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Seryl-tRNA synthetase from *Escherichia coli*: functional evidence for cross-dimer tRNA binding during aminoacylation

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ABSTRACT

***Escherichia coli* seryl-tRNA synthetase (SerRS) is a homo-dimeric class II aminoacyl-tRNA synthetase. Each subunit is composed of two distinct domains: the N-terminal domain is a 60 Å long, arm-like coiled coil structure built up of two antiparallel α-helices, whereas the C-terminal domain, the catalytic core, is an α–β structure overlying a seven-stranded antiparallel β-sheet. Deletion of the arm-like domain (SerRS Δ35–97) does not affect the amino acid activation step of the reaction, but reduces aminoacylation activity by more than three orders of magnitude. In the present study, it was shown that the formation of heterodimers from two aminoacylation defective homodimers, the N-terminal deletion and an active site mutant (SerRS E355Q), restored charging activity. The aminoacylation activity in a mixture containing the heterodimers was compared to that of solutions containing the same concentrations of homodimer. The activity of the mixture was eight times higher than the activities of the homodimer solutions, and reached 50% of the theoretical value that would be expected if 50% of the mixture was in the heterodimer form and assuming that a heterodimer contains only one active site. These results are in full agreement with the structural analysis of *E.coli* SerRS complexed with its cognate tRNA and provide functional evidence for the cross-dimer binding of tRNA in solution.**

INTRODUCTION

The fidelity of protein biosynthesis rests on the specific attachment of amino acids to their cognate tRNA species. This process is catalysed by the aminoacyl-tRNA synthetases, which discriminates with remarkable selectivity amongst many structurally similar tRNAs and amino acids. In spite of their common catalytic function, synthetases have long been known to be diverse in subunit structure, polypeptide size and amino acid sequence. Sequence analysis and X-ray crystallography of seryl-tRNA synthetase have shown that the 20 aminoacyl-tRNA synthetases can be partitioned into two distinct classes of 10 members each (1,2). Each class is characterised by different short sequence motifs and a distinctive topology of the active site (class I: Rossmann fold, class II: antiparallel fold). In addition class II synthetase show a clear modular domain structure, allowing their sub-classification (3). In addition to the catalytic core, these enzymes contain N-terminal or C-terminal extra domains. The diversity of structure and location of these extra domains make them candidates to act as tRNA binding domains. Crystallographic structures of the *E.coli* (4) and *Thermus thermophilus* seryl-tRNA synthetase–$tRNA^{Ser}$ complexes (5) show that the tRNA molecule binds across the dimer. In contrast to the glutaminyl-tRNA synthetase–$tRNA^{Gln}$ (6) and aspartyl-tRNA synthetase–$tRNA^{Asp}$ (7) complexes, the anticodon stem and loop of $tRNA^{Ser}$ do not contact the seryl enzyme. It contacts the T loop and the long extra-arm of the tRNA via its N-terminal arm. The importance of this N-terminal domain for aminoacylation activity and specificity has very recently been demonstrated (8). Mutant studies on $tRNA^{Ser}$ have also confirmed the importance of this arm by arm recognition of synthetase and tRNA (9). A similar cross-binding mechanism has been proposed for *Bacillus stearothermophilus* tyrosyl-tRNA synthetase (TyrRS) (10), a dimeric class I synthetase, on the basis of heterodimer studies. Below, we describe the formation of aminoacylation-active seryl-tRNA synthetase heterodimers from two aminoacylation defective enzymes and provide functional evidence for cross-dimer tRNA binding.

