

Chapman University

Chapman University Digital Commons

Biology, Chemistry, and Environmental Sciences
Faculty Articles and Research

Science and Technology Faculty Articles and
Research

6-10-2010

Redox Status Affects the Catalytic Activity of Glutamyl-tRNA Synthetase

Assaf Katz
Universidad de Chile

Ranat Banerjee
The Ohio State University

Merly de Armas
Universidad de Chile

Michael Ibba
Chapman University, ibba@chapman.edu

Omar Orellana
Universidad de Chile

Follow this and additional works at: https://digitalcommons.chapman.edu/sees_articles

 Part of the [Amino Acids, Peptides, and Proteins Commons](#), [Biochemistry Commons](#), [Cellular and Molecular Physiology Commons](#), [Molecular Biology Commons](#), [Nucleic Acids, Nucleotides, and Nucleosides Commons](#), and the [Other Biochemistry, Biophysics, and Structural Biology Commons](#)

Recommended Citation

Katz, A., Banerjee, R., de Armas, M. Ibba, M. and Orellana, O. (2010) Redox status affects the catalytic activity of glutamyl-tRNA synthetase. *Biochem. Biophys. Res. Comm.* **398**, 51-55. <https://doi.org/10.1016/j.bbrc.2010.06.031>

This Article is brought to you for free and open access by the Science and Technology Faculty Articles and Research at Chapman University Digital Commons. It has been accepted for inclusion in Biology, Chemistry, and Environmental Sciences Faculty Articles and Research by an authorized administrator of Chapman University Digital Commons. For more information, please contact laughtin@chapman.edu.

Redox Status Affects the Catalytic Activity of Glutamyl-tRNA Synthetase

Comments

NOTICE: this is the author's version of a work that was accepted for publication in *Biochemical and Biophysical Research Communications*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Biochemical and Biophysical Research Communications*, volume 398, in 2010. <https://doi.org/10.1016/j.bbrc.2010.06.031>

The Creative Commons license below applies only to this version of the article.

Creative Commons License



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 4.0 License](https://creativecommons.org/licenses/by-nc-nd/4.0/).

Copyright

Elsevier

Published in final edited form as:

Biochem Biophys Res Commun. 2010 July 16; 398(1): 51–55. doi:10.1016/j.bbrc.2010.06.031.

Redox status affects the catalytic activity of glutamyl-tRNA synthetase

Assaf Katz^a, Rajat Banerjee^b, Merly de Armas^a, Michael Ibba^{b,c}, and Omar Orellana^{a,*}

^a Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Chile

^b Department of Microbiology and Center for RNA Biology, The Ohio State University, Columbus, OH 43210-1292, USA

^c Ohio State Biochemistry Program, The Ohio State University, Columbus, OH 43210-1292, USA

Abstract

Glutamyl-tRNA synthetases (GluRS) provide Glu-tRNA for different processes including protein synthesis, glutamine transamidation and tetrapyrrole biosynthesis. Many organisms contain multiple GluRSs, but whether these duplications solely broaden tRNA specificity or also play additional roles in tetrapyrrole biosynthesis is not known. Previous studies have shown that GluRS1, one of two GluRSs from the extremophile *Acidithiobacillus ferrooxidans*, is inactivated when intracellular heme is elevated suggesting a specific role for GluRS1 in the regulation of tetrapyrrole biosynthesis. We now show that, *in vitro*, GluRS1 activity is reversibly inactivated upon oxidation by hemin and hydrogen peroxide. The targets for oxidation-based inhibition were found to be cysteines from a SWIM zinc-binding motif located in the tRNA acceptor helix-binding domain. tRNA^{Glu} was able to protect GluRS1 against oxidative inactivation by hemin plus hydrogen peroxide. The sensitivity to oxidation of *A. ferrooxidans* GluRS1 might provide a means to regulate tetrapyrrole and protein biosynthesis in response to extreme changes in both the redox and heme status of the cell via a single enzyme.

Keywords

Glutamyl-tRNA synthetases; Heme; Hydrogen peroxide; Zn²⁺-binding site; *Acidithiobacillus ferrooxidans*

1. Introduction

The aminoacyl-tRNA synthetases are an ancient family of proteins. Their primary role is to provide aminoacyl-tRNA for protein synthesis, although they have also been found to provide activated amino acids to an increasing number of processes outside translation [1]. One example is GluRS. During protein synthesis glutamyl-tRNA is used both to translate glutamate codons and sometimes as a precursor for glutamyl-tRNA synthesis depending on the genome context. In cells containing glutamyl-tRNA synthetase (GlnRS), a discriminating GluRS specifically aminoacylates tRNA^{Glu} with glutamate. Conversely, when there is no GlnRS a structurally distinct non-discriminating (ND) GluRS mischarges tRNA^{Gln} with glutamate. The resulting Glu-tRNA^{Gln} is excluded from translation, and instead is transformed into Gln-

*Corresponding author. Address: Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago 8380453, Chile. Fax: +56 2 7355580. oorellan@med.uchile.cl (O. Orellana).

tRNA^{Gln} by a tRNA dependent amidotransferase [2,3]. Outside of protein biosynthesis, Glu-tRNA^{Glu} serves as the first substrate for the biosynthesis of tetrapyrroles in organisms that carry out this process by the C₅ pathway (archaea, chloroplasts and most bacteria). The glutamate moiety of Glu-tRNA^{Glu} is transformed to glutamate semialdehyde by the glutamyl-tRNA reductase (GluTR) and is subsequently transformed to δ -aminolevulinic acid (ALA), the universal precursor of tetrapyrroles, by the glutamate semialdehyde amidotransferase [4].

