Archives of Biochemistry and Biophysics 529 (2013) 122–130

Contents lists available at SciVerse ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

# Substrate recognition and fidelity of maize seryl-tRNA synthetases

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ARTICLE INFO

*Article history:*
Received 12 October 2012
and in revised form 28 November 2012
Available online 7 December 2012

*Keywords:*
Aminoacyl-tRNA synthetase
tRNA
Editing
Plant
Mitochondria
Chloroplast

ABSTRACT

Aminoacyl-tRNA synthetases (aaRSs) catalyze the attachment of amino acids to their cognate tRNAs to establish the genetic code. To obtain the high degree of accuracy that is observed in translation, these enzymes must exhibit strict substrate specificity for their cognate amino acids and tRNAs. We studied the requirements for $tRNA^{Ser}$ recognition by maize cytosolic seryl-tRNA synthetase (SerRS). The enzyme efficiently recognized bacterial and eukaryotic $tRNAs^{Ser}$ indicating that it can accommodate various types of $tRNA^{Ser}$ structures. Discriminator base G73 is crucial for recognition by cytosolic SerRS. Although cytosolic SerRS efficiently recognized bacterial $tRNAs^{Ser}$, it is localized exclusively in the cytosol. The fidelity of maize cytosolic and dually targeted organellar SerRS with respect to amino acid recognition was compared. Organellar SerRS exhibited higher discrimination against tested non-cognate substrates as compared with cytosolic counterpart. Both enzymes showed pre-transfer editing activity implying their high overall accuracy. The contribution of various reaction pathways in the pre-transfer editing reactions by maize enzymes were different and dependent on the non-cognate substrate. The fidelity mechanisms of maize organellar SerRS, high discriminatory power and proofreading, indicate that aaRSs in general may play an important role in translational quality control in plant mitochondria and chloroplasts.

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## Introduction

The fidelity of protein synthesis relies upon the interpretation of the genetic code by aminoacyl-tRNA synthetases (aaRSs)[1]. Each enzyme from aaRS family catalyzes covalent attachment of the cognate amino acid to the specific set of tRNA isoacceptors in a two-step aminoacylation reaction [1]. The amino acid is first activated by ATP to form an enzyme-bound aminoacyl-adenylate (aa-AMP) intermediate, accompanied by the release of pyrophosphate. Subsequently, the amino acid moiety of the intermediate is transferred to the 3′-terminal ribose of the cognate tRNA, leading to the synthesis of aminoacyl-tRNA and release of AMP.

To obtain the high degree of accuracy that is observed in translation (error rate of one misincorporated amino acid per $10^3$–$10^4$ codons), these enzymes must exhibit strict substrate specificity for their cognate amino acids and tRNAs [1,2]. Selection of cognate tRNA proceeds with high accuracy based on binding and reaction kinetics; in both cases the complexity of tRNA molecules allows establishment of many contacts between the tRNA and the protein either in the ground or in the transition state [3,4]. However, at the level of amino acid binding and selection step (prior to activation), a number of aaRSs are unable to discriminate rigorously against amino acids or their analogs of closely related structures. To avoid potential mistakes and maintain the fidelity of protein synthesis, about half of aaRSs evolved complex editing mechanisms [5,6]. Mistakes by aaRSs are generally cleared either by hydrolysis of the non-cognate aminoacyl-adenylate intermediate in the synthetic site (pre-transfer editing) or, if non-cognate aminoacyl moiety is transferred to tRNA, by hydrolysis of the mischarged aminoacyl-tRNA within a distinct editing domain (post-transfer editing). In some cases, hydrolysis of the non-cognate aminoacyl-adenylate intermediate may be stimulated by tRNA (tRNA-dependent pre-transfer editing). Selective release of the non-cognate aminoacyl-adenylate from the synthetic site may also contribute to the pre-transfer editing mechanism. Released aa-AMP may non-enzymatically hydrolyze in solution. AaRS editing deficiencies cause mistranslation and can have significant physiological effects, mainly due to an increased level of misfolded proteins [7–9].

