Cloning and characterization of the gene for *Escherichia coli* seryl-tRNA synthetase

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ABSTRACT

Seryl-tRNA synthetase is the gene product of the serS locus in *Escherichia coli.* Its gene has been cloned by complementation of a serS temperature sensitive mutant K28 with an *E.coli* gene bank DNA. The resulting clones overexpress seryl-tRNA synthetase by a factor greater than 50 and more than 6% of the total cellular protein corresponds to the enzyme. The DNA sequence of the complete coding region and the 5'- and 3' untranslated regions was determined. Protein sequence comparison of SerRS with all available aminoacyl-tRNA synthetase sequences revealed some regions of significant homology particularly with the isoleucyl- and phenylalanyl-tRNA synthetases from *E.coli.*

INTRODUCTION

Aminoacyl-tRNA synthetases are a structurally diverse set of enzymes (1) performing the same primary catalytic function viz. the aminoacylation of their cognate tRNAs. The primary structures of 16 of these enzymes from both prokaryotic an eukaryotic sources have been determined (see 2 for references). Only 4 crystalline aminoacyl-tRNA synthetases are under active investigation (3-6) and high resolution X-ray structures are available for the tyrosyl enzyme from *Bacillus stearothermophilus* (3) and a tryptic fragment of the methionyl enzyme from *Escherichia coli* (4).

Although all the aminoacyl-tRNA synthetases exhibit certain common properties such as binding of ATP, aminoacid and tRNA, there is no apparent concensus homology in the primary structures of the aminoacyl-tRNA synthetases so far sequenced. Webster et al (7) describe a sequence alignment of segments of polypeptide chain at the N-terminii of 4 enzymes which might be part of the catalytic core. However other synthetases, like the histidyl enzyme, do not show strong alignment in this region. More recently Hountondji et al (2) have aligned 11 amino acid segments from 11 enzymes.

Escherichia coli seryl-tRNA synthetase, SerRS, is an α_2-dimer with a subunit molecular weight of about 50,000 which has been purified to apparent homogeneity (8,9). The size of the subunit corresponds approximately to that of the catalytic core for aminoacyl-tRNA synthetases proposed by Jasin et al (10). Mutants for thermosensitive SerRS gene have been described and the structural gene has been localized on the *E.coli* chromosome at position 20 min. near serC, the gene for phosphohydroxy-pyruvate transaminase (11).

In this paper we report the cloning, overexpression and sequencing of serS as well as the purification of its gene product.

MATERIALS and METHODS
Bacterial Strains.

The serS temperature sensitive strain K28 (*phoA4, serC13, serS14, relA1, pit-10, spoT1 tonA22, T^R, supD32*) was obtained from the E.coli Genetic Stock Center, Yale University, New Haven through Dr.B.Bachmann. Strain CSH26 (*recA; (recA)Δ(pro lac)Δthi ara rpsL*) was provided by Dr.N. Mackman. Strain P678-54 (*F⁻ minA, minB, thr leu, thi, ara, lacY, gal, malA, xyl, mtl, tonA, rpsL, supE*)) for minicell preparation was donated by Dr.W.Goebel. Strain JM101 for M13 DNA sequencing was obtained from Amersham.

Gene library.

The gene bank DNA of E.coli K12 strain 1100 was a generous gift from A.Wittinghofer (12).

Enzymes and Chemicals.

Calf intestine alkaline phosphatase, *E.coli* DNA polymerase I (Klenow fragment), restriction enzymes, T4 DNA ligase, lysozyme, DNaseI and unfractionated *E.coli* tRNA were all from Boehringer. Octyl glucoside (practical, lot 14711) was a gift from Pfanstiehl Laboratories, Waukegan, Illinois. N-octyl-β-D-glucopyranoside was obtained from Bachem, Bubendorf. [^{14}C]L-Serine was obtained from Amersham. Sepharose Cl-6B was from Pharmacia and AcA 44 was from IBF. All other chemicals were of analytical quality.

Cloning of the serS gene.

K28 was transformed with a portion of the gene library DNA using the method described by Hanahan (13). The transformation mixture was incubated at 44°C on modified LB ampicillin plates (50μg/ml) containing only 0,05% NaCl as recommended by Clarke et al.(11), in order to use the temperature sensitive marker. A small fraction of the mixture was incubated at 28°C to determine the total number of transformants.

Aminoacylation assay for seryl-tRNA synthetase activity.