A number of bacterial genomes encode two different versions of GluRS, consistent with the different possible roles of the enzyme. For example, the existence in *Helicobacter pylori* of two GluRSs that each recognize tRNA^{Glu} or tRNA^{Gln}, respectively, allows for a high degree of tRNA specificity during aminoacylation with Glu [2,3]. Conversely, in the extremophile *Acidithiobacillus ferrooxidans*, an acidophilic bacterium that contains two GluRSs, all tRNA^{Glu} isoacceptors and at least one of all the tRNA^{Gln} species are aminoacylated by GluRS1, while GluRS2 preferentially aminoacylates one of the tRNA^{Gln} isoacceptors and to a lower extent the tRNA^{Glu} isoacceptors [2,5].

Acidithiobacillus ferrooxidans has received attention due to its role in the bioleaching of minerals both in natural and man-made environments. *A. ferrooxidans* obtains reducing power from the oxidation of ferrous ions or reduced sulfur compounds using oxygen as the final electron acceptor. To compensate for the low reducing power of some of these substrates, *A. ferrooxidans* produces an extremely high number of respiratory chain components per cell. The level of these respiratory chain components, including cytochromes and, thus, heme depends greatly on the growth conditions. For example, heme content in *A. ferrooxidans* cultured in medium with ferrous ions is 1.8 times higher than in S^o and up to 5.8 times the level in *Escherichia coli* cultured in Luria broth [6–8]. Given the high levels of heme in *A. ferrooxidans* and since the biosynthesis of the molecule in this organism occurs via the C₅ pathway, the ability of GluRS to provide considerable amounts of Glu-tRNA outside of protein synthesis is critical. In this context the duplication of GluRS may provide a means to ensure the adequate provision of substrate for the C₅ pathway without adversely affecting protein biosynthesis.

The observation that GluRS2 preferentially aminoacylates tRNA^{Gln} suggests that GluRS1 is the main enzyme responsible for supplying Glu-tRNA^{Glu} for heme biosynthesis. Addition of ALA to *A. ferrooxidans* cultures increased the intracellular levels of heme and greatly reduced the activity of GluRS1 [8]. Heme, as well as some of its biosynthesis intermediates, is known to generate oxidative radicals [4,9,10] leading us to speculate that GluRS1 might be regulated by oxidative reactions. This idea is supported by the observation that the inactivation of GluRS1 by addition of ALA to the culture medium is partially prevented if the medium is also supplemented with glutathione, a known thiol reducing agent [8]. The fact that some cysteines from *E. coli* GluRS may be oxidized *in vivo* [11] provides further support to the idea that GluRS1 from *A. ferrooxidans* might be a target for *in vivo* oxidation. The objective of this study was to evaluate *in vitro* the effect of oxidative agents in the activity of GluRS1 and determine the molecular mechanism involved in this process. Our results indicate that specific zinc-coordinating cysteine residues in GluRS1 are sensitive to oxidation in a reversible manner. Both tRNA and ATP protected GluRS1 from oxidative inactivation. Thus, these findings led us to propose that redox modulation of GluRS1 might provide a means to control both heme and protein biosyntheses in response to stress via a single enzyme.

2. Materials and methods

2.1. Overexpression, purification and mutagenesis of GluRS1

Overproduction and purification of the recombinant protein GST-GluRS1 was carried out as described [2], and the GST tag removed by digestion with thrombin (Sigma, St. Louis, MO)

according to the manufacturer's instructions. Purified protein was dialyzed against a buffer containing 20mM Hepes KOH, pH 7.2, 10mM MgCl₂, 50mM KCl and 50% glycerol. 0.5 mM DTT was added to the dialysis buffer as required. When necessary, DTT was removed using Bio-Gel P-10 Micro Bio-Spin columns (Bio-Rad). The GluRS1 C183A encoding gene was obtained via site-directed mutagenesis as described [12] using the following primers: ERS1_C183A_fd (5'-CCG ACC TAC AAT TTC **GCC** GTG GTG GTG GAT GAC-3') and ERS1_C183A_rv (5'-GTC ATC CAC CAC CAC **GGC** GAA ATT GTA GGT CGG -3').

2.2. Oxidation and reduction of GluRS1

GluRS1 free of DTT was incubated in reaction buffer (100 mM Hepes KOH, pH 7.2, 30 mM KCl, 12 mM MgCl₂) in the presence of varying amounts of H₂O₂. When indicated, the reaction mixture also contained one or more of 10 μM hemin, 50 μM glutamate, 5 mM ATP or 2.6 μM *in vitro* transcribed *A. ferrooxidans* tRNA^{Glu₂} [5]. At indicated times, aliquots were removed for enzymatic activity measurements or quantification of reduced cysteines. In the latter case, excess oxidizing agent was removed using Bio-Gel P-10 Micro Bio-Spin columns (Bio-Rad). DTT or β-mercaptoethanol was added to the mixture as necessary, and aliquots were taken at the corresponding times for activity measurements. The oxidation reactions were performed at 37 °C unless otherwise stated.