SerRS enzymes from all three domains of life do not possess editing domain and therefore were considered non-editing aaRS enzymes [10]. Investigation of specificity of amino acid recognition revealed weak misactivation of threonine by archeal SerRS from

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[1] *Abbreviations used:* aaRS, aminoacyl-tRNA synthetase; SerRS, seryl-tRNA synthetase; ZmcSerRS, maize (*Zea mays*) cytosolic SerRS; ZmoSerRS, maize (*Zea mays*) organellar SerRS; EcSerRS, *Escherichia coli* SerRS; aa-AMP, aminoacyl-adenylate; IPTG, isopropyl-β-d-thiogalactopyranoside; TLC, thin layer chromatography; SerHX, serine hydroxamate.

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http://dx.doi.org/10.1016/j.abb.2012.11.014

*Methanosarcina barkeri* [11,12], as well as weak misactivation of both threonine and cysteine by the yeast cytosolic enzyme [13]. In case of the yeast cytosolic SerRS, these misactivated near-cognate amino acids were shown to be cleared by the pre-transfer editing within the synthetic site of the enzyme [13]. This provided the first example of proofreading activity of aaRS naturally lacking a separate editing domain in all domains of life and it opened a completely new perspective showing that aaRS may possess hydrolytic correction even in the absence of the specialized domain.

The fidelity of amino acid recognition by plant aaRS has not been extensively studied. This issue is not only important from the functional and evolutionary point of view, but it should also be considered in biotechnology, namely in recombinant protein production in plant cytosol or chloroplasts. Errors in translation could potentially affect the quality of produced proteins, which is especially relevant in the case of therapeutic recombinant proteins. Several transgenic maize plants have been developed for various biopharming applications [14,15]. Findings that yeast and archaeal SerRSs showed moderate or low misactivation of near-cognate amino acids, prompted us to explore the fidelity of maize cytosolic and dually targeted organellar SerRSs.

Recognition of tRNA by aaRSs is facilitated by both positive and negative identity elements contained within the tRNA structure that ensure binding to the proper enzyme [3]. Recognition studies performed with the components of serylation systems from different species, revealed that some, but not all, determinants have been conserved during evolution [16,17]. The main recognition element required for productive tRNA:synthetase complex formation is a long variable arm of $tRNA^{Ser}$, present in all organisms except in animal mitochondria. Length and orientation of the variable arm were shown to be important for proper recognition of $tRNA^{Ser}$ by SerRS enzymes [18–20]. Structural studies of bacterial tRNAs with long variable arm (type II tRNAs) have shown that orientation of the variable arm is influenced by the number of unpaired nucleotides between the variable stem and the nucleotide at position 48 [21]. Furthermore, orientation of the variable arm of bacterial $tRNAs^{Ser}$ is maintained through its tertiary interactions with base G20B in the loop of D-arm [22]. Another major identity region of $tRNA^{Ser}$ is the acceptor arm comprising the discriminator base. Although strictly conserved in all $tRNAs^{Ser}$, the role and significance of the discriminator base G73 varies in different organisms, even within the eukaryotic domain [16]. Thus far, the importance of G73 for plant serylation has not been studied.

## Material and methods

### *Complementation of E. coli strain KL229*

The cDNA encoding maize ZmcSerRS was amplified by PCR using pET28bZmcSerRS [23] as a template and cloned into pET15b. The gene for *Escherichia coli* SerRS was amplified by PCR from *E. coli* genomic DNA and cloned into pET15b. *E. coli* strain KL229 carries a mutated *serS* gene responsible for the temperature-sensitive phenotype [24]. KL229 cells were transformed with pET15bZmcSerRS, pET15bEcSerRS or empty pET15b and tested for growth at permissive (30 °C) and nonpermissive temperature (34 °C) on M9/glucose minimal plates supplemented with ampicillin (100 μg/ml), thymine (200 μg/ml) and isopropyl-β-D-thiogalactopyranoside (IPTG) (2 mM) where indicated. The addition of IPTG ensures the derepression of the T7 promoter, which is followed by the *lac* operator in the pET15b vector. As already demonstrated [25], the T7 promoter allows a low level of transcription even in bacterial strains that do not contain T7 RNA polymerase.