Cells were lysed by incubating for 30min in a buffer containing 60mM Tris-HCl (pH7.4), 2mM EDTA, 1mg/ml lysozyme. After addition of 0.04% sodium deoxycholate, 10mM $MgCl_2$ and 10mg of DNAse I, the crude extract was centrifuged for 5min. and the supernatant assayed. The reaction mixture contained 60mM. Tris-HCl (pH7.5), 10mM $MgCl_2$, 0.5mM DTE, 2mM ATP, 1mg/ml unfractionated tRNA, 20μM [^{14}C]L-Serine (173mCi/mmol) and enzyme fraction. Incubation was for 10min at 37°C, or for the ts-mutant, at the temperature indicated. The reaction was stopped with TCA and the reaction mixture put on a Whatman GF-A filter. Filters were washed dried and counted.

Analysis of plasmid-specific proteins by minicells.

The minicell producing strain P678-54 was transformed with plasmids of serS positive clones. Minicells were purified as described by Lewy (14) and labelled with 15μCi [^{35}S]-methionine assay medium for 45 min. Cells were centrifuged, lysed in electrophoresis sample buffer (15). TCA precipitable counts were collected on Whatman GF-A filters. Samples were electrophoresed on 12% SDS-polyacrylamide gels (15). [^{14}C]-labelled marker proteins (Amersham) were run in a separate lane. Gels were dried and autoradiographed.

Analysis of serS-specific proteins by immunblotting.

E.coli strains were grown in LB at 37°C to stationary phase. Samples (1ml) of the cell suspensions were centrifuged and the cells lysed in 0.5ml electrophoresis sample buffer containing SDS. Fractions (10μl) were separated on 12% SDS-polyacrylamide gels, proteins were electrophoretically transfered to nitrocellulose paper (16). The nitrocellulose paper was treated with a 1:200 diluted rabbit antiserum raised against highly purified seryl-tRNA synthetase (see below). Horseradish peroxidase conjugated anti-rabbit IgG was used as previously described (17). The coloration was performed with diaminobenzidine and H_2O_2 in the presence of nickel and cobalt ions (16).

DNA manipulations.

DNA manipulations were as described by Manniatis et al (18). DNA sequence was determined by the dideoxy-chain termination method of Sanger et al. (19), using M13mp8, M13mp9, M13mp18 and M13mp19 (20, 21).

Determination of polypeptide N-terminal sequence.

The N-terminal sequence was determined by Dr.Rainer Frank (EMBL, Heidelberg) by sequential Edmann degradation and identification of the first 25 amino acids by HPLC.

Isolation of seryl-tRNA synthetase.

CSH26 pSerS2 was cultured in 17litres LB supplemented with ampicillin (25mg/l) at 37°C using a 500ml overnight culture in the same medium as the innoculum. After 7 hours inncubation an A_{600} of 5.2 was reached and the bacteria (82g wet cell paste) were harvested.

The isolation procedure was essentially that previously used for the isolation of EF-Tu (22). The cell paste (82g) in 230ml buffer A (0.051 Tris-HCl, 0.5mM DTE, 10μM PMSF, 1mM NaN$_3$, 1mM EDTA pH7.6 at room temperature) was lysed with lysozyme, the lysate was made 10mM in MgCl$_2$ and then treated with sodium deoxycholate and DNase I. All subsequent steps were carried out at 4°C. The clarified lysate was applied to a column of Sepharose CL-6B (2.6cm x 30cm) equilibrated in buffer B (buffer A with 10mM MgCl$_2$ but without EDTA). The column was washed with 100ml buffer B and developed with a 3000ml linear gradient of 0-0.5M KCl in buffer B. Active fractions were detected by aminoacylation assays using the conditions of Katze and Konigsberg (8). The most highly active fractions were pooled (100ml) and solid ammonium sulphate (47.2g) added. The precipitated protein was recovered by centrifugation, taken up in 7ml buffer B and applied to a column of AcA 44 (2.6cm x 140cm) equilibrated in buffer B and the column developed with the same buffer. The most highly active fractions were pooled (50ml), ammonium sulphate added to 50% saturation and left overnight at 4°C. The precipitate was removed by centrifugation and the supernatant adjusted to 60% saturation in ammonium sulphate to precipitate the enzyme. By SDS-PAGE the enzyme was more than 95% pure and had a mobility corresponding to an M_r of 50,000. Approximately 180mg of enzyme was obtained which corresponded, by the assay of Katze and Konigsberg (8) to a yield of 52% but with twice the specific activity.