2.3. tRNA aminoacylation

Reactions were carried out as described [2] in a reaction buffer containing 5 mM ATP, 2.6 μM *in vitro* transcribed tRNA^{Glu₂} and 50 μM glutamate. Data is presented either as nM Glu-tRNA/s or as a percentage of GluRS1 activity compared to the enzyme treated in the same conditions without the addition of oxidizing agents. All reactions were carried out at 37 °C.

2.4. Cysteine quantification

Reduced cysteine content was analyzed as previously described [13]. Briefly, the reaction was carried out at pH 7.27 in a solution containing 0.1 M Tris-HCl, 1 mM EDTA and 500 μM 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB). Changes in absorbance at 412 nm due to the release of nitro benzoic acid (TNB⁻) were recorded in a Jenway 6305 spectrophotometer. Cysteine content was assumed to be equal to TNB⁻ concentration and calculated using a molar extinction coefficient of 14,150 M⁻¹ cm⁻¹ for native conditions and 13,700 M⁻¹ cm⁻¹ in the presence of 0.2% SDS.

2.5. Circular dichroism spectroscopy

2.5 μM GluRS1 in 50 mM Tris-HCl, pH 7.23, 0.5 mM EDTA, was incubated for 50 min at 37 °C in the presence or absence of 300 μM H₂O₂ and 10 μM hemin. The circular dichroism (CD) spectra were measured at 25 °C in an Aviv 62A DS spectropolarimeter (Aviv, Lake Wood, NJ). Spectra were recorded five times for each sample from 200 to 250 nm with step-size of 1 nm in a 1 mm pathlength cuvette with 1 nm bandwidth and 5 s averaging time. The protein only spectra were obtained by subtracting the CD signal of buffer. Experiments were repeated 3 times [14].

2.6. Zinc determinations

Zn²⁺ content of GluRS1 was analyzed by ICP-mass spectrometry at the Trace Element Research Laboratory of the Ohio State University, Columbus, Ohio, USA. For Zn²⁺ release analysis, 0.1 μM 4-(2-pyridylazo)resorcinol (PAR) was added to the solution and change in absorbance at 500 nm due to complex formation with released Zn²⁺ was measured [15]. The amount of released Zn²⁺ was then calculated using a calibration curve with known amounts of zinc acetate in similar buffer conditions. The data is presented as the ratio of the amount of

Zn²⁺ released to the total Zn²⁺ release by the oxidized enzyme denatured in the presence of 8 M urea. All Zn²⁺ determinations were performed at room temperature (~25 °C).

3. Results

3.1. Cysteine oxidation inactivates GluRS1

Previous data suggested that oxidation of GluRS1 might regulate tetrapyrrole biosynthesis [8], prompting us to directly investigate the effect of hydrogen peroxide (H₂O₂) on the activity of the enzyme. Ten minutes incubation with 300 μM H₂O₂ reduced GluRS1 activity by more than 90%. Addition of 10 μM hemin, equivalent to a typical physiological heme concentration and a level that on its own has no effect on GluRS1 activity [8,16], significantly increased both the sensitivity of the enzyme to H₂O₂ and the rate of inactivation (Fig. 1A). The inactivation of the enzyme by H₂O₂, either in the presence or in the absence of hemin, was partially reversed by subsequent incubation with DTT (not shown) or β-mercaptoethanol (Fig. 1B) suggesting that cysteine residues are the primary targets for oxidative inactivation of GluRS1. Partial recovery of the enzymatic activity by treatment with DTT or β-mercaptoethanol suggests that cysteines might be oxidized to disulfide bonds or sulfenic acid [11].

Acidithiobacillus ferrooxidans GluRS1 is predicted to contain four cysteines clustered in the catalytic domain. Amino acid sequence alignments of GluRS from different sources revealed that cysteines 98, 100 and 125 together with glutamate 127 (Fig. S1) from *A. ferrooxidans* GluRS1 are equivalent to the characteristic residues of the SWIM domain from *E. coli* GluRS, which is known to bind Zn²⁺ [14]. The presence of one Zn²⁺ ion per molecule in *A. ferrooxidans* GluRS1 was confirmed by ICP-mass spectrometry (see below). Replacement of C125A at the SWIM domain led to a mostly insoluble enzyme, as for several SWIM domain replacements in *E. coli* GluRS [17]. The recovered soluble fraction of GluRS1 C125A showed ~10% of the specific activity of the wild type enzyme (13.8 vs 118.6 pM Glu-tRNA/(s × nM enzyme), respectively).

Outside the SWIM domain the sequence alignment indicated that Cys183 is likely equivalent to Ala190 from *Thermus thermophilus* GluRS. The crystal structure of *T. thermophilus* GluRS (pdb 1N78) [18] indicates that Ala190 is located in a cleft near the active site, thus Cys183 of *A. ferrooxidans* may have the potential to impact activity upon oxidation. Replacement of *A. ferrooxidans* GluRS1 Cys183 by alanine had no effect on the enzymatic activity, nor did the C183A variant show any alteration compared to wild type in sensitivity to hemin/H₂O₂ and in ability to recover activity after thiol reduction (Fig. S2). These findings indicate that Cys183 is not a target for inactivation of GluRS1 by oxidizing agents, lending further support to the idea that the target(s) of oxidation should be one or more cysteine(s) in the putative Zn²⁺-binding domain.

