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Original Scientific Article

Efficiently Activated Serine Analogue is Not Transferred to Yeast tRNA^{Ser}

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Abstract. Covalent attachment of cognate amino acid to the cognate tRNA is a prerequisite for the faithful synthesis of proteins in the cell. Aminoacylation of tRNA, catalyzed by aminoacyl-tRNA synthetases (aaRSs), proceeds by a two-step reaction whereby amino acid is first activated and then transferred to the 3'-ribose of tRNA. Serine hydroxamate (SerHX) is an interesting analogue of serine as it exhibits antimicrobial activity due to its inhibition of serylation in yeast and *Escherichia coli*. SerHX also mimics a noncognate substrate of yeast seryl-tRNA synthetase (ScSerRS) since it is efficiently activated and edited by this enzyme. However, whether this analogue is also transferred to tRNA during the second step of aminoacylation was not previously known. Here we show, for the first time, that aminoacylation of yeast tRNA with SerHX does not occur at a measurable rate, suggesting that the transfer is less tolerable toward SerHX than the activation step.

Keywords: serine hydroxamate, seryl-tRNA synthetase, tRNA, transfer step, amino acid activation

INTRODUCTION

During translation, genetic information that has been transcribed to messenger RNA (mRNA) is used for the synthesis of the corresponding protein. The position of the amino acid in the synthesized protein is determined by recognition between the codon of mRNA and the anticodon of aminoacylated transfer RNA (tRNA), a molecule that brings along the cognate amino acid covalently attached to its 3'-end. The accuracy of protein biosynthesis relies on these two events: codon-anticodon recognition at the ribosome and covalent attachment of the cognate amino acid to the cognate tRNA (*i.e.* aminoacylation). Aminoacylation of tRNA proceeds via a two-step reaction catalyzed by a group of enzymes called aminoacyl-tRNA synthetases (aaRS, reviewed in Refs. 1 and 2). Firstly, the amino acid is activated via an ATP-dependent pathway to produce a noncovalently bound aminoacyl-adenyate intermediate. In the second step, the amino acid is transferred from the aminoacyl-adenylate to the 2'- or 3'-OH group of the 3'-terminal ribose of the bound cognate tRNA. Both steps, activation and transfer, are catalyzed by the same enzyme within the same active site located in the catalytic domain. Based on mutually exclusive sequence motifs that reflect distinct topologies of the active sites, aaRSs are divided into two unrelated classes, I and II, consisting of 11 and 10 members, respectively.[3,4] While substrate binding occurs in a class-dependent manner, the mechanism of catalysis is strictly conserved between the two families.[1,5]

Aminoacylation of tRNA by a cognate amino acid is a prerequisite for faithful protein biosynthesis. Nevertheless, some aaRS cannot discriminate between similar amino acids with appropriate accuracy (overall error rate in protein biosynthesis is less than 1 in 3000),[6] and thus may activate structurally similar noncognate amino acids. However, in order to clear misactivated amino acids, these enzymes have developed sophisticated hydrolytic editing. Noncognate amino acid may be eliminated through pre-transfer editing where hydrolysis of misacyl-adenylate occurs. Additionally, the transfer of noncognate amino acid to tRNA may occur followed by hydrolysis of misacyl-tRNA (post-transfer editing).[7] For example, *Escherichia coli* isoleucyl- and valyl-tRNA synthetases (IleRS and ValRS, respectively) activate noncognate valine and threonine only two orders of magnitude less efficiently than cognate isoleucine and valine, respectively.[7] However, efficient editing prevents the incorporation of valine or threonine at the place of isoleucine or valine, respectively, during protein biosynthesis.[8]

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Serine hydroxamate (SerHX) is an analogue of serine having a hydroxamate group instead of carboxylate. Its potential as a microbial agent was recognized early on as it inhibits growth of *Escherichia coli*,[9] primary through the inhibition of *E. coli* seryl-tRNA synthetase.[10] Seryl-tRNA synthetases (SerRS) catalyze the attachment of serine to $tRNA^{Ser}$ isoacceptors and thus participate in protein biosynthesis in the cell. SerHX was found to be a competitive inhibitor of *E. coli* SerRS. Interestingly, the enzyme has a higher apparent affinity for SerHX than it does for its natural substrate ($K_I = 30$ μmol dm^{-3}, K_m(Ser) = 50 μmol dm^{-3}).[10] Findings that SerHX has antimetabolite activity in *E. coli* have stimulated parallel studies in different organisms. SerHX was shown to be activated by *Saccharomyces cerevisiae* seryl-tRNA synthetase (ScSerRS)[11] and to inhibit serylation by ScSerRS both *in vitro* and *in vivo*. However, the obtained inhibition constant regarding serine (K_I) was 90-fold higher than for *E. coli* SerRS.[12] Additionally, inhibition studies performed on SerRSs from different organisms revealed that SerRSs from all domains of life are competitively inhibited by SerHX, albeit with different affinities.[13]