The enzyme was crystallized from ammonium sulphate solutions containing 0.5 or 1.0% octyl glucoside. Details of the procedure are described elsewhere (23).

RESULTS AND DISCUSSION
Cloning of serS.

A gene library from E.coli (strain 1100) was used which contained chromosomal fragments of about 5kb generated by partial digestion with Sau3A and HpaII cloned into pBR322 ClaI and BamHI sites (12). The temperature sensitive mutant for SerRS (strain K28) was transformed with the library DNA and grown at 44°C on LB ampicillin plates under low salt

Table 1: Seryl-tRNA synthetase activity.

STRAINS	28 °C	37 °C	44 °C
K28	0.18		0.00
K28 pSerS1	4.60		3.60
CSH26		0.26	
CSH26 pSerS1		13.10	
CSH26 pSerS2		14.00	

The enzyme activities are given in nmol/min/mg. Crude extract preparation and assay conditions are described under METHODS. The given temperature indicates the temperature of cell growth and assay.

conditions. The 36 clones which grew at the non-permissive temperature were isolated (the total number of transformants at 28°C was 2×10^6). The sizes of the plasmids in these clones were all in the range of 9-10 kb.

The seryl-tRNA synthetase activity of one of the isolated clones (pSerS1) was measured by the aminoacylation assay and compared with that of the ts-mutant (K28) and the wild type (CSH26) at different temperatures (see table 1). A 25 fold overproduction of SerRS activity by the clone K28 pSerS1 compared with K28 at 28°C clearly indicates a gene dose effect and that pSerS1 contains the
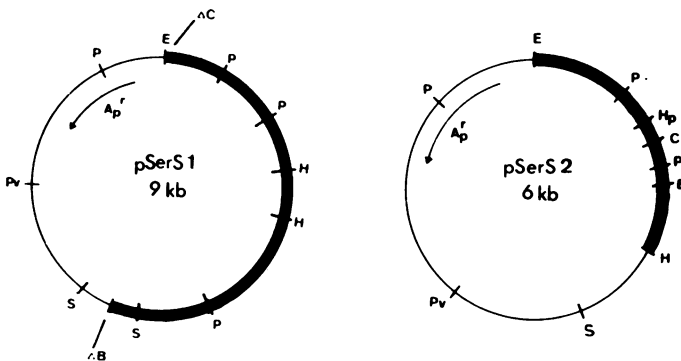
Figure 1: Restriction map of plasmid pSerS1 and pSerS2.
The gene for ampicillin resistance on pBR322 is marked by an arrow. Thick lines represent the inserted chromosomal DNA. Abbreviations for restriction enzymes are: B-BamHI, C-ClaI, E-EcoRI, H-HindIII, Hp-HpaI, P-PstI, Pv-PvuII, S-SalI. Restriction sites of ClaI(C) and BamHI (B) of pBR322 have been lost by the insertion of the chromosomal fragment. Plasmid pSerS2 contains the EcoRI-HindIII fragment of pSerS1
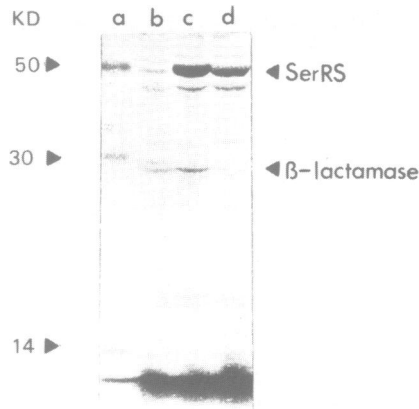
Figure 2: SDS-PAGE analysis of minicell proteins.

Minicells were prepared as described in METHODS, proteins were separated on a 12% SDS-polyacrylamide gel. Molecular weight markers were [^{14}C] methylated proteins from Amersham; 14K lysozyme, 30K carbonic anhydrase, 50K ovalbumin (the methylated ovalbumin from Amersham exhibited only one band of M_r 50,000 and not, as expected, two bands of 46,000 and 50,000). Lane a, marker proteins; lane b, minicells pBR322; lane c, minicells pSerS1; lane d, minicells pSerS2.

whole serS gene. The comparison of wild type (CSH26) with wild type containing pSerS1 at 37°C, shows a more than a 50 fold overproduction of the synthetase activity in the clone.