MATERIALS AND METHODS

Bacterial alkaline phosphatase was from Appligene and T4 DNA ligase was purchased from Boehringer Mannheim; 2′ deoxynucleoside 5′-triphosphates, DEAE–Sepharose CL6B Sepharose-4B were from Pharmacia; AcA 44 was from IBF; [^{3}H]ATP (0.4 Ci/mmol), L-[U-^{14}C]serine (228 mCi/mmol), [α-^{35}S]dATP (410 Ci/mmol) and Tub® DNA polymerase were from Amersham; pET16b vector and host strain HMS174 (DE3) were from

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Novagen; inorganic pyrophosphatase and unfractionated *E.coli* tRNA were from Sigma; oligonucleotides and restriction enzymes were from Eurogentec; Quiagen PCR purification kit was from Quiagen GmbH; Sep-Pac Vac 1 cc Cartidges (Accell Plus QMA) were from Waters. All other chemicals were of analytical quality.

Construction and overexpression of *serS* mutant genes

Deletion of the N-terminal arm-like structure: SerRS Δ35–97. The construction of the clone overexpressing this N-terminal deletion mutant is described in (8).

Construction of the active site mutant: SerRS E355Q. The SerRS E355Q mutant was constructed by site-directed mutagenesis using polymerase chain reaction (PCR), essentially as described in (11). The plasmid pSerS2, which contains the *E.coli serS* gene cloned in pBR322 (12), was used as template, and the following mutagenic $P_{1.2}$ and flanking ($P_{2.1}$ and $P_{3.1}$) primers were used:

$P_{1.2}$: 5′-CAGGAAGAGATCTG*ACGGTAGGTGTTCT-3′
$P_{2.1}$: 5′-ACCATCCCTAACCTGCCTGC-3′
$P_{3.1}$: 5′-CGGACGCAGAACTTCTGGTAC-3′

The asterisk above the mutagenic primer indicates the altered base (C→G) in the non-coding strand. The first PCR was carried out in 100 µl reaction buffer, using 10 ng of supercoiled DNA, 200 pmol each of $P_{1.2}$ and $P_{2.1}$, 40 nmol of each dNTP and 2.0–2.5 U of Tub DNA polymerase.

DNA amplification was carried out for 30 cycles of 1 min denaturation at 94°C, 2 min annealing at 37°C and 3 min elongation at 72°C. The 759 bp PCR product (PCR 1) was separated from unincorporated primers using a Quiagen PCR purification kit and was used as a primer in the second reaction. Conditions for the second PCR were identical to those of the first, except that the reaction mixture contained 100 pmol $P_{3.1}$ and ~250 ng of PCR 1. The second PCR product (960 bp) was purified as above, digested with *Kpn*I and *Bgl*II, and the 685 bp *Kpn*I–*Bgl*II fragment was cloned in pSerS2-1 (8) from which the wild-type *Kpn*I–*Bgl*II fragment had previously been removed. Plasmid DNA from several clones was then sequenced to test for the presence of the mutation; plasmid DNA from a clone containing the desired mutation was then sequenced completely between the *Kpn*I and *Bgl*II sites, to ensure the absence of additional mutations introduced during the PCR. The mutant *serS* gene was finally inserted into a pET vector (13) to overexpress the protein (8) in the host strain HMS 174 (DE3) (14). The overexpressed SerRS corresponded to 30% of the total soluble protein.

Purification of SerRS mutant enzymes

The mutant enzymes were purified from 18 l of bacterial culture in three fractionation steps following a modified scheme of Leberman (8). The proteins were fractionated on DEAE–Sepharose CL6B column, followed by a 40% ammonium sulphate precipitation. The supernatant was then applied to a Sepharose-4B column with a gradient from 1.5 to 0.5 M ammonium sulphate and finally a AcA 44 chromatography was used. The enzymes were at least 95% pure as judged by electrophoresis on SDS–polyacrylamide gels.

Seryl-AMP formation

The rate of seryl-AMP formation by SerRS E355Q was assayed in 40 µl reaction mixture containing 10 µM enzyme. The K_m value for serine was obtained at a fixed [^{3}H]ATP concentration of 100 µM (0.4 Ci/mmol) by varying the serine concentrations from 0 to 10 mM. The serine concentration was fixed at 10 µM and ATP concentration varied from 10 to 100 µM to determine the K_m for ATP. The reaction buffer used contained 50 mM MOPS, pH 7, 10 mM $MgCl_2$ and 0.05 U inorganic pyrophosphatase. The seryl-[^{3}H]AMP formed was quantified as previously described (8).