The possible protective effect of GluRS substrates during oxidative inactivation was investigated. Glutamate provided no protection against oxidation, while saturating concentrations of tRNA^{Glu} or ATP, both protected the enzyme from oxidative inactivation by hemin and H₂O₂ (Fig. 2). Since all substrates bind far from the SWIM domain, these results suggest that upon binding of tRNA or ATP, a conformational change might take place in the enzyme that protects the distant cysteine(s) from oxidation. The SWIM motif is located in the CP1 insertion of GluRS [17,19]. In addition, previous studies have shown specific binding of the tRNA acceptor stem to the CP1 insertion of other GluRSs, an interaction which may also hinder the access of oxidizing agents to the Zn²⁺-binding cysteines [18].

3.2. Assessment of the redox status of cysteine residues in GluRS1

Since only reduced cysteines are sensitive to chemical modification by DTNB, we used this reagent to assess the redox status of cysteines in GluRS1. All four cysteines from reduced and denatured (0.2% SDS) GluRS1 reacted with DTNB. Fully inactive (99%) GluRS1 retained ~1 reduced cysteine indicating that almost three cysteines are oxidized (Fig. 3A). Under non-denaturing conditions, approximately 2.6 cysteines are accessible to the reagent in the reduced enzyme (Fig. 3B). As DTNB is in excess over the concentration of cysteines, the modification reaction kinetics can be treated as pseudo first order for each cysteine. The Guggenheim linearized plot [20] (Fig. 3C) revealed that there are fast and slow reacting cysteines in GluRS1. Upon full inactivation by incubation with 400 μM H_2O_2 , one reduced cysteine which reacted slowly with DTNB remained indicating that ~1.6 accessible cysteines per GluRS1 were oxidized (Fig. 3B and C). To verify that cysteine oxidation did not denature the enzyme we performed a far-UV CD spectra of the reduced and oxidized GluRS1. The results showed that the structure of the enzyme is maintained. Differences in the far-UV CD spectra of reduced and oxidized GluRS1 in the 205–225 nm region suggest a subtle change in the α -helix content of the enzyme [21] (Fig. S3).

3.3. Zn^{2+} ion release and modulation of GluRS1 activity

In light of the fact that readily oxidizable cysteines in the Zn^{2+} -binding motif are important for GluRS1 activity, we investigated whether the release of Zn^{2+} critically impacts the enzyme. ICP-mass spectrometry revealed that *A. ferrooxidans* GluRS1 contains 1.03 Zn^{2+} per molecule. While more than 80% inactivation is observed upon oxidation by H_2O_2 , only ~30% of the bound Zn^{2+} is released from the oxidized GluRS1 (Fig. 4). Also, the kinetics of inactivation is significantly faster than the release of the metal. In addition, after the treatment of the oxidized enzyme with a reducing agent, the proportion of activity recovered is also greater than the proportion of reabsorbed ion. These data suggest that although oxidation of cysteines induces release of Zn^{2+} , ion loss is not the sole cause of enzyme inactivation.

4. Discussion

4.1. Role of zinc in the function of *A. ferrooxidans* GluRS1

Zn^{2+} binds to a number of aminoacyl-tRNA synthetases, including GluRS from some species such as *E. coli* and *Bacillus subtilis* [14,17]. *E. coli* GluRS contains a Zn^{2+} ion coordinated to three cysteines and one histidine forming a so called SWIM motif in the catalytic domain of the enzyme [14]. We found that *A. ferrooxidans* GluRS1 contains one Zn^{2+} ion per enzyme. Sequence alignments predict that a relatively canonical SWIM motif (C-X-C-X₂₄-C-X-E) forms a Zn^{2+} -binding site in *A. ferrooxidans* GluRS1. The crystal structure of GluQRS (pdb 1NZJ), a paralog of the GluRS catalytic domain, indicates that this enzyme binds Zn^{2+} with a slightly different motif which uses the same cysteines, but a different non-cysteine-binding amino acid (C-X-C-X₁₁-Y-X₃-C) [22]. This motif is also observed in GluRS1 from *A. ferrooxidans* (Fig. S1). Thus, while our data clearly indicate that cysteines are involved in Zn^{2+} binding, it is still an open question exactly which residues are the ligands of the ion in *A. ferrooxidans* GluRS1. *A. ferrooxidans* GluRS2 also contains the two predicted Zn^{2+} -binding motifs (C-X-C-X₂₄-C-X-H or C-X-C-X₂₀-Y-X₃-C) (Fig. S1), but the presence of the ion as well as the enzyme sensitivity to H_2O_2 has not been determined.