To date, SerHX has primarily been explored as a competitive inhibitor of serylation in order to reveal either mechanistic implications or to determine its antimicrobial activity. Recently we showed that ScSerRS efficiently recognizes SerHX and edits it through the hydrolysis of formed noncognate aminoacyl-adenylate (pre-transfer editing).[14] This shows that the SerHX analogue behaves as a noncognate substrate of yeast SerRS. In order to explore how SerHX efficiently mimics a real serine substrate in the activation and transfer to tRNA, we employed a new method using ^{32}P-labeled tRNA that allows direct tracking of SerHX-$tRNA^{Ser}$ formation. In order to compare noncognate reactions in eukaryotic and archaeal systems, we have performed our research on both yeast SerRS and atypical SerRS from methanogenic archaea *Methanosarcina barkeri* (mMbSerRS).

EXPERIMENTAL

General

The overexpression and purification of ScSerRS and mMbSerRS has been described.[15,16] $tRNA^{Ser}$ from *S. cerevisiae* ($SctRNA^{Ser}$) was prepared by run off *in vitro* transcription of the BstNI-digested DNA template and purified by denaturing electrophoresis on 6 % polyacrylamide gel as described.[17]

Preparation of $tRNA^{Ser}$ by Ion-Exchange Chromatography

$tRNA^{Ser}$ from *M. barkeri* ($MbtRNA^{Ser}$) was transcribed *in vitro* from pUC18 carrying the $MbtRNA^{Ser}$ gene, inserted between T7 promoter and NsiI restriction site. The transcription template was prepared by NsiI digestion, according to the manufacturer's instructions. DNA was purified from the mixture by phenol/chloroform extraction and ethanol-precipitated. *In vitro* transcription was performed in a reaction containing 40 mmol dm^{-3} Tris-HCl (pH = 8.0), 20 mmol dm^{-3} $MgCl_2$, 2 mmol dm^{-3} spermidine, NTPs (6 mmol dm^{-3} each) and 0.1 mg ml^{-1} DNA template in a presence of 5 mmol dm^{-3} dithiothreitol (DTT), 50 μmol dm^{-3} bovine serum albumin (BSA), 20 mmol dm^{-3} GMP, 13 U ml^{-1} ribonuclease inhibitor (Rnasin), and 8 mU ml^{-1} inorganic pyrophosphatase (PPase). Following the addition of 0.1 mg ml^{-1} T7-RNA polymerase, reaction was incubated for 4 h at 37 ºC. The DNA template and large RNA transcripts were precipitated from the transcription mixture with 8 % polyethylene-glycol 8000 in the presence of 0.5 mol dm^{-3} NaCl (10 min at room temperature). The supernatant was obtained by centrifugation at 10 000 × g for 10 min, dialyzed against buffer A (40 mmol dm^{-3} Tris-HCl (pH = 7.5), 20 mmol dm^{-3} $MgCl_2$, and 200 mmol dm^{-3} NaCl) and applied to the DEAE-cellulose column (4 ml). Elution of tRNA was performed with 2 volumes of 0.3 mol dm^{-3}, 0.4 mol dm^{-3}, 0.6 mol dm^{-3}, and 0.8 mol dm^{-3} NaCl (in a buffer of the same composition as buffer A). Fractions containing tRNA were pooled and ethanol-precipitated.