Aminoacylation reaction

The reaction mixture contained 50 mM Tris–HCl pH 7.6, 10 mM $MgCl_2$, 20 mg/ml unfractionated *E.coli* tRNA, 0.2 mM spermidine, 20 µM [^{14}C]serine 160 mCi/mmol and 400 nM SerRS E355Q. The [^{14}C]serine concentration was 20 µM and the ATP concentration was varied from 2 µM to 1 mM to determine the K_m value for ATP. For the K_m value for serine the ATP concentration was 2 mM and the [^{14}C]serine concentration was varied from 0 to 320 µM. After 10 min incubation at 37°C, the reaction was quenched on Whatman GFA filter pre-wetted with 20 µl 5% TCA. Filters were washed three times with 5% TCA, once with ethanol, once with ethanol/ether and finally ether, then dried and counted.

In vitro fomation of heterodimers

Samples (200 µl) of 20 nM SerRS E355Q, 20 nM SerRS Δ35–97 and a mixture of 10 nM of each, were treated with 0.4 M urea and dialysed overnight against a buffer containing 64 mM Tris–HCl pH 7.6, 10 mM $MgCl_2$, 1 mM DTT and 10 µM PMSF.

Ten µl of each sample were used to determine aminoacylation activity and the same volume was loaded on a non-denaturing PAGE. The stacking gel was 3% polyacrylamide in 24% saccharose, the running gel 8% polyacrylamide in 0.3 M Tris–HCl, pH 8.7 and 17% saccharose. The electrophoresis buffer was 40 mM Tris, 0.5 M glycine, pH 8.4 at the cathode and 4 mM Tris, 50 mM glycine, pH 8.4 at the anode. Electrophoresis was carried out at 100 V. The gel was stained with Coomassie Brilliant Blue G250. Bands of the non-denaturing gel were cut out and loaded on a 8% SDS–PAGE. Samples were preapared by heating to 100°C in 1 × SDS gel loading buffer (15).

RESULTS

In a recent paper we described the kinetic constants for the wild-type SerRS and for a N-terminal deletion mutant (SerRS Δ35–97) in which the two antiparallel α-helices forming an arm-like structure were replaced by a glycine residue (8).

The mutant enzyme has nearly wild-type kinetic constants for the first step of the aminoacylation reaction. The K_{app} value for L-serine is 64 mM for the wild type enzyme and 60 mM for the mutant. The K_{app} value for the ATP is 0.068 and 0.106 mM respectively for the wild type and the mutant. The rate constant is 0.34 s^{-1} for the wild type and 0.26 s^{-1} for the mutant enzyme. In contrast to the serine activation reaction the aminoacylation of $tRNA^{Ser}$ is dramatically affected in the N-terminal deletion mutant. The K_m for $tRNA^{Ser}$ is increased by a factor of 30 and the k_{cat} is 1000 times smaller for the SerRS Δ35–97 compared to the wild type enzyme.

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                          A4          H11     C2 LOOP L3   C3
                        ββββββββββββ αααααα   βββ          βββ ββ
SRSEC 351   :   NTYREISSCSNVWDFQARRMQARCRS-KS--DKKTRLVHT
SRSTT 341   :   GRYRETHSCSALLDWQARRANLRYRD-PE---GRVRYAYT
SRSBS 345   :   DTYREISSCSNFEAFQARRANIRFRREAK---GKPEHVHT
SRSCB 345   :   NKYREISSCSNCEDFQARRIQARWRNPKT---GKPELLHT
SRSSC 362   :   KEYKELVSCSNCTDYQSRNLEIRCGI-KKMEDREKKYVHC
SRSCH  81*  :   GAFRELVSCSNCTDYQARRLRIRYGQTKKMMDKVEF-VHM
                    Δ
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Figure 1. Alignment of seryl-tRNA synthetase (SerRS) sequences in the region of residue Glu 355. SerRS from *E.coli* (SRSEC, P09156*), from *T.thermophilus* (SRSTT, P34945*), from *Bacillus subtilis* (SRSBS, gi|467403*), from *Coxiella burnetii* (SRSCB, X75627*), from yeast (SRSSC, P07284*) and from Chinese hamster (SRSCH, P26636*). The conserved glutamic acid residue (E355 in SRSEC) and secondary structure elements for SRSEC are indicated. Strictly conserved residues are in bold. *GenBank/EMBL accession numbers.