It is known that Zn^{2+} is essential for the proper binding of glutamate to GluRS, consistent with a structural communication between the Zn^{2+} -binding motif and the active site [14]. Our work now provides further support for such a role for the Zn^{2+} -binding domain, since oxidation of the metal-binding site cysteine of GluRS1 significantly impaired catalysis. Also, binding of ATP or tRNA protects the distant cysteines of the SWIM motif. The Zn^{2+} -binding site is part of the CP1 insertion in the Rosmann fold of the catalytic domain [17,19]. The CP1 insertion

is predicted to be involved in the transduction of the conformational change promoted by the binding of the anticodon of tRNA to the anticodon-binding domain [23]. This conformational change is required for the activation of glutamate by ATP. Thus, we propose that the inactivation observed upon oxidation of cysteines at the Zn²⁺-binding motif from *A. ferrooxidans* GluRS1, might be due to an impairment of the structural communication between the anticodon-binding domain and the active site. This interpretation is consistent with the observation that Zn²⁺ release from the *E. coli* enzyme affects the affinity of GluRS for glutamic acid only in the presence of tRNA [14].

It has been shown that some proteins that participate in translation are oxidized *in vivo* under oxidative stress inhibiting translation or modifying its fidelity [11,24,25]. Whether *A. ferrooxidans* GluRS1 is reversibly inactivated *in vivo* by the cellular redox status is a question still to be answered. Also, whether redox sensitivity is a common feature of all GluRSs and GluQRSs that contain Zn²⁺, or is confined to certain extremophiles such as *A. ferrooxidans* is an open question.

4.2. Reversible oxidation of cysteine and the regulation of heme biosynthesis

Heme and its tetrapyrrole precursors are known to be toxic because of their capacity to generate reactive radicals [4,9,10], thus, cellular levels of heme must be finely tuned. While it has been shown in several organisms that GluTR, which is involved in the biosynthesis of ALA, is the first enzyme directly committed to the C₅ pathway [4,26], our previous results led us to speculate that in *A. ferrooxidans* GluRS1 might be the first regulated enzyme of the pathway [8].

The inactivation of GluRS1 upon exposure of *A. ferrooxidans* to conditions that increase the intracellular levels of heme [8] is counteracted by the presence of a reducing agent (glutathione) in the culture medium suggesting that an oxidative event modulates GluRS1 activity. The data presented here revealed that hemin at concentrations near to the reported intracellular levels has a synergistic effect on the oxidation and inactivation of GluRS1 by H₂O₂. As the redox status and the activity of GluRS1 are recovered after treatment of the oxidized enzyme with reducing agents, we speculate that the cellular activity of GluRS1 might be controlled by changes in the redox status induced by heme, and possibly other oxidizing agents, in a reversible process. As *A. ferrooxidans* proliferate in the bioleaching environment, Fe²⁺ is transformed to the oxidizing agent Fe³⁺, exposing the bacterium to oxidative stress [27] and enormous variations in the available reducing power [28]. Thus, the reversible inactivation of GluRS1 might represent a possible regulatory mechanism by which the enzyme could rapidly respond to environmental conditions that alter the cellular redox status and heme requirements.

Beyond the immediate effect on GluRS activity, redox modulation may also more broadly fine tune heme biosynthesis. An interaction between GluRS and GluTR has been proposed [29, 30]. Our data indicate that a complex between GluRS and GluTR from *A. ferrooxidans* is also formed (O. Orellana, D. Pezoa, P. Alamos, unpublished results), which could potentially channel Glu-tRNA directly for tetrapyrrole synthesis. Another possible function of the complex could be to protect GluRS1 and GluTR from inactivation or degradation. Previous reports suggest that GluTR may be regulated by protein degradation in *A. ferrooxidans* [8] as well as in *Salmonella typhimurium* [26]. Answering the question as to whether the stability of the complex is altered by the cellular redox status and/or the levels of heme could shed light on the global mechanisms of regulation of tetrapyrrole synthesis in *A. ferrooxidans* and other γ -proteobacteria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Hervé Roy for helpful discussions. A.K. was recipient of fellowships from Conicyt, Chile and Universidad de Chile. This work was supported by Grant Fondecyt 1070437 from Conicyt, Chile (O.O.) and Grant GM65183 from the National Institutes of Health, USA (M.I.).

Abbreviations

GluRS	glutamyl-tRNA synthetases
GlnRS	glutaminyl-tRNA synthetase
ND	non-discriminating
GluTR	glutamyl-tRNA reductase
ALA	δ -aminolevulinic acid
DTNB	5,5'-dithio-bis(2-nitrobenzoic acid)
TNB ⁻	nitro benzoic acid
PAR	4-(2-pyridylazo)resorcinol
H ₂ O ₂	hydrogen peroxide
CD	circular dichroism

