Biochemical and Biophysical Research Communications 398 (2010) 51–55

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Redox status affects the catalytic activity of glutamyl-tRNA synthetase

Assaf Katz [a], Rajat Banerjee [b], Merly de Armas [a], Michael Ibba [b,c], 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

ARTICLE INFO

Article history:
Received 28 May 2010
Available online 10 June 2010

Keywords:
Glutamyl-tRNA synthetases
Heme
Hydrogen peroxide
Zn^{2+}-binding site
Acidithiobacillus ferrooxidans

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.

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 glutaminyl-tRNA synthesis depending on the genome context. In cells containing glutaminyl-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-$tRNA^{Gln}$ 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 C_5 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° 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

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_2O_2, hydrogen peroxide; CD, circular dichroism.

* 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.
E-mail address: oorellan@med.uchile.cl (O. Orellana).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.06.031

the biosynthesis of the molecule in this organism occurs via the C_5 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 C_5 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 20 mM Hepes KOH, pH 7.2, 10 mM $MgCl_2$, 50 mM 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 $MgCl_2$) in the presence of varying amounts of H_2O_2. 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* $tRNA^{Glu}_2$ [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 $tRNA^{Glu}_2$ 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′-dithiobis(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 H_2O_2 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

Zn^{2+} content of GluRS1 was analyzed by ICP-mass spectrometry at the Trace Element Research Laboratory of the Ohio State University, Columbus, Ohio, USA. For Zn^{2+} 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 Zn^{2+} was measured [15]. The amount of released Zn^{2+} 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 Zn^{2+} released to the total Zn^{2+} release by the oxidized enzyme denatured in the presence of 8 M urea. All Zn^{2+} 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 (H_2O_2) on the activity of the enzyme. Ten minutes incubation with 300 μM H_2O_2 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 H_2O_2 and the rate of inactivation (Fig. 1A). The inactivation of the enzyme by H_2O_2, 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

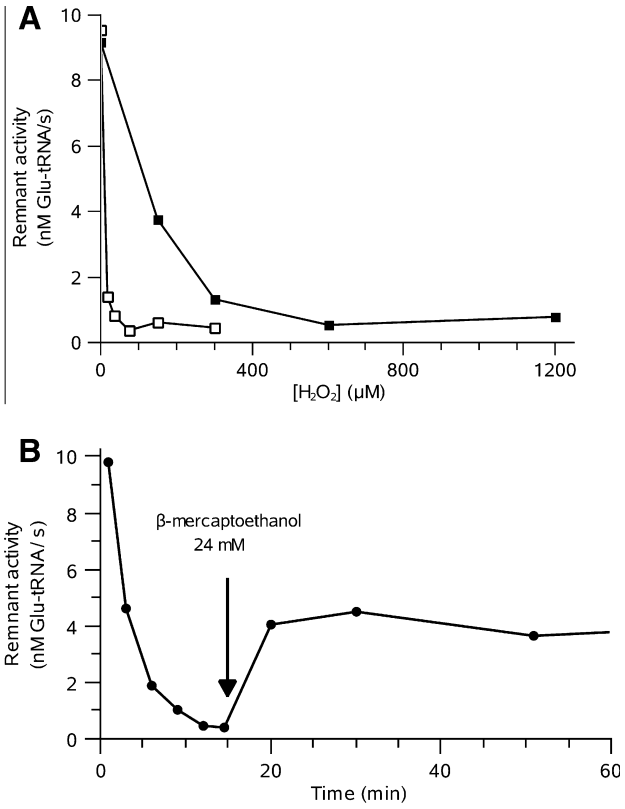

Fig. 1. H_2O_2 inactivates GluRS1. (A) 0.25 μM GluRS1 was incubated at 37 °C in the presence (□) or absence (■) of 10 μM hemin and variable concentrations of H_2O_2. After 10 min of incubation the activity of a 0.1 μM GluRS1 aliquot was measured. (B) 0.25 μM GluRS1 was incubated in the presence of 10 μM hemin plus 60 μM H_2O_2. 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.