Labelling of tRNA and Misacylation with SerHX

Radiolabelling of the 3'-terminus of tRNA was performed using tRNA nucleotidyltransferase to exchange the endogenous A76 of tRNA with [α-^{32}P]ATP. This was performed by incubating 4 μmol dm^{-3} $tRNA^{Ser}$ with tRNA nucleotidyltransferase, 1 μmol dm^{-3} [α-^{32}P]ATP (specific activity 3000 Ci mmol^{-1}), and 5 mmol dm^{-3} sodium pyrophosphate in buffer containing 50 mmol dm^{-3} Tris (pH = 7.5), 20 mmol dm^{-3} $MgCl_2$, and 0.5 mmol dm^{-3} DTT at 37 °C for 1 min to remove unlabeled endogenous A76. Following this, 0.1 U μl^{-1} thermostable inorganic pyrophosphatase was added and the mixture was incubated at 37 °C for an additional 2 min. The pyrophosphatase degrades the free pyrophosphate and stimulates tRNA nucleotidyltransferase that catalyses addition of [α-^{32}P]ATP to tRNA. Following phenol/chloroform extraction, the sample was passed through two consecutive Bio-Spin P-30 columns (Bio-Rad) to remove phenol and unreacted [α-^{32}P]ATP. Finally, the reaction was dialyzed against water.

$SctRNA^{Ser}$ was carefully renatured prior to use in aminoacylation by heating for 5 min at 80 °C, slow cooling to 70 °C followed by the addition of $MgCl_2$ to the final concentration of 10 mmol dm^{-3}, cooling at RT for 20 min and placing on ice. Attachment of the unlabeled DL-SerHX to ^{32}P-labeled $SctRNA^{Ser}$ was measured by the aminoacylation assay carried out at 30 °C in

a reaction mixture containing 50 mmol dm^{-3} Tris (pH = 7.5), 15 mmol dm^{-3} $MgCl_2$, 5 mmol dm^{-3} ATP, 0.01 U μl^{-1} inorganic pyrophosphatase, 4 mmol dm^{-3} DTT, and 5 μmol dm^{-3} unlabelled SctRNA^{Ser}, approximately 20 nmol dm^{-3} ^{32}P-labeled SctRNA^{Ser}, 500 nmol dm^{-3} ScSerRS, and 200 mmol dm^{-3} DL-SerHX.

MbtRNA^{Ser} was carefully renatured prior to use in aminoacylation by heating for 5 min at 75 °C, followed by addition of $MgCl_2$ to the final concentration of 5 mmol dm^{-3}, and placing on ice. The reaction with mMbSerRS was performed at 37 °C in the reaction mixture containing 50 mmol dm^{-3} Hepes (pH = 7.6), 15 mmol dm^{-3} $MgCl_2$, 25 mmol dm^{-3} KCl, 5 mmol dm^{-3} ATP, 0.01 U μl^{-1} inorganic PPase, 4 mmol dm^{-3} DTT and 5 μmol dm^{-3} unlabelled MbtRNA^{Ser}, approximately 20 nmol dm^{-3} ^{32}P-labeled MbtRNA^{Ser}, 500 nmol dm^{-3} mMbSerRS, and 200 mmol dm^{-3} DL-SerHX.

All reactants were preincubated at a given temperature prior to the reaction, and the reaction was started with the addition of DL-SerHX solution to the premixed solution of reactants. Reactions were stopped by placing 1.5 μl of the reaction mixture in a microtiter well containing 3 μl of 0.05 mg ml^{-1} P1 nuclease in 300 mmol dm^{-3} sodium acetate (pH = 5.2). After 15 min of incubation at 37 °C, thin-layer chromatography (TLC) was employed to separate ^{32}P-labeled aminoacyl-adenylate (aa-AMP) from ^{32}P-labeled AMP. The ratio of aa-AMP to AMP is equivalent to the ratio of aminoacylated versus non-aminoacylated tRNA in the reaction. The sample (1.5 μl) was spotted on polyethyleneimine (PEI) cellulose plates prewashed with water and developed in 100 mmol dm^{-3} ammonium acetate and 5 % acetic acid. The dried plates were exposed on an imaging plate overnight, scanned using the Typhoon PhosphorImager, and quantified using ImageQuant software.