Determination of kinetic parameters for the mutant SerRS E355Q

Active site mutant SerRS E355Q. Glu 355 of SerRS is an active site residue which is not part of a characteristic class II motif (1). It is, however, conserved in all seryl-tRNA synthetase sequences studied so far (12,16–18) and is part of a β-strand (A4) situated between motif 2 and motif 3 in the primary sequence. An alignment of different SerRS sequences in this region is shown in Figure 1. In the homologous *T.thermophilus* structure, the corresponding residue interacts with the 3′-OH of the ribose of the substrate ATP and the active site Mg^{2+} ion (19). To eliminate or to reduce at least significantly the aminoacylation activity of SerRS, this residue was changed by PCR-driven site-directed mutagenesis from Glu to Gln. To determine the rate constant and K_m values for L-serine and ATP in the reaction of seryl AMP formation for the SerRS E355Q, we initially used the same conditions previously described (8). No seryl-AMP formation could be detected in the absence of pyrophosphatase. Pyrophosphate formed in the amino acid activation stage is known to be a potent inhibitor of aminoacylation and cleavage of pyrophosphate by inorganic pyrophosphatase can substantially increase the activity of aminoacyl tRNA synthetases (20). Therefore, we re-determined the kinetic parameter in the presence of pyrophosphatase. In this case, kinetic parameters can be measured according to the Michaelis–Menten equation.

Serine activation activity of the active site mutant. The K_{app} values for the mutant enzyme SerRS E355Q were increased by more than two orders of magnitude compared to the wild type enzyme (Table 1). Since the K_{app} values for the wild type enzyme were determined in the absence of inorganic pyrophosphatase, the difference in the K_{app} values for serine and ATP may be even larger. The rate constants for the mutant enzyme were also strongly decreased (more than two orders of magnitude). This leads to a dramatically decreased catalytic efficiency of the mutant enzyme.

Aminoacylation reaction for the active site mutant. Aminoacylation activity was determined as described in Materials and Methods. Table 2 shows that the k_{cat} values for the mutant enzyme are 100 times decreased compared to the wild type enzyme. The K_m values for serine (135 compared to 53 μM for the SerRS wild type) and ATP (43 compared to 25 μM for SerRS wild type) were slightly increased. This seems contradictory to the K_{app} values obtained for the first step of the reaction. One explanation is that mutant enzyme has zero aminoacylation activity and the residual activity measured is due to a 1% contamination by the chromosomal encoded wild type SerRS. In contrast to the amino acid activation reaction, the K_m and k_{cat} values in the aminoacylation reaction are measured in a turn-over reaction.

Table 1. Kinetic parameter for wild type SerRS and SerRS E355Q in the first step of the reaction (seryl-AMP formation)

Enzyme	Substrate	*Kapp* (μM)	Rate constant (s^{-1})	Relative rate constant/K_{app} ($s^{-1}\mu M^{-1}$)
SerRS E355Q	serine	8790	8×10^{-4}	1.7×10^{-5}
	ATP	22	4.8×10^{-4}	4.2×10^{-6}
SerRS[a]	serine	64	0.34	1
	ATP	0.068	0.35	1

[a]Indicates that the kinetic parameters for the wild type SerRS had to be determined in presence of pyrophosphatase (8).

