tRNA are indicated. Lanes: 1 and 2, *E. coli* MC4100 (wild type); 3 and 4, WL400 (*selD*::*cat*); 5 and 6, WL400 carrying pBR322; 7 and 8, WL400 carrying pMN330. Cells were grown anaerobically in the absence (lanes 1, 3, 5, and 7) or presence (lanes 2, 4, 6, and 8) of nitrate (0.5%, wt/vol).
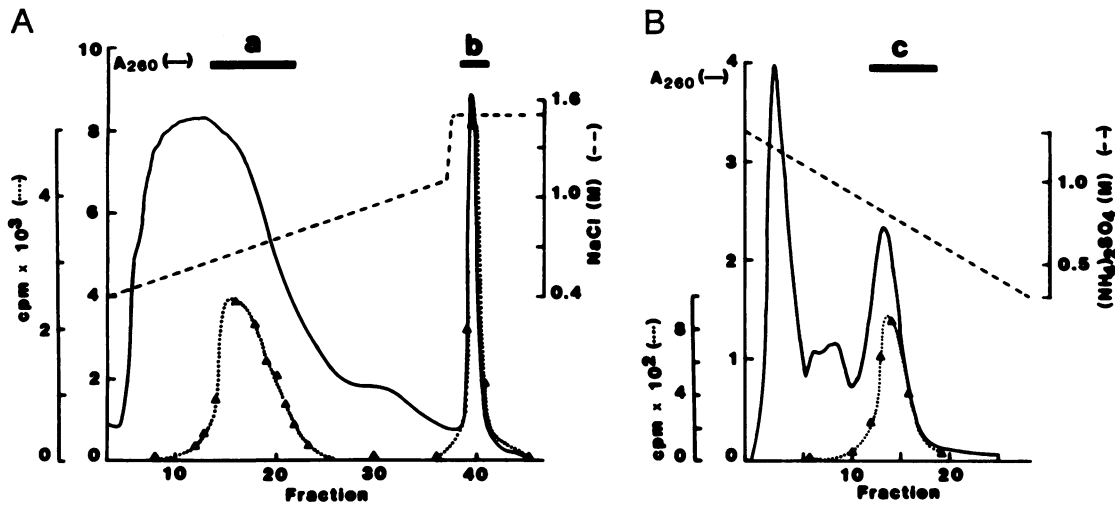
FIG. 5. Purification of tRNA$_{UCA}$ and cognate serine-inserting tRNA. (A) Chromatography of bulk tRNA on BD-cellulose. ▲, Serine acceptor activity. Pool a, tRNA$_{UCA}$; pool b, the serine-inserting isoacceptor species. (B) Chromatography of partially purified tRNA$_{UCA}$, corresponding to pool a in A, on Sepharose 4B using a decreasing salt gradient. ▲, Serine acceptor activity. Pool c contains essentially pure tRNA$_{UCA}$.

contained extracts with overproduced *selA* and *selD* gene products as well as sodium selenide. L-Serine charged to serine-inserting tRNA was not recognized as a substrate.

DISCUSSION

The proposed pathway of selenocysteine biosynthesis in *E. coli*, as suggested by previous work (5, 6) and by the results presented above, is depicted in Fig. 7. Biosynthesis starts with an L-serine residue aminoacylated to the *selC* gene
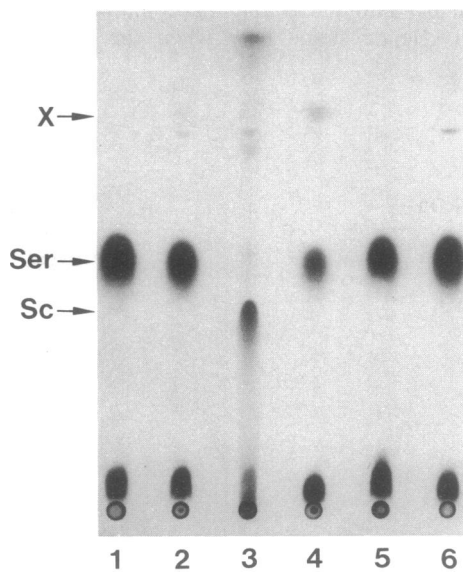
FIG. 6. Conversion of seryl-tRNA$_{UCA}$ to selenocysteinyl-tRNA$_{UCA}$. Autoradiograph of a thin-layer chromatogram of the deacylation products from tRNA$_{UCA}$ (lanes 1–5) and from cognate serine isoacceptor tRNA (lane 6). Seryl-tRNA$_{UCA}^{Ser}$ (lanes 1–5) was incubated with the following: lane 1, 450 pmol of Na$_2$Se; lane 2, S100/G-25 extracts of WL60153/pGP1-2/pWL187 (*selA*⁺) and WL60153/pGP1-2/pMN340 (*selD*⁺) (50 μg of protein from each extract); lane 3, as in lane 2 plus 450 pmol of Na$_2$Se; lane 4, 100 μg of an S100/G-25 extract of WL60153/pGP1-2/pWL187 (*selA*⁺) plus 450 pmol of Na$_2$Se; lane 5, 100 μg of a S100/G-25 extract of WL60153/pGP1-2/pWL340 (*selD*⁺) plus 450 pmol of Na$_2$Se. Lane 6, seryl-tRNA^{Ser} (serine-inserting) was incubated as indicated for lane 3. The migration positions of serine (Ser) and selenocysteine (Sc) are indicated. X denotes an unidentified compound formed by SelA-containing extracts in the absence of SelD protein.