References

- Banerjee R, Chen S, Dare K, et al. TRNAs: cellular barcodes for amino acids. *FEBS Lett* 2010;584:387–395. [PubMed: 19903480]
- Salazar JC, Ahel I, Orellana O, et al. Coevolution of an aminoacyl-tRNA synthetase with its tRNA substrates. *Proc Natl Acad Sci USA* 2003;100:13863–13868. [PubMed: 14615592]
- Skouloubris S, Ribas de Pouplana L, De Reuse H, et al. A noncognate aminoacyl-tRNA synthetase that may resolve a missing link in protein evolution. *Proc Natl Acad Sci USA* 2003;100:11297–11302. [PubMed: 13679580]
- O'Brian MR, Thöny-Meyer L. Biochemistry, regulation and genomics of haem biosynthesis in prokaryotes. *Adv Microb Physiol* 2002;46:257–318. [PubMed: 12073655]
- Levicán G, Katz A, Valenzuela P, et al. A tRNA^{Glu} that uncouples protein and tetrapyrrole biosynthesis. *FEBS Lett* 2005;579:6383–6387. [PubMed: 16271718]
- Inglede WJ. *Thiobacillus ferrooxidans*: the bioenergetics of an acidophilic chemolithotroph. *Biochim Biophys Acta* 1982;683:89–117. [PubMed: 6295474]
- Yarzabal A, Basseur G, Bonnefoy V. Cytochromes *c* of *Acidithiobacillus ferrooxidans*. *FEMS Microbiol Lett* 2002;209:189–195. [PubMed: 12007804]
- Levicán G, Katz A, de Armas M, et al. Regulation of a glutamyl-tRNA synthetase by the heme status. *Proc Natl Acad Sci USA* 2007;104:3135–3140. [PubMed: 17360620]
- Aft RL, Mueller GC. Hemin-mediated oxidative degradation of proteins. *J Biol Chem* 1984;259:301–305. [PubMed: 6323403]
- Kumar S, Bandyopadhyay U. Free heme toxicity and its detoxification systems in human. *Toxicol Lett* 2005;157:175–188. [PubMed: 15917143]
- Leichert LI, Jakob U. Protein thiol modifications visualized in vivo. *PLoS Biol* 2004;2:e333. [PubMed: 15502869]
- Roy H, Ling J, Irnov M, et al. Post-transfer editing *in vitro* and *in vivo* by the beta subunit of phenylalanyl-tRNA synthetase. *EMBO J* 2004;23:4639–4648. [PubMed: 15526031]
- Liu M, Huang Y, Wu J, et al. Effect of cysteine residues on the activity of arginyl-tRNA synthetase from *Escherichia coli*. *Biochemistry* 1999;38:11006–11011. [PubMed: 10460155]

14. Banerjee R, Dubois DY, Gauthier J, et al. The zinc-binding site of a class I aminoacyl-tRNA synthetase is a SWIM domain that modulates amino acid binding via the tRNA acceptor arm. *Eur J Biochem* 2004;271:724–733. [PubMed: 14764088]
15. Hunt JB, Neece SH, Ginsburg A. The use of 4-(2-pyridylazo)resorcinol in studies of zinc release from *Escherichia coli* aspartate transcarbamoy. *Anal Biochem* 1985;146:150–157. [PubMed: 3887984]
16. Garrick MD, Scott D, Kulju D, et al. Evidence for and consequences of chronic heme deficiency in Belgrade rat reticulocytes. *Biochim Biophys Acta* 1999;1449:125–136. [PubMed: 10082971]
17. Liu J, Gagnon Y, Gauthier J, et al. The zinc-binding site of *Escherichia coli* glutamyl-tRNA synthetase is located in the acceptor-binding domain. *J Biol Chem* 1995;270:15162–15169. [PubMed: 7797500]
18. Sekine S, Nureki O, Dubois DY, et al. ATP binding by glutamyl-tRNA synthetase is switched to the productive mode by tRNA binding. *EMBO J* 2003;22:676–688. [PubMed: 12554668]
19. Liu J, Lin SX, Blochet JE, et al. The glutamyl-tRNA synthetase of *Escherichia coli* contains one atom of zinc essential for its native conformation and its catalytic activity. *Biochemistry* 1993;32:11390–11396. [PubMed: 8218204]
20. Niebergall PJ, Sugita ED. Utilization of the Guggenheim method in kinetics. *J Pharm Sci* 1968;57:1805–1808. [PubMed: 5684763]
21. Van Holde, KE.; Johnson, C.; Ho, PS. *Principles of Physical Biochemistry*. 1. Prentice Hall; Upper Saddle River, NJ: 1998.
22. Campanacci V, Dubois DY, Becker HD, et al. The *Escherichia coli* YadB gene product reveals a novel aminoacyl-tRNA synthetase like activity. *J Mol Biol* 2004;337:273–283. [PubMed: 15003446]
23. Sethi A, Eargle J, Black AA, et al. Dynamical networks in tRNA:protein complexes. *Proc Natl Acad Sci USA* 2009;106:6620–6625. [PubMed: 19351898]
24. Shenton D, Smirnova JB, Selley JN, et al. Global translational responses to oxidative stress impact upon multiple levels of protein synthesis. *J Biol Chem* 2006;281:29011–29021. [PubMed: 16849329]
25. Ling J, Söll D. Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site. *Proc Natl Acad Sci USA* 2010;107:4028–4033. [PubMed: 20160114]
26. Wang L, Elliott M, Elliott T. Conditional stability of the HemA protein (glutamyl-tRNA reductase) regulates heme biosynthesis in *Salmonella typhimurium*. *J Bacteriol* 1999;181:1211–1219. [PubMed: 9973348]
27. Rodrigues VD, Martins PF, Gaziola SA, et al. Antioxidant enzyme activity in *Acidithiobacillus ferrooxidans* LR maintained in contact with chalcopyrite. *Process Biochem* 2010;45:914–918.
28. Rawlings DE, Tributsch H, Hansford GS. Reasons why '*Leptospirillum*'-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. *Microbiology* 1999;145:5–13. [PubMed: 10206710]
29. Jahn D. Complex formation between glutamyl-tRNA synthetase and glutamyl-tRNA reductase during the tRNA-dependent synthesis of 5-aminolevulinic acid in *Chlamydomonas reinhardtii*. *FEBS Lett* 1992;314:77–80. [PubMed: 1451806]
30. Paravisi S, Fumagalli G, Riva M, et al. Kinetic and mechanistic characterization of *Mycobacterium tuberculosis* glutamyl-tRNA synthetase and determination of its oligomeric structure in solution. *FEBS J* 2009;276:1398–1417. [PubMed: 19187240]