Table 2. K_{cat} values for SerRS E355Q compared to the wild type enzyme in the aminoacylation reaction

Enzyme	substrate	k_{cat} (s^{-1})
SerRS E355Q	serine	<0.007
	ATP	<0.008
SerRS WT	serine	0.8
	ATP	0.8

To test this hypothesis we used the seryl adenylate analogue 5′-*O*-[*N*-(L-seryl)-sulfamoyl] adenosine (Ser-AMS) (19) as an inhibitor of the aminoacylation reaction. The aminoacylation reaction was carried out as described in Materials and Methods, and Ser-AMS was added to final concentrations ranging from 0 to 400 nM. Figure 2 shows the titration of 10 nM wild type enzyme and 200 nM SerRS E355Q with Ser-AMS inhibitor. The inhibition profiles for both enzymes were similar. A Ser-AMS concentration of 40 nM was sufficient to inhibit 50% activity of 10 nM wild type or 69% of 200 nM SerRS E355Q enzyme. For the mutant enzyme, this inhibitor concentration was lower than the enzyme concentration. Therefore, the inhibition of the mutant enzyme can at least partly be explained by the inhibition of a contaminating wild type activity. Considering that the affinity of Ser-AMS is similar for both enzymes, the upper level for the content of wild type enzyme can be estimated at 3.6%.

Strategy to test cross-dimer tRNA binding during aminoacylation

Mutant studies on $tRNA^{Ser}$ have demonstrated the importance of the extra arm for synthetase recognition (9,21) and have led, together with structural information (22), to the model proposed by Asahara *et al.* (21), where each of the two tRNA molecules binds to two monomers of the synthetase (cross binding). In this model, the extra arm is in contact with the N-terminal arm like domain of one subunit, whereas the acceptor stem of the same molecule binds to the active site (core region) of the other subunit.

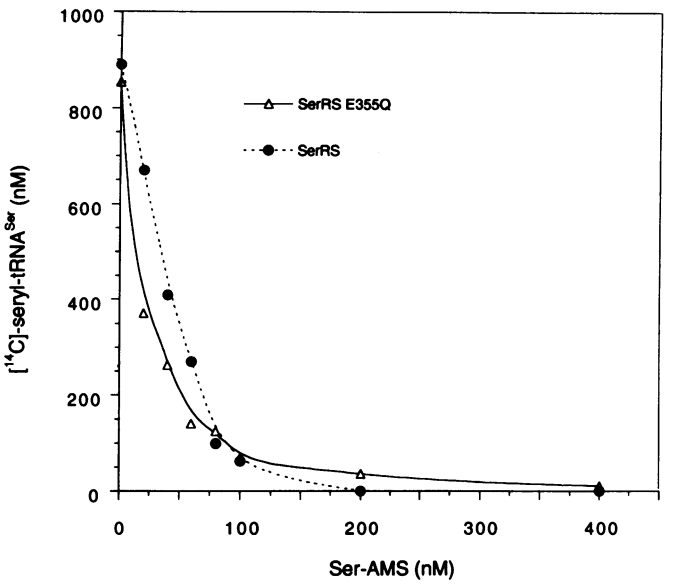

Figure 2. Inhibition of aminoacylation reaction by the seryl-adenylate analogue 5′-*O*-[*N*-(L-seryl)-sulfamoyl] adenosine (Ser-AMS). Aminoacylation activity of 10 nM wild type SerRS and 200 nM SerRS E355Q was determined in the presence of increasing concentrations of Ser-AMS ranging from 0 to 400 nM.