Subcloning of serS.

The coding region for seryl-tRNA synthetase is expected, on the basis of the reported molecular weight of the *E.coli* enzyme (8), to consist of approximately 1300 bp. Since pSerS1 contains an insert of about 5kb, the gene was subcloned. A restriction map of pSerS1 was constructed (Fig.1), and a 2kb EcoRI-HindIII fragment of pSerS1 was cloned into pBR322. The resulting clone, pSerS2, showed, like pSerS1, an overproduction of SerRS activity of more than 50 fold indicating that the EcoRI-HindIII fragment contained the whole serS gene.

Expression of serS in minicells.

To analyse plasmid specific expression of proteins in the clones the minicell system was used. Minicell producing strain P678-54 was transformed with pSerS1 and pSerS2. Minicells were prepared as described in METHODS. An autoradiogram of the [^{35}S]-methionine labelled proteins in minicells is shown in Fig.2. The predominant band at an apparent M_r of 47,000 corresponds to the
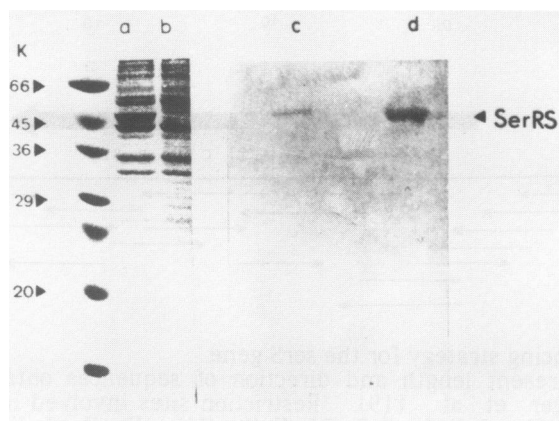
Figure 3: Expression of SerRS in normal cells and identification of the enzyme by immunoblotting.
MW standards: 20K, trypsin inhibitor; 29K, carbonic anhydrase; 36K, glyceraldehyde-3phosphate dehydrogenase; 45K, ovalbumin; 66K, bovine serum albumin. CSH26 pSerS2 (lane a) and CSH26 (lane b) were grown to stationary phase, cells were lysed in Laemmli buffer (15) and proteins analysed on a 12% SDS-polyacrylamide gel. Additionally separated proteins of CSH26 (lane c) and CSH26 pSerS2 (lane d) were electrophoretically transfered to a nitrocellulose filter (16) and treated with a 1:200 dilution of rabbit antiserum raised against purified SerRS. The binding of antibodies was detected by the horseadish peroxidase system (17) as described under METHODS.

subunit molecular weight of the 95,000 -103,000 α_2-dimer reported for SerRS (8,9).

Expression of serS in normal E.coli cells.

To estimate the quantity of SerRS expressed in pSerS2 a comparison was made of the total protein pattern of CSH26 with the clone CSH26 pSerS2 by SDS-PAGE (Fig.3). The clone expresses very strongly a protein M_r 47,000 (Fig.3a), corresponding to the value found for the purified enzyme, whereas the strain CSH26 without plasmid does not (Fig.3b). The expression of this protein seems to be higher than the expression of elongation factor Tu, which represents about 6% of the total cellular protein under standard conditions (24). The proteins, separated by SDS-PAGE, were transferred to nitrocellulose and were tested by immunoblotting using rabbit antiserum against highly purified SerRS. With CSH26 cells, a specific reaction was observed (Fig.3c) with a 47K protein indicating that the protein overproduced in CSH26 pSerS2 has the same molecular weight as that produced in normal cells. This result together with the expression of SerRS in minicells and the >50 fold enhancement of
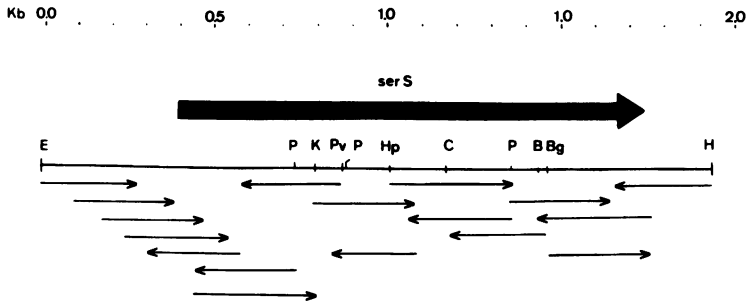
Figure 4: Sequencing strategy for the serS gene.
Arrows represent length and direction of sequences obtained by the method of Sanger et al. (19). Restriction sites involved in sequencing; B=BamHI, Bg=BglII, C=ClaI, E=EcoRI, H=HindIII, Hp=HpaI, K=KpnI, P=PstI, Pv=PvuII. The large arrow indicates the coding region for seryl-tRNA synthetase.