Amino Acid Activation by mMbSerRS

Activation of serine and DL-SerHX was investigated by ATP-PP_i exchange at 37 °C. The reaction mixture contained 100 mmol dm^{-3} Hepes (pH = 7.0), 20 mmol dm^{-3} $MgCl_2$, 25 mmol dm^{-3} KCl, 1 mmol dm^{-3} potassium pyrophosphate (^{32}P-PP_i, 0.002–0.01 mCi ml^{-1}), and 4 mmol dm^{-3} ATP. A total of 100 nmol dm^{-3} of enzyme was used for the cognate reaction and between 0.5 and 1.5 μmol dm^{-3} for the activation of DL-SerHX. DL-SerHX was used in a range of 10–250 μmol dm^{-3}. ^{32}P-ATP was separated from ^{32}P-PP_i by thin-layer chromatography and quantified as described previously.[18]

RESULTS

Purification of tRNA by Ion-Exchange Chromatography

The standard procedure for the preparation of tRNA *in vitro* transcripts involves several denaturation steps. Proteins are removed by phenol/chloroform extraction followed by purification of the transcript on denaturing urea polyacrylamide gel. The procedure is time-consuming (elution from the gel usually takes 20 hours) and results in a denatured purified tRNA sample. Though tRNA is usually carefully renatured prior to experiments, renaturation will generally yield a tRNA sample that is a mixture of conformations with different biochemical activity or no activity at all.[19] RNA structures possess extraordinarily high stability in physiological buffers, mainly due to the highly favourable free energy of stacking of the nucleotide bases in the folded structure and the ability of counterions to effectively neutralize phosphate repulsion. However, the downside of a very stable native structure is that non-native states of RNA are often also very stable. Therefore, denaturing procedures could form stable denatured tRNA conformers that could lead to a conformationally heterogeneous population of the purified tRNA sample. Use of such sample could significantly complicate interpretation of biochemical and kinetic data.

In order to design a "mild" purification protocol, we used polyethylene-glycol 8000 to fractionally precipitate the DNA template and large RNA transcripts from the transcription mixture, followed by ion-exchange chromatography on DEAE-cellulose column to remove T7-RNA polymerase and unincorporated nucleotides. For MbtRNA^{Ser}, no significant increase in activity was observed compared to the MbtRNA^{Ser} purified on urea denaturing gels. However, this may depend on the particular tRNA sequence, suggesting either that MbtRNA^{Ser} is not prone to forming unreactive conformers or that the applied renaturation procedure is very good. The other significant disadvantage of the "gel method" is that it is time-consuming. The developed protocol is significantly faster than the gel protocol, resulting in purified *in vitro* transcripts from the gene in just one day.

Several other procedures for tRNA purification under the native conditions are known from the literature. One of the common methods is affinity chromatography, whereby elongation factor EF-Tu or some other RNA binding proteins are immobilized on the column matrix.[20–22] Advantage of the method that relies on significantly higher affinity of the EF-Tu toward aminoacylated than nonaminoacylated tRNA [20,21] is that only active, aminoacylable tRNA *in vitro* transcripts are obtained during the purification step. On the other hand, Keift and Batey[22] added an affinity tag on the RNA construct. The tag contains two elements: a variant of the hepatitis delta virus ribozyme that is activated by imidazole and a hairpin loop from a termostable signal recognition particle RNA that forms a high-affinity and kinetically stable complex with *Thermotoga maritima* Ffh-M domain protein. The problem of nonspecific