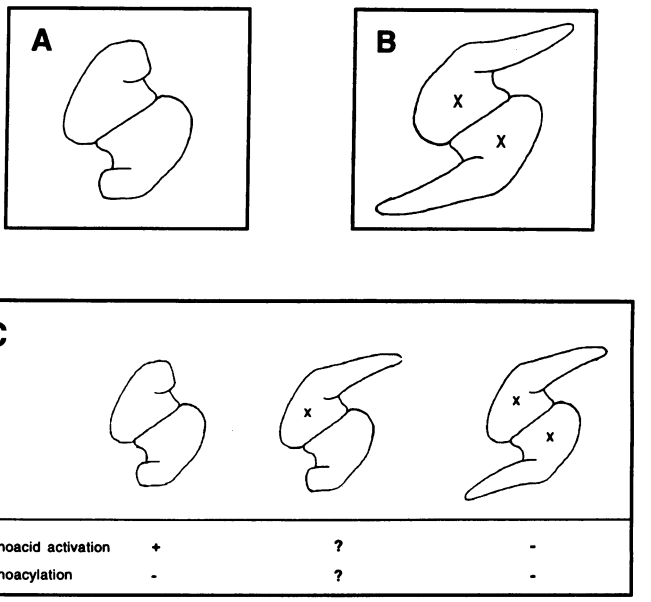

Figure 3. Strategy to test the cross-dimer mode of tRNA binding. (**A**) Homodimer SerRS Δ35–97 with N-terminal deletion (**B**) Homodimer SerRS E355Q. The x marks the mutation in the active site. (**C**) Heterodimer containing solution. The N-terminal truncated form (A) is aminoacylation defective, but activates serine like wild type enzyme. The active site mutant (B) shows a strongly decreased catalytic efficiency in serine activation leading to a dramatically reduced aminoacylation activity. If the tRNA(s) bind to each subunit separately no additional activity should be observed after heterodimer formation. In the case of tRNA cross-dimer binding however a heterodimer composed of an intact active site (N-terminal-truncated subunit) and a subunit containing a tRNA binding domain (with a defective active site) should lead to a significant increase in aminoacylation activity. The schematic drawing of wild-type SerRS was taken from (21) and modified.

The basic features of this model were confirmed by the crystallographic structures of *E.coli* and *T.thermophilus* SerRS complexed with its cognate tRNA (4,5).

To test the cross-binding of the tRNA, two aminoacylation defective enzymes were used (Fig. 3). The N-terminal truncated form activates serine as well as the wild type enzyme but has only residual aminoacylation activity. The active site mutant shows a strongly decreased catalytic efficiency in serine activation, leading to a dramatically reduced aminoacylation activity. If the tRNA(s) bind to each subunit separately no additional activity should be observed after heterodimer formation. In the case of tRNA cross-dimer binding, however, a heterodimer composed of an intact active site and a subunit containing a tRNA binding domain should lead to a significant increase in aminoacylation activity.

In vitro formation of heterodimers

Heterodimers of the two aminoacylation defective mutant proteins were formed by urea treatment as described in Materials and Methods. The appearance of an intermediate band in the urea treated mixture compared to the two mutant proteins after non-denaturing PAGE indicated the formation of heterodimers (Fig. 4). The band with the lowest mobility corresponded to the homodimer SerRS E355Q and that with the highest mobility to SerRS Δ35–97. The intermediate band on the non-denaturing gel was cut out and subjected to SDS–PAGE. Two bands of equal intensities with mobilities corresponding to the homodimers subunits of the two mutant proteins were observed, confirming the identity of the heterodimer form. Only ~20% of the total protein could be found in the heterodimer form and not 50% as expected indicating that the heterodimer form is slightly disfavoured or that the subunit exchange has not reached equilibrium. Even with urea concentrations up to 2 M, the ratio between hetero- and homodimers did not increase.

Aminoacylation activity of SerRS heterodimers

The aminoacylation reaction was performed as described in Materials and Methods. As positive control wild type SerRS was used. The aminoacylation activity for 0.2 pmol homodimers SerRS E355Q and SerRS Δ35-97 was compared to 0.2 pmol of the mixture containing heterodimers (Fig. 5). In agreement with the proposed model, the activity in the heterodimer mixture was eight times higher than the activities of the homodimer solutions and reached ~30% of the theoretical value one would expect if 50% of the mixture had been in the heterodimer form containing only one active site.