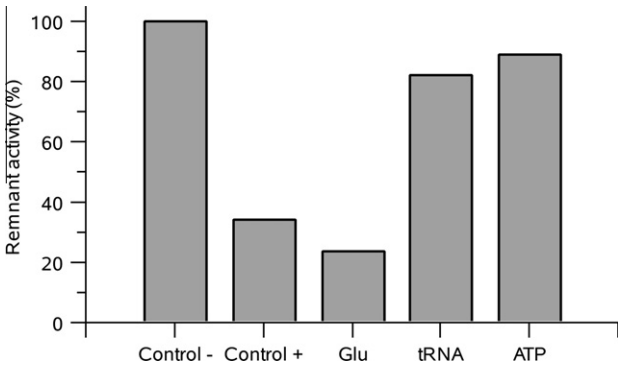

Fig. 2. GluRS1 is protected from oxidation by tRNA and ATP. The enzyme was incubated at 37 °C for 8 min. in the absence (control −) or presence (control +) of 10 μM hemin plus 300 μM H_2O_2. Where indicated, the enzyme was incubated in the presence of the oxidation mixture plus saturating concentrations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

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 Zn^{2+} [14]. The presence of one Zn^{2+} 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 x 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/H_2O_2 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 Zn^{2+}-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 H_2O_2 (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 Zn^{2+}-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

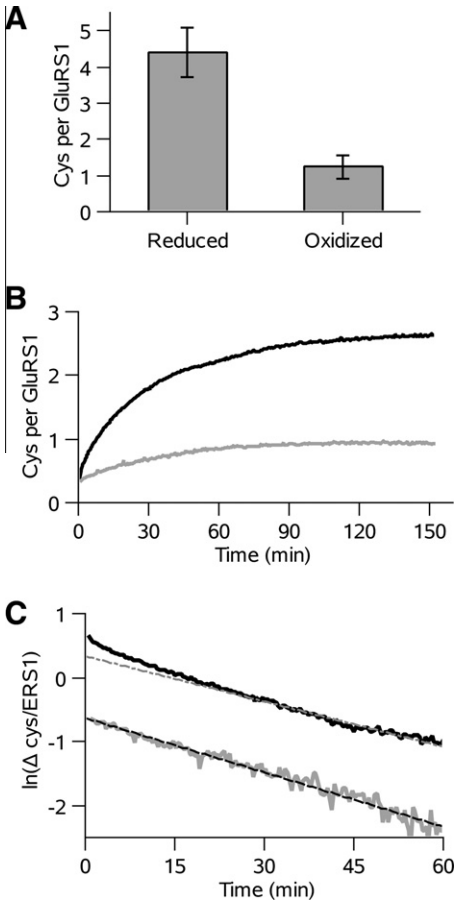

Fig. 3. DTNB reactivity of cysteines from GluRS1. (A) Number of reduced cysteines per molecule of denatured (0.2% SDS) GluRS1 either reduced or oxidized in the presence of 400 μM H_2O_2 as determined by DTNB reactivity. (B) GluRS1 was incubated in the presence (gray) or absence (black) of 400 μM H_2O_2. 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.

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.

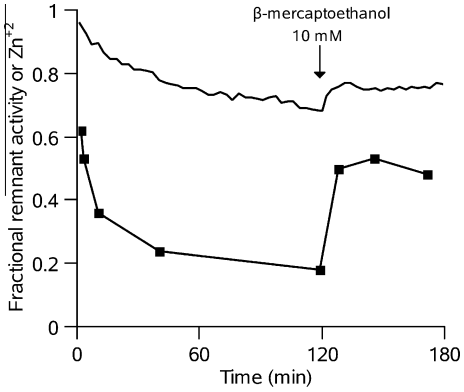

Fig. 4. Oxidation of GluRS1 induces partial Zn^{2+} release. GluRS1 was incubated with PAR (Zn^{2+} indicator) and in the presence of 500 μM H_2O_2. After 2 h of incubation 10 mM β-mercaptoethanol was added (↓). Changes in Zn^{2+} concentration were recorded through variations in absorbance at 500 nm (graph line with no symbol). At given time points, aliquots were taken to measure the remaining GluRS1 activity (■).

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-X_{24}-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-X_{11}-**Y**-X_3-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-X_{24}-C-X-H or C-X-C-X_{20}-Y-X_3-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 Zn^{2+}-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 Zn^{2+} 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 Zn^{2+}, 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 C_5 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 H_2O_2. 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, Fe^{2+} is transformed to the oxidizing agent Fe^{3+}, 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.

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.).

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.