SerRS activity clearly shows that the EcoRI-HindIII fragment in pSerS2 contains the whole structural gene.

Isolation of SerRS.

The isolation of the enzyme was easily accomplished using only two chromatography steps over a period of three days. The180mg obtained from 82g of cell paste was at least 95% pure as judged by SDS-PAGE and Coomassie blue staining.

Sequencing of serS.

We sequenced the gene following the method of Sanger et al.(19). The sequencing strategy is shown in Fig.4,and the sequence obtained in Fig.5. The region sequenced included the 336bp of the 5'untranslated region, the 1290bp of the coding region and148bp of the 3'-untranslated region of serS. The N-terminal sequence of the protein was confirmed by independent protein sequencing by sequential Edman degradation and amino acid identification by HPLC of the first 25 residues. In Fig.5 only the sense strand for serS is shown. Nucleotide position 1 is arbitrarily set at the beginning of serS. At position -9 to -6 there is a short sequence (AGGA) which is complementary to the 3' end of 16S r-RNA and probably used as ribosome binding site (25). A possible promotor region (26) is underlined in Fig.5 (position -115/-143). Between residues 1313 and 1336 there is a region of two-fold symmetry, just 20bp after the TAA stop codon for the synthetase. This is probably the RNA polymerase termination site for serS (27). Interestingly there is another open reading frame on the opposite strand from position 852 to position 315;

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                                             ......tgacgtgccgttcatttgcg

          taatgcgccgacgaaattaatgaaggaaatgggctacgggcaggaatatcgttacgctcatgatgaggcaaacgcttat

          gctgccggtgaggtttacttcccgccggaaatagcacaaacacgctattatttcccgacaaacaggggccttgaaggca      -237

          agattggcgaaaagctcgcctggctggctgaacaggatcaaaatagccccataaaacgctaccgttaatgttatcgttg

          cggtaatgttgttactgtatccctgtggtcgcaggctgtggccacatctcccatttaattcgataagcacaggataagc      -79
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  1      M   L   D   P   N   L   L   R   N   E   P   D   A   V   A   E   K   L   A   R
         ATG CTC GAT CCC AAT CTG CTG CGT AAT GAG CCA GAC GCA GTC GCT GAA AAA CTG GCA CGC      60

 21      R   G   F   K   L   D   V   D   K   L   G   A   L   E   E   R   R   K   V   L
         CGG GGC TTT AAG CTG GAT GTA GAT AAG CTG GGC GCT CTT GAA GAG CGT CGT AAA GTA TTG     120

 41      Q   V   K   T   E   N   L   Q   A   E   R   N   S   R   S   K   S   I   G   Q
         CAG GTC AAA ACG GAA AAC CTG CAA GCG GAG CGT AAC TCC CGA TCG AAA TCC ATT GGC CAG     180

 61      A   K   A   R   G   E   D   I   E   P   L   R   L   E   V   N   K   L   G   E
         GCG AAA GCG CGC GGG GAA GAT ATC GAG CCT TTA CGT CTG GAA GTG AAC AAA CTG GGC GAA     240

 81      E   L   D   A   A   K   A   E   L   D   A   L   Q   A   E   I   R   D   I   A
         GAG CTG GAT GCA GCA AAA GCC GAG CTG GAT GCT TTA CAG GCT GAA ATT CGC GAT ATC GCG     300

101      L   T   I   P   N   L   P   A   D   E   V   P   V   G   K   D   E   N   D   N
         CTG ACC ATC CCT AAC CTG CCT GCA GAT GAA GTG CCG GTA GGT AAA GAC GAA AAT GAC AAC     360

121      V   E   V   S   R   W   G   T   P   R   E   F   D   F   E   V   R   D   H   V
         GTT GAA GTC AGC CGC TGG GGT ACC CCG CGT GAG TTT GAC TTT GAA GTT CGT GAC CAC GTG     420