DISCUSSION

In this paper, we describe the formation of aminoacylation active seryl-tRNA synthetase heterodimers from two aminoacylation defective enzymes, and provide functional evidence for the requirement of cross-dimer tRNA binding during aminoacylation.The results are in full agreement with the structure of *E.coli* SerRS complexed with its cognate tRNA (4) and confirm the model suggested by Asahara and co-workers (21). In this model the extra-arm of one $tRNA^{Ser}$ molecule is in contact with the N-terminal arm-like domain, whereas the acceptor stem of the same molecule binds to the active site (core region) of the other subunit. The importance of the N-terminal arm-like SerRS

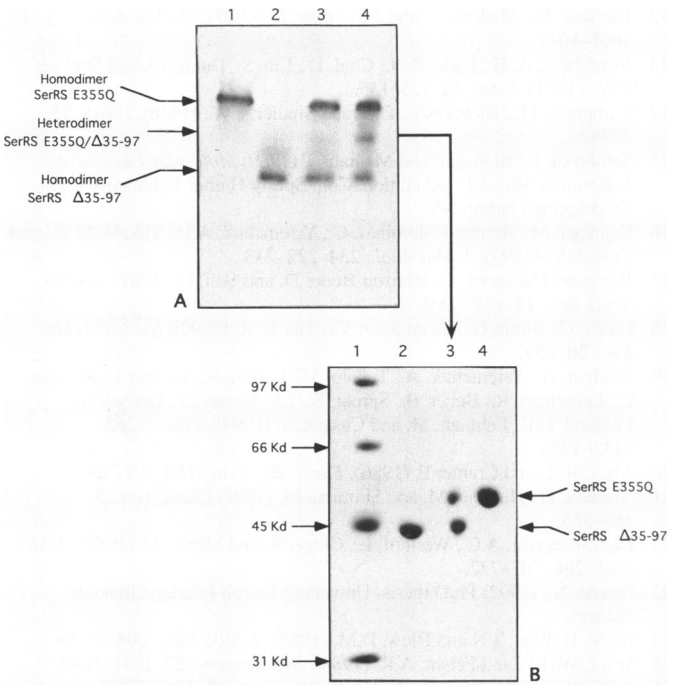

Figure 4. *In vitro* formation of heterodimers. (**A**) Non denaturing PAGE. Lane 1: 0.2 pmol of SerRS E355Q, lane 2: 0.2 pmol of SerRS Δ35–97, lane 3: 0.2 pmol of SerRS E355Q and SerRS Δ35–97. Lane 4 contains SerRS E355Q and SerRS Δ35–97 treated with 0.4 M urea and then dialysed (for details see Materials and Methods). An extra band with intermediate mobility compared to the two mutant enzymes can be observed. (**B**) SDS–PAGE. The intermediate band (lane 3) was cut out and loaded on to an SDS–PAGE gel. Lanes 2 and 4 show the monomers corresponding to SerRS Δ35–97 and SerRS E355Q, respectively. The presence of the two types of monomers in lane 3 confirms the heterodimer formation.