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.bbrc.2010.06.031.

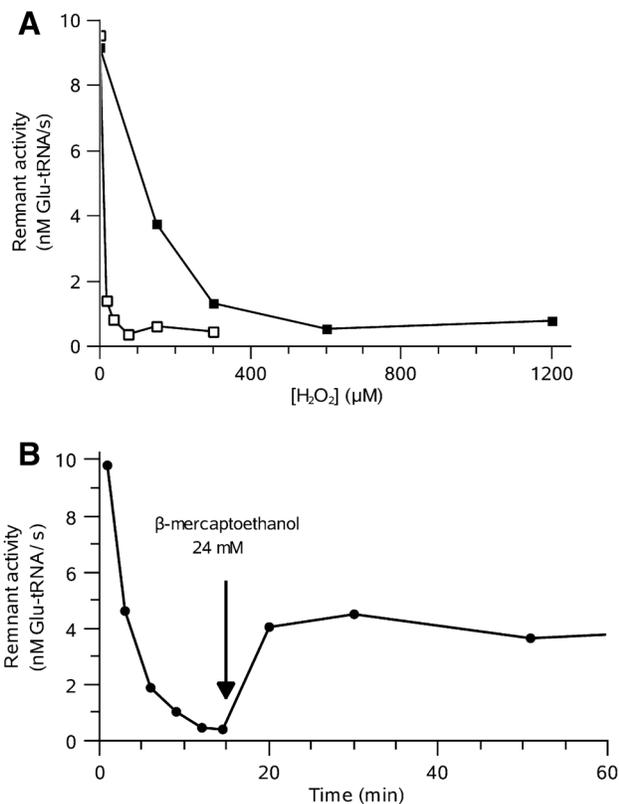


Fig. 1. H₂O₂ inactivates GluRS1. (A) 0.25 μM GluRS1 was incubated at 37 °C in the presence (□) or absence (■) of 10 μM hemin and variable concentrations of H₂O₂. After 10 min of incubation the activity of a 0.1 μM GluRS1 aliquot was measured. (B) 0.25 μM GluRS1 was incubated in the presence of 10 μM hemin plus 60 μM H₂O₂. At given time points the activity of a 0.1 μM GluRS1 aliquot was measured. After 15 min of oxidation, 24 mM of β-mercaptoethanol was added (↓) and the remaining activity was measured.

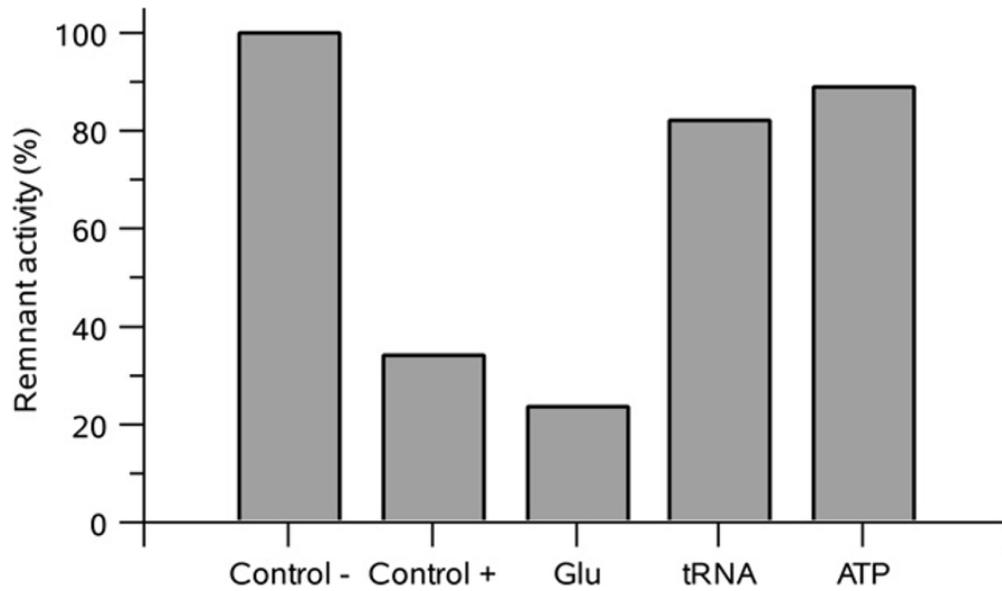


Fig. 2. GluRS1 is protected from oxidation by tRNA and ATP. The enzyme was incubated at 37 °C for 8 min. in the absence (control -) or presence (control +) of 10 μ M hemin plus 300 μ M H_2O_2 . Where indicated, the enzyme was incubated in the presence of the oxidation mixture plus saturating concentrations of either glutamate (50 μ M), tRNA (2.6 μ M) or ATP (5 mM). After incubation the activity of a 0.068 μ M GluRS1 aliquot was measured.