141      T   L   G   E   M   H   S   G   L   D   F   A   A   A   V   K   L   T   G   S
         ACG CTG GGT GAA ATG CAC TCT GGC CTC GAC TTT GCA GCT GCA GTT AAG CTG ACT GGT TCC     480

161      R   F   V   V   M   K   G   Q   I   A   R   M   H   R   A   L   S   Q   F   M
         CGC TTT GTG GTA ATG AAA GGG CAG ATT GCT CGC ATG CAC CGC GCA CTG TCG CAG TTT ATG     540

181      L   D   L   H   T   E   Q   H   G   Y   S   E   N   Y   V   P   Y   L   V   N
         CTG GAT CTG CAT ACC GAA CAG CAT GGC TAC AGT GAG AAC TAT GTT CCG TAC CTG GTT AAC     600

201      Q   D   T   L   Y   G   T   G   Q   L   P   K   F   A   G   D   L   F   H   T
         CAG GAC ACG CTG TAC GGT ACG GGT CAA CTG CCG AAA TTT GCT GGC GAT CTG TTC CAT ACT     660

221      R   P   L   E   E   E   A   D   T   S   N   Y   A   L   I   P   T   A   E   V
         CGT CCG CTG GAA GAA GAA GCA GAC ACC AGT AAC TAT GCG CTG ATC CCA ACG GCA GAA GTT     720

241      P   L   T   N   L   V   R   G   E   I   I   D   E   D   D   L   P   I   K   M
         CCG CTG ACT AAC CTG GTG CGC GGT GAA ATC ATC GAT GAA GAT GAT CTG CCA ATT AAG ATG     780

261      T   A   H   T   P   C   F   R   S   E   A   G   S   Y   G   R   D   T   R   G
         ACC GCC CAC ACC CCA TGC TTC CGT TCT GAA GCC GGT TCA TAT GGT CGT GAC ACC CGT GGT     840

281      L   I   R   M   H   Q   F   D   K   V   E   M   V   Q   I   V   R   P   E   D
         CTG ATC CGT ATG CAC CAG TTC GAC AAA GTT GAA ATG GTG CAG ATC GTG CGC CCA GAA GAC     900

301      S   M   A   A   L   E   E   M   T   G   H   A   E   K   V   L   Q   L   L   G
         TCA ATG GCG GCG CTG GAA GAG ATG ACT GGT CAT GCA GAA AAA GTC CTG CAG TTG CTG GGC     960

321      L   P   Y   R   K   I   I   L   C   T   G   D   M   G   F   G   A   C   K   T
         CTG CCG TAC CGT AAA ATC ATC CTT TGC ACT GGC GAC ATG GGC TTT GGC GCT TGC AAA ACT    1020

341      Y   D   L   E   V   *   W   I   P   A   Q   N   T   Y   R   E   I   S   S   C   S
         TAC GAC CTG GAA GTA TGG ATC CCG GCA CAG AAC ACC TAC CGT GAG ATC TCT TCC TGC TCC    1080

361      N   V   W   D   F   Q   A   R   R   M   Q   A   R   C   R   S   K   S   D   K
         AAC GTT TGG GAT TTC CAG GCA CGT CGT ATG CAG GCA CGT TGC CGC AGC AAG TCG GAC AAG    1140

381      K   T   R   L   V   H   T   L   N   G   S   G   L   A   V   G   R   T   L   V
         AAA ACC CGT CTG GTT CAT ACC CTG AAC GGT TCT GGT CTG GCT GTT GGT CGT ACG CTG GTT    1200

401      A   V   M   E   N   Y   Q   Q   A   D   G   R   I   E   V   P   E   V   L   R
         GCA GTA ATG GAA AAC TAT CAG CAG GCT GAT GGT CGT ATT GAA GTA CCA GAA GTT CTG CGT    1260

421      P   Y   M   N   G   L   E   Y   I   G
         CCG TAT ATG AAC GGA CTG GAA TAT ATT GGC taatacccaattttctgaatctaaaaacgcctgcgggcg    1330
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          ctttttttgtctcccttTgataccgaacaataattactcctcacttacacgtaatactactttcgagtgaaaatctacct    1410

          atctctttgattttcaaattattcgatgtatacaagcctatatagcgaactgctatagaaataattacacaatacggtttg   1490

          ttactggaatcaatcgtgagcaagctt......                                                  1570
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Figure 5: DNA sequence of serS and derived aminoacid sequence for seryl-tRNA synthetase.