product, tRNA$_{UCA}^{Ser}$. After activation at the hydroxyl group, the leaving group is replaced by a selenol moiety to yield selenocysteinyl-tRNA$_{UCA}^{Ser}$. In eukaryotes, it has been unequivocally demonstrated that the active serine derivative is O^3-phosphoseryl-tRNA (28, 29); the evidence presented by Mizutani *et al.* (30) that *E. coli* selenocysteine formation also involves O-phosphorylation of L-serine is less compelling. Experiments have yet to be presented which show that (*i*) the *in vitro* phosphorylation reaction is dependent on the presence of the *selC*-encoded tRNA and (*ii*) the phosphorylated product is identical with *O*-phosphoserine ester-bonded to tRNA (30).

Mutant studies (5, 6) had suggested that the *selA* gene product is specifically involved in the conversion of L-serine into selenocysteine. SelA might be the first enzyme to act on seryl-tRNA$_{UCA}$, since L-serine was consumed and an as yet unidentified compound appeared (Fig. 6, lanes 2 and 4) when the extracts in the *in vitro* conversion system contained overexpressed SelA and lacked SelD protein. Our conversion system did not contain ATP and the extracts employed were purified by gel filtration to remove low molecular weight compounds. Some carryover of ATP from the charging reaction or its resynthesis, however, cannot be ruled out completely. A detailed analysis of the serine-activation step, therefore, must await the purification of the SelA protein.

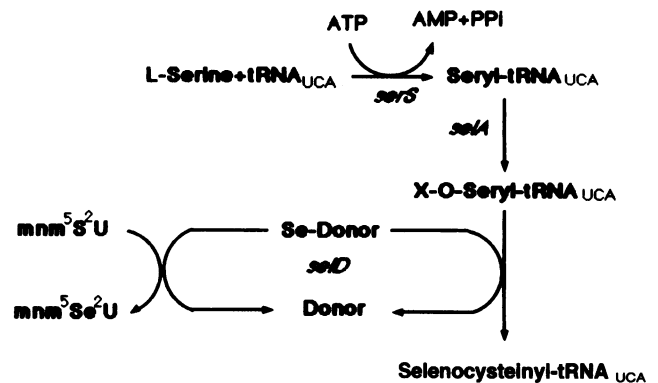In contrast to SelA, the SelD protein is required for incorporating selenium both into selenocysteine and into

FIG. 7. Pathway of selenocysteine biosynthesis and suggested role of the *selD* gene product. mnm⁵S²U, 5-methylaminomethyl-2-thiouridine; X-O-seryl-, serine residue activated at the hydroxyl group; *serS*, structural gene for serine–tRNA ligase.

mnm^{5}Se^{2}U (7). Kramer and Ames (31) have described a mutant ("selA1") from *Salmonella typhimurium* that exhibits a phenotype identical to that of *E. coli* MB08. Its defect could be complemented by plasmid pMN330, which carries the *E. coli selD* gene (32). The *selA1* locus of *S. typhimurium*, therefore, is functionally equivalent to the *E. coli selD* locus, although these loci are situated at different sites on the chromosomes of these closely related organisms (8, 31). Interestingly, the *Salmonella selA1* and the *E. coli selD* mutant effectively took up selenite from the medium and incorporated selenium nonspecifically into protein, as both selenocysteine and selenomethione (32). These facts preclude that the *selD* gene has a function in selenite uptake or in its intracellular reduction to selenide (32). Rather, the *in vitro* results have demonstrated that SelD is required for the enzymatic conversion of L-seryl-tRNA$_{UCA}$ to selenocysteinyl-tRNA; therefore, an analogous role in tRNA selenation also seems obvious. From a purely theoretical basis, it seems implausible to assume that the SelD protein recognizes both substrates for selenation, the 2-thiouridine precursor of mnm^{5}Se^{2}U (33) and X-O-seryl-tRNA$_{UCA}$. In accordance with previous arguments (32) we therefore propose that SelD has a function in delivering reduced selenium to two specific enzymes catalyzing the individual reactions. If this is the case, we have to postulate the existence of at least two additional *sel* gene products, one specific for selenating the X-O-serine residue and another for the sulfur/selenium exchange at the modified uridine. As yet, no specific mutants are available that are blocked in either of these reactions.

The extracts used in the *in vitro* synthesis of selenocysteine contained overexpressed SelA and SelD but, due to a *selB* deletion mutation in strain WL60153, lacked SelB protein. This provides evidence that SelB is not required for selenocysteinyl-tRNA formation. Indeed, recent experiments have shown that SelB is a translation factor specific for the insertion of selenocysteine into protein (34).

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