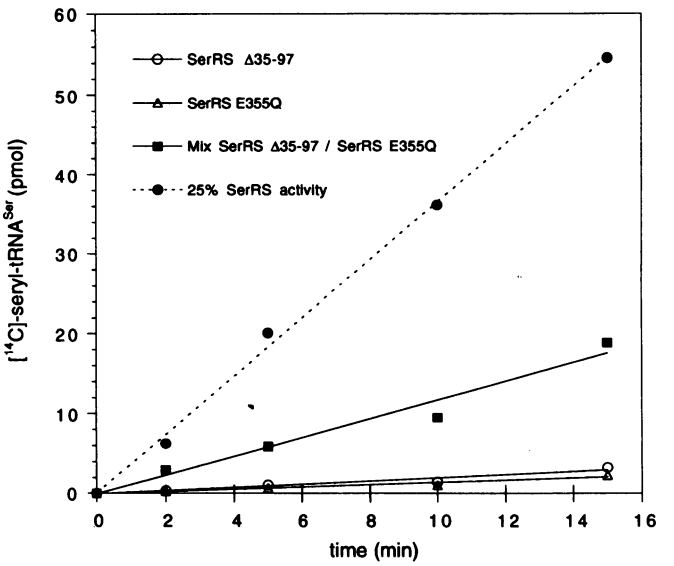

Figure 5. Aminoacylation activity of the heterodimer. A heterodimer containing solution from two aminoacylation defective enzymes obtained after urea treatment was assayed for aminoacylation activity and compared to solutions of the same concentration (20 nM) of homodimers and wild type enzymes. The heterodimer containing solution shows a >8-fold increased activity. The theoretical value of 25% wild-type activity is indicated.

domain for aminoacylation activity and specificity has recently been demonstrated (8).

Heterodimers generated by urea treatment of heterodimer solutions have already been used to study the interaction of a class I synthetase (TyrRS) with its cognate tRNA and to provide evidence for cross-dimer tRNA binding. Carter and co-workers (10) observed a transition from the native TyrRS dimer to the unfolded monomer at around 6 M urea. For the seryl enzyme, much lower urea concentrations (0.4 M) are sufficient for heterodimer formation (dimer dissociation). Some heterodimer formation was even observed in the absense of urea. This could indicate that the number of interactions leading to dimer formation are limited. In the crystal structure of the native SerRS, however, 30 hydrogen bonds and 14 salt bridges can be identified in the dimer interface (23).

Aminoacyl-tRNA synthetases have been classified according to their ATP-binding topology (1,2): class I enzymes (such as the tyrosyl-tRNA synthetase) possess a Rossmann fold, whereas the active site of class II enzymes (such as seryl-tRNA synthetase) is built around an antiparallel β-sheet. The mode of recognition of the acceptor stem appears to be very different for the two classes of synthetases. Class I enzymes approach the tRNA acceptor stem from the minor groove side; class II synthetases bind the tRNA from the major groove side (6,7). Nevertheless, cross-dimer tRNA binding exists for the tyrosyl and the seryl enzymes belonging to different classes of synthetases. Both enzymes are dimers of two identical subunits. Their cognate tRNAs are type 2 tRNAs with long extra arms and interact with both subunits of the synthetases. This shows that the tRNA cross-dimer binding of the tyrosyl and the seryl enzymes seems to be influenced by the structure of their tRNA substrate. If tRNA cross-dimer binding can be demonstrated also for leucyl-tRNA synthetase (class I) with its type 2 tRNA substrate, is an open question. The crystal structure of tyrosyl-tRNA synthetase (TyrRS) from *B.stearothermophilus* has been determined at 2.3 Å resolution (24). The C-terminal domain, for which it has not been possible to trace the polypeptide chain, extends for ~34 Å above the central α-helical domain. It seems to play a role in tRNA recognition similar to that of the N-terminal arm-like domain of SerRS. Kinetic analysis of heterodimers formed between variant enzymes with defective tyrosine activation or tRNA aminoacylation showed that one molecule of $tRNA^{Tyr}$ interacts with the N-terminal region of one subunit and the C-terminal region of the other subunit in the dimer (10). In contrast to SerRS, the tyrosyl enzyme forms only one molecule of amino acid adenylate per dimer due to an asymmetry of the free enzyme in solution (25) and only one molecule of $tRNA^{Tyr}$ binds to each dimer of TyrRS (26,27). Important elements in the discrimination between $tRNA^{Ser}$ and $tRNA^{Tyr}$ are bases dictating the orientation of the long extra arm. Aminoacylation of $tRNA^{Tyr}$, in contrast to that of $tRNA^{Ser}$, is sensitive to changes in the anticodon bases (28). Experiments of cross-linking between *E.coli* $tRNA^{Tyr}$ and TyrRS indicates also that parts of the anticodon stem are close to the enzyme (29). To summarize, the seryl and the tyrosyl enzymes belonging to two different classes of synthetases share a common mode of interaction with their cognate tRNAs (cross-dimer binding), even if there are significant differences in the details of their interactions.

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