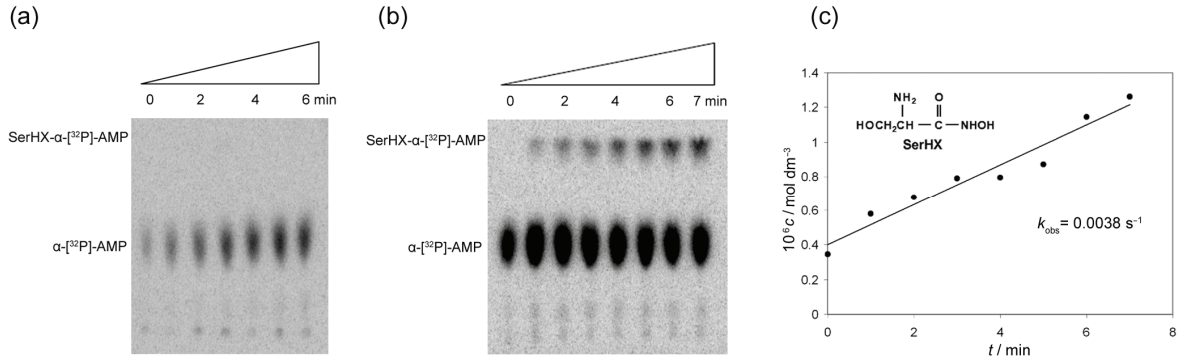

Figure 1. Thin-layer chromatograms of a reaction time course showing separation of SerHX-[^{32}P]-adenylate and [^{32}P]-adenylate in a typical aminoacylation reaction containing DL-serine hydroxamate, (a) ScSerRS and SctRNA^{Ser} or (b) mMbSerRS and MbtRNA^{Ser}. (c) Representative plot of SerHX-MbtRNA^{Ser} concentration *vs.* time obtained with 500 nmol dm^{-3} mMbSerRS. Following quantification of the intensities of SerHX-[^{32}P]-adenylate and [^{32}P]-adenylate using ImageQuant, the concentration of SerHX-MbtRNA^{Ser} formed at each time point was calculated by dividing the intensity of the SerHX-[^{32}P]-adenylate spot by the total intensity.

addition of nucleotides to the 3'-end by T7 RNA polymerase is largely overcame by using cis-acting ribozyme.

Transfer of Serine Hydroxamate to yeast tRNA^{Ser}

SerHX, an analogue of serine, is efficiently activated by yeast SerRS.[11,14] The obtained catalytic constant (k_{cat}) is slightly decreased in comparison with the cognate reaction, while a significant increase is observed for the Michaelis constant (K_m). Therefore, at high concentrations of SerHX, the enzyme activates SerHX almost as efficiently as cognate serine. Whether this efficiently activated analogue is transferred from SerHX-adenylate to the cognate tRNA^{Ser} is therefore an intriguing question, which long remained unanswered likely due to the lack of commercially available radioactively labelled SerHX. However, we adapted a relatively new method[23,24] for monitoring aminoacylation kinetics, that relies on ^{32}P-labeled tRNA and therefore can be used with nonlabelled amino acid substrates. Using this method, we were able to directly track the aminoacylation of tRNA^{Ser} by SerHX under saturating conditions of this analogue (when activation is rapid). Interestingly, no significant signal corresponding to aminoacylated tRNA (Figure 1a) was detected. Therefore, it can be concluded that the rate of SerHX-tRNA^{Ser} formation is beneath the detection limits of this method. This result is surprising, as SerHX behaves as a good substrate during activation step. Therefore, one may suspect that activated SerHX is well positioned within the active site for the next step to occur. Based on indirect evidence, a previous study concluded that SerHX is transferred to SctRNA^{Ser}.[11] However, this activity was not detected using the direct kinetic steady-state assay.

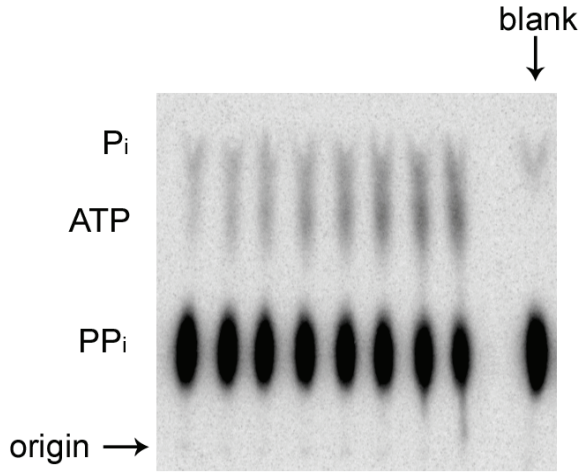

Figure 2. Thin-layer chromatogram of a reaction time course showing separation of [^{32}P]-PP$_i$ and [^{32}P]-ATP in a typical ATP/PP$_i$ exchange reaction containing DL-serine hydroxamate. The last lane (marked as blank) is control reaction in which DL-serine hydroxamate was omitted. Since separation of [^{32}P]-P$_i$ and [^{32}P]-ATP signals was poor, we quantified them together and calculated the rate of their cumulative formation. To calculate the rate of [^{32}P]-ATP formation, we substracted the rate of [^{32}P]-P$_i$ formation obtained in control reaction from the cumulative rate of [^{32}P]-P$_i$ and [^{32}P]-ATP formation.