Only the sequence of the sense strand is shown. Numbering of the DNA sequence starts with ATG, the first codon for the gene product. Possible regulatory sequences like -35 and -10 transcription control elements (26), ribosome binding site (25) and stop of transcription signal (27) are underlined.

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SerRS (E.coli)        51 R H S R - S K S I G Q  60
MetRS (E.coli)       329 H G R K H S K S R G T 339
MetRS (S.cerevisiae) 522 E H G K F S K S R G U 532
IleRS (E.coli)       599 Q G R K H S K S I G H 609
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Figure 6. Homology between residues 51-60 of SerRS and peptides implicated in the binding of the 3'-adenosine of cognate tRNAs of aminoacyl-tRNA synthetases.

Only alignments for methionyl-tRNA sythetase (*E.coli* and yeast) and isoleucyl-tRNA synthetase (*E.coli*) are shown; for alignments with other synthetases see Hountondji et al (2).

comparison of the sequence of the corresponding hypothetical polypeptide with all sequences contained in the National Biomedical Research Foundation data bank showed no significant homology with any listed protein.

Aminoacid composition and codon usage.

The aminoacid composition of the protein sequence derived from the serS DNA sequence was compared with the amino acid analysis of the enzyme isolated by Katze and Konigsberg (8). The agreement between the two sets of values is good, with an average difference in the composition for each aminoacid of less than 3% and a maximum difference of less than 10%.

The codon usage of the serS gene resembles in general that of highly expressed proteins (28) with exception of Phe codons where UUU is prefered in the serS sequence. For serine a rather equal distribution of all 6 possible codons is found.

Homology with other aminoacyl-tRNA synthetases.

For a group of enzymes that perform essentially the same function, the aminoacyl-tRNA synthetases, with the exception of corresponding enzymes from different sources, exhibit remarkably little homology in their primary structures. Only a limited number of holmologies have been detected of rather limited extent and which do not span the whole range of enzymes so far sequenced (2,7,29 - 34).

Sequence homology searches were carried out on the aminoacyl-tRNA synthetase sequences in the NBRF and EMBL data banks, and on the aminoacyl-

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SerRS   78 L G E E L D R R K R E L D - - R L Q R E I R D I R L T I P H - L
β-PheRS 68 U Q Q R L H R R K R E L E S R R L H R - - R L R R E T I D U S L
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Figure 7. Alignment of sequences of regions in the N-terminal portions of SerRS and the β-subunit of *E.coli* phenylalanyl-tRNA synthetase (38) Identities and conservative differences ar indicated by the boxed sequences.

Figure 8. Alignment of sequences from SerRS and *E.coli* isoleucyl-tRNA synthetase (7).
Identities and conservative differences are indicated by the boxed sequences.

tRNA synthetase data bank at the Ecole Polytechnique with the assistance of Dr.Philippe Dessen. A correspondance was found between residues 50 to 60 of the SerRS sequence and the set of homologous sequences described for other aminoacyl-tRNA synthetases (2). This sequence (Figure 6) contains a lysine residue at position 56 which in corresponding sequences of the E.coli methionyl- and tyrosyl-tRNA synthetase has been implicated in the binding of the CCA end of their cognate tRNAs (35,36).

A more extensive region of homology was found between SerRS and the β-subunit of *E.coli* phenylalanyl-tRNA synthetase (Figure 7). Both these regions are situated towards the N-terminii of the respective proteins and are about 30 residues long. With 15 identities out of 29 amino acids and a further 5 conservative differences, this represents the largest homology so far observed between dissimilar aminoacyl-tRNA synthetases. Looking more closely at the N-terminal regions of all available aminoacyl-tRNA sequences other, less extensive homologies to these regions were found. A secondary structure prediction based on the method of Chou and Fasman (37) for the first 120 amino acid residues of SerRS and the β-subunit of phenylalanyl-tRNA synthetase (38) showed that both the homologous regions for these enzymes shown in Figure 7 have a high probability of being α-helices.

An interesting observation is the homology between a region of about 40 amino acids in the N-terminal portion of SerRS, and 2 regions situated in the C-terminal portion of *E.coli* isoleucyl-tRNA synthetase (7) separated by a 19 amino acid residue gap (Figure 8). For SerRS, this region includes the portion of the polypeptide chain shown in Figure 7.

ACKNOWLEDGEMENTS

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