### *Supression of bacterial amber mutation*

*E. coli* strain JR 104 (F′ *trpA*(*UAG*)*211*/*glyV55* Δ(*tonB-trpAB*) *argE*(*UAG*) *rpoB*) was previously described [26]. Suppression of *trpA* amber mutations in strain JR104 was tested in cells cotransformed with pET15b plasmid carrying the gene for maize or bacterial SerRS and pTech plasmid carrying the gene for tyrosine-specific amber suppressor *supF* or its mutated variant *supF*A73G [27]. Supressor $tRNAs^{Tyr}$ were previously cloned in pTech plasmid under control of the *lpp* promoter and the *rrnC* terminator [28]. Cotransformed cells were plated on selective M9 minimal glucose plates supplemented with IPTG (2 mM), arginine (40 μg/ml), chloramphenicol (20 μg/ml) and ampicilline (100 μg/ml), and grown at 30 °C for 20 h.

### *Green fluorescent protein targeting analysis in maize protoplasts*

To determine subcellular localization of ZmcSerRS, ZmcSerRS-GFP fusion construct was prepared. ZmcSerRS coding sequence was amplified by PCR using pSKZmcSerRS [29] as a template and cloned in frame with GFP(S65T) coding sequence, behind 35S CaMV promoter in pTH-2 vector [30]. Protoplasts were isolated from greening leaves of 10-day-old maize seedlings and transformed by PEG-mediated transformation according to the protocol described in [31]. The transiently transformed protoplasts were analyzed with confocal laser scanning microscope Leica TCS SP2 (Leica, Solms, Germany). To stain mitochondria, protoplasts were incubated with 0.5 μM MitoTracker Red CM-H2XRos (Molecular Probes) for 30 min and washed before analysis by confocal microscopy. Both the GFP fluorophore and chlorophyll were excited with argon laser at 488 nm. GFP fluorescence and far-red chlorophyll fluorescence were collected between 500–535 nm and 650–700 nm, respectively, in two separate channels. MitoTracker Red CM-H2XRos fluorescence was detected between 600 and 610 nm with excitation at 578 nm. The programs LCSLite and Adobe Photoshop were used for post-acquisition image processing.

### *ATP–$PP_i$ exchange*

Proteins ZmcSerRS and ZmoSerRS were overexpressed and purified as previously described [23,32]. The samples of amino acids and serine hydroxamate (SerHX) were checked by mass spectrometry and contamination with serine was not detected. ATP–$PP_i$ exchange was measured at 37 °C in 100 mM HEPES pH 7.0, 20 mM $MgCl_2$, 25 mM KCl, 4 mM ATP and 1 mM [$^{32}$P]$PP_i$ (0.002–0.01 mCi/ml). Amino acid and analog concentrations varied between 0.1 and 10 × $K_M$. Due to low solubility, the concentration of cysteine in reactions with ZmoSerRS was varied between 0.1 and 5 × $K_M$. Enzyme concentrations used were 100–200 nM in reactions with serine, 500 nM in reactions with D,L-SerHX and 1–2 μM in reactions with threonine and cysteine. The reactions were stopped with sodium acetate (pH 5.0) and SDS (final concentrations 333 mM and 0.067%, respectively). The generated [$^{32}$P]ATP was separated from the remaining [$^{32}$P]$PP_i$ by thin layer chromatography (TLC) on polyethyleneimine plates, using 750 mM $KH_2PO_4$ pH 3.5 and 4 M urea as developer. The obtained signal was visualized on Storm PhosphorImager and then quantified using ImageQuant software. Kinetic parameters were determined by fitting the data directly to the Michaelis–Menten equation using nonlinear regression. The parameters were obtained from three independent measurements.

### *Aminoacyl-adenylate synthesis assay*

Aminoacyl-adenylate synthesis assay was done in 50 mM HEPES pH 7.0, 25 mM KCl, 20 mM $MgCl_2$, 0.5 mM ATP (0.01–0.1 mCi/ml). Concentration of serine, D,L-SerHX, threonine

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