Activation of Serine Hydroxamate and its Transfer to tRNA^{Ser} in Methanogenic Archaea

In order to compare the activation of the analogue between methanogenic archaea and yeast, the ATP-PP$_i$ exchange assay was performed using mMbSerRS in the presence of serine and DL-SerHX (Table 1, Figure 2). Contrary to the yeast enzyme, mMbSerRS

Table 1. Kinetic parameters for the activation of serine and DL-serine hydroxamate (DL-SerHX) determined by ATP-PP_i exchange

	K_m/ μmol dm^{-3}	k_{cat} / s^{-1}	$\frac{k_{cat}/K_m}{s^{-1}\ \mu mol^{-1}\ dm^3}$	Discrimination factor[(a)]
		mMbSerRS		
Ser	44.93 ± 7.7	3.09 ± 0.19	0.069	1
DL-SerHX	40.8 ± 10.2	0.036 ± 0.005	0.00088	0.0128
		ScSerRS[(b)]		
Ser	500	3.9	0.0078	1
DL-SerHX	4230	1.56	0.00037	0.0474

[(a)] Discrimination factor is defined as $[1 / (k_{cat} / K_m)_{rel}]$
[(b)] From Ref. 14

has a significantly decreased turnover (k_{cat}) for SerHX activation than that obtained with serine, while K_m(SerHX) remains almost the same as K_m(Ser). Therefore, if K_m values are taken as a measure of enzyme:substrate affinity, binding of SerHX by mMbSerRS is very efficient though kinetically unproductive. In order to determine whether weakly activated SerHX is transferred to $MbtRNA^{Ser}$ in methanogenic archea, we labelled tRNA with ^{32}P[α-ATP] and monitored its aminoacylation with SerHX by mMbSerRS. The observed catalytic constant ($k_{obs} = 0.004\ s^{-1}$; Figure 1b and c) shows that the transfer of SerHX occurs slowly though at a measurable rate. This is completely opposite to the result obtained using ScSerRS, where efficiently activated SerHX is not transferred to $SctRNA^{Ser}$ at a measurable rate.

DISCUSSION

Aminoacylation of tRNA proceeds by a two-step reaction; formation of aminoacyl-adenylate intermediate is followed by a transfer of the activated amino acid to tRNA. Pre-steady state kinetic analysis has shown that the slowest step in overall aminoacylation catalyzed by ScSerRS is serine activation. The transfer of serine from seryl-adenylate to tRNA and the dissociation of $Ser\text{-}tRNA^{Ser}$ from the enzyme occur significantly faster than activation.[25] Interestingly, in the presence of SerHX it seems that ScSerRS behaves in the opposite way and processes serine analogue, SerHX, much better during activation than during aminoacylation. Moreover, data obtained in the direct kinetic steady-state assay show that the yeast enzyme does not aminoacylate $tRNA^{Ser}$ with SerHX at a measurable rate, suggesting that the transfer of SerHX is significantly more constrained than its activation. Furthermore, the obtained k_{obs} for $SerHX\text{-}tRNA^{Ser}$ formation by mMbSerRS is an order of magnitude lower than the turnover for SerHX activation ($0.004\ s^{-1}$ and Table 1, respectively) while the ratio of k_{cat}(activation)/k_{cat}(acylation) for cognate serine is around 4.[26] However, it should be noted that steady-state rate constants for aminoacylation and activation are not directly kinetically comparable due to slightly different reaction mixtures and the presence or absence of tRNA within. Moreover, steady-state kinetics have serious limitations and the obtained cumulative steady-state catalytic constants should be carefully used in mechanistical interpretations. Nevertheless, the presented data strongly suggest that the transfer step is less tolerable toward SerHX than activation.

It was shown previously that the observed rate constant for the transfer step depends on the nature of the present tRNA.[27] k_{obs} was shown to decrease when noncognate tRNA or cognate tRNA bearing mutations in identity elements were used in an assay designed to measure only the transfer step. This leads to the conclusion that only interactions of the enzyme with the cognate tRNA induce an optimal conformation able to provide full stabilization of the transition state during the transfer reaction. On the other hand, reactions of noncognate amino acids have been studied for highly editing synthetases, as a part of a proofreading mechanism that clears misactivated and/or misacylated noncognate amino acids.[7] Both IleRS and ValRS are known for their inability to efficiently reject smaller and isosteric noncognate valine and threonine, respectively. Therefore, the accuracy of aminoacylation is raised to the appropriate level by the additional proofreading activities displayed by these enzymes. Comparison of the cognate and noncognate reactions reveals that both IleRS and ValRS enzymes activate noncognate valine and threonine with similar turnover numbers as cognate isoleucine and valine, respectively.[28,29] Contrary to that, K_m values were two orders of magnitude higher for noncognate substrates. Additionally, pre-steady state analysis has shown that rates of transfer of efficiently activated noncognate valine and threonine to appropriate $tRNA^{Ile}$ and $tRNA^{Val}$, respectively, are similar to the

transfer rates obtained for cognate reactions.[28,29] On the other hand, nonediting glutaminyl-tRNA synthetase (GlnRS) achieves a major portion of the selectivity through the amino acid activation with significantly impaired turnover (three orders of magnitude) and K_m values (two orders of magnitude).[18] Though the transfer step was not directly measured, it can be concluded that it may be impaired, since binding of noncognate glutamate in a distinct orientation results in its disposition within the active site.