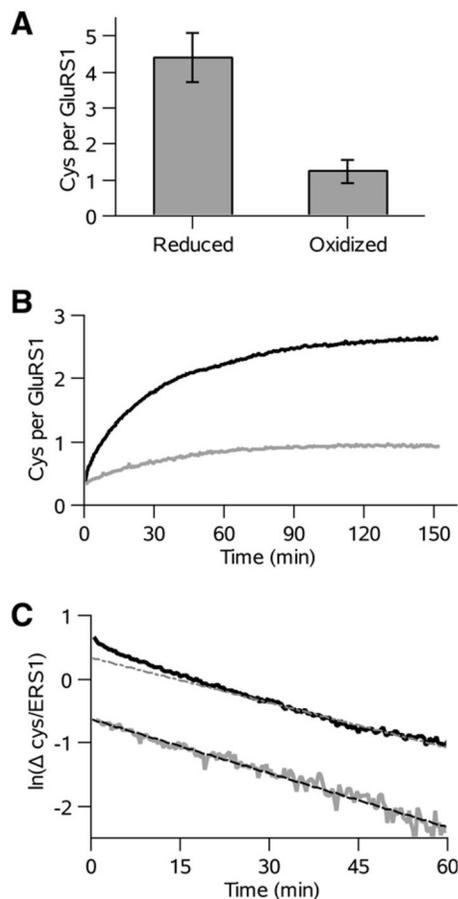


Fig. 3. DTNB reactivity of cysteines from GluRS1. (A) Number of reduced cysteines per molecule of denatured (0.2% SDS) GluRS1 either reduced or oxidized in the presence of 400 μ M H₂O₂ as determined by DTNB reactivity. (B) GluRS1 was incubated in the presence (gray) or absence (black) of 400 μ M H₂O₂. The resulting enzyme was treated with DTNB and the reaction was followed by absorbance at 412 nm. (C) Guggenheim plot of the data from (B). Discontinuous lines indicate the linear zone of the plot lines.

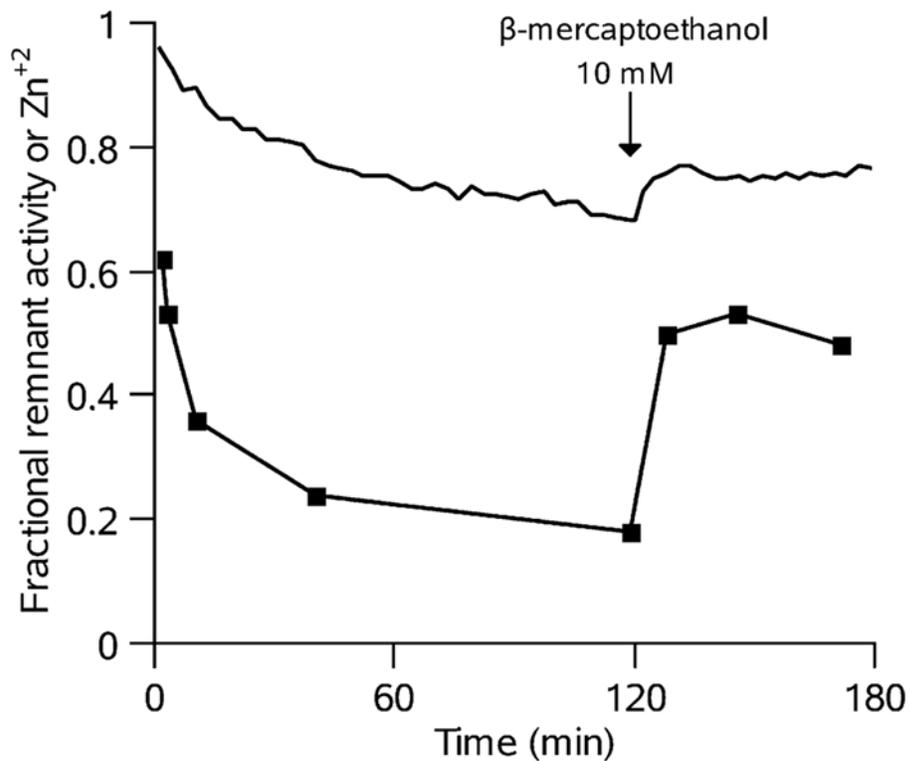


Fig. 4. Oxidation of GluRS1 induces partial Zn²⁺ release. GluRS1 was incubated with PAR (Zn²⁺ indicator) and in the presence of 500 μ M H₂O₂. After 2 h of incubation 10 mM β -mercaptoethanol was added (\downarrow). Changes in Zn²⁺ concentration were recorded through variations in absorbance at 500 nm (graph line with no symbol). At given time points, aliquots were taken to measure the remaining GluRS1 activity (\blacksquare).