The unexpected result that ScSerRS does not aminoacylate $tRNA^{Ser}$ with SerHX at a detectable level may be explained by our recent findings that the yeast enzyme possesses pre-transfer editing activity towards the SerHX analogue.[14] The obtained catalytic constant for hydrolysis of SerHX-adenylate was shown to be 0.04 s^{-1} suggesting that transfer of SerHX may not be observed if it is slower. An ongoing study on the accuracy of aminoacylation in methanogenic archaea, shows that mMbSerRS may also hydrolyze SerHX-adenylate, though at a significantly slower rate than the yeast counterpart (k_{obs} is about 0.008 s^{-1}, IGS, unpublished data). This may explain an obvious discrepancy between yeast and methanogenic enzymes concerning formation of SerHX-$tRNA^{Ser}$.

CONCLUSIONS

Serine hydroxamate (SerHX), an analogue of serine, mimics a noncognate substrate of seryl-tRNA synthetase (SerRS).[11,12,14,30] It is activated in an ATP-dependent manner to yield SerHX-adenylate and, surprisingly, it is also removed by the pre-transfer hydrolytic proofreading activity recently discovered for SerRS.[14] However, participation of the analogue in overall aminoacylation has been ambiguous. The present study gives the first evidence that SerHX is not transferred to $tRNA^{Ser}$ at a measurable rate, despite its efficient activation by the ScSerRS. The lack of detectable aminoacylation may be a consequence of faster hydrolytic activity toward SerHX-adenylate. In that case, aminoacylation occurring at a rate of 0.04 s^{-1} (k_{obs} for pre-transfer editing)[14] or less would still be about two orders of magnitude slower than activation. This strongly suggests that the transfer step is less tolerable toward SerHX than activation. This is an interesting finding, as it can easily be imagined that binding of the SerHX-adenylate (efficiently formed within the active site of SerRS) is driven by the AMP moiety, common to cognate and noncognate aminoacyl-adenylates, and therefore is well positioned for the nucleophilic attack of tRNA. Therefore, it seems that the carbonyl carbon atom is dispositioned due to the presence of an extra NH group within the SerHX-adenylate (Figure 1c). Since SerHX, bearing a hydroxamate group instead of carboxylate, is still efficiently activated at saturating conditions, it can be concluded that the enzyme's active site can accommodate an extra bond length during activation but not during transfer. Thus, it seems that structural flexibility is not an inherent feature of the SerRS's active site, as it is lost during the second step of aminoacylation.

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SAŽETAK

Efikasno aktivirani analog serina ne prenosi se na kvaščevu tRNA^{Ser}

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Kovalentno povezivanje pripadnog para aminokiseline i tRNA preduvjet je točne sinteze proteina u stanici. Aminoaciliranje tRNA, katalizirano aminoacil-tRNA-sintetazama (aaRSs), događa se u dva uzastopna koraka; prvo dolazi do aktivacije aminokiseline koja se zatim prenosi na 3'-ribozu tRNA. Serin hidroksamat (SerHX) zanimljiv je analog serina koji posjeduje antimikrobnu aktivnost uslijed inhibicije serilacije u kvascu i bakteriji *Escherichia coli*. SerHX se također može smatrati nepripadnim supstratom kvaščeve seril-tRNA-sintetaze (SerRS) budući da se efikasno aktivira dok se nastali SerHX-adenilat čak i hidrolizira pomoću tog enzima. No, da li se navedeni analog također i prenosi na tRNA prilikom drugog koraka aminoacilacije, još nije pokazano. Ovdje, po prvi put, pokazujemo da se aminoacilacija kvaščeve tRNA^{Ser} sa SerHX ne događa mjerljivom brzinom što upućuje na to da je korak prijenosa manje tolerantan prema serin-hidroksamatu u odnosu na korak aktivacije.