References

[1] R. Banerjee, S. Chen, K. Dare, et al., TRNAs: cellular barcodes for amino acids, FEBS Lett. 584 (2010) 387–395.
[2] J.C. Salazar, I. Ahel, O. Orellana, et al., Coevolution of an aminoacyl-tRNA synthetase with its tRNA substrates, Proc. Natl. Acad. Sci. USA 100 (2003) 13863–13868.
[3] S. Skouloubris, L. Ribas de Pouplana, H. De Reuse, et al., A noncognate aminoacyl-tRNA synthetase that may resolve a missing link in protein evolution, Proc. Natl. Acad. Sci. USA 100 (2003) 11297–11302.
[4] M.R. O'Brian, L. Thöny-Meyer, Biochemistry, regulation and genomics of haem biosynthesis in prokaryotes, Adv. Microb. Physiol. 46 (2002) 257–318.
[5] G. Levicán, A. Katz, P. Valenzuela, et al., A $tRNA^{Glu}$ that uncouples protein and tetrapyrrole biosynthesis, FEBS Lett. 579 (2005) 6383–6387.
[6] W.J. Ingledew, *Thiobacillus ferrooxidans*: the bioenergetics of an acidophilic chemolithotroph, Biochim. Biophys. Acta 683 (1982) 89–117.
[7] A. Yarzabal, G. Brasseur, V. Bonnefoy, Cytochromes *c* of *Acidithiobacillus ferrooxidans*, FEMS Microbiol. Lett. 209 (2002) 189–195.
[8] G. Levicán, A. Katz, M. de Armas, et al., Regulation of a glutamyl-tRNA synthetase by the heme status, Proc. Natl. Acad. Sci. USA 104 (2007) 3135–3140.
[9] R.L. Aft, G.C. Mueller, Hemin-mediated oxidative degradation of proteins, J. Biol. Chem. 259 (1984) 301–305.
[10] S. Kumar, U. Bandyopadhyay, Free heme toxicity and its detoxification systems in human, Toxicol. Lett. 157 (2005) 175–188.
[11] L.I. Leichert, U. Jakob, Protein thiol modifications visualized in vivo, PLoS Biol. 2 (2004) e333.
[12] H. Roy, J. Ling, M. Irnov, et al., Post-transfer editing *in vitro* and *in vivo* by the beta subunit of phenylalanyl-tRNA synthetase, EMBO J. 23 (2004) 4639–4648.
[13] M. Liu, Y. Huang, J. Wu, et al., Effect of cysteine residues on the activity of arginyl-tRNA synthetase from *Escherichia coli*, Biochemistry 38 (1999) 11006–11011.
[14] R. Banerjee, D.Y. Dubois, J. Gauthier, 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. 271 (2004) 724–733.
[15] J.B. Hunt, S.H. Neece, A. Ginsburg, The use of 4-(2-pyridylazo)resorcinol in studies of zinc release from *Escherichia coli* aspartate transcarbamoy, Anal. Biochem. 146 (1985) 150–157.
[16] M.D. Garrick, D. Scott, D. Kulju, et al., Evidence for and consequences of chronic heme deficiency in Belgrade rat reticulocytes, Biochim. Biophys. Acta 1449 (1999) 125–136.
[17] J. Liu, Y. Gagnon, J. Gauthier, et al., The zinc-binding site of *Escherichia coli* glutamyl-tRNA synthetase is located in the acceptor-binding domain, J. Biol. Chem. 270 (1995) 15162–15169.
[18] S. Sekine, O. Nureki, D.Y. Dubois, et al., ATP binding by glutamyl-tRNA synthetase is switched to the productive mode by tRNA binding, EMBO J. 22 (2003) 676–688.
[19] J. Liu, S.X. Lin, J.E. Blochet, et al., The glutamyl-tRNA synthetase of *Escherichia coli* contains one atom of zinc essential for its native conformation and its catalytic activity, Biochemistry 32 (1993) 11390–11396.
[20] P.J. Niebergall, E.D. Sugita, Utilization of the Guggenheim method in kinetics, J. Pharm. Sci. 57 (1968) 1805–1808.
[21] K.E. Van Holde, C. Johnson, P.S. Ho, Principles of Physical Biochemistry, first ed., Prentice Hall, Upper Saddle River, NJ, 1998.
[22] V. Campanacci, D.Y. Dubois, H.D. Becker, et al., The *Escherichia coli* YadB gene product reveals a novel aminoacyl-tRNA synthetase like activity, J. Mol. Biol. 337 (2004) 273–283.
[23] A. Sethi, J. Eargle, A.A. Black, et al., Dynamical networks in tRNA:protein complexes, Proc. Natl. Acad. Sci. USA 106 (2009) 6620–6625.
[24] D. Shenton, J.B. Smirnova, J.N. Selley, et al., Global translational responses to oxidative stress impact upon multiple levels of protein synthesis, J. Biol. Chem. 281 (2006) 29011–29021.
[25] J. Ling, D. Söll, Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site, Proc. Natl. Acad. Sci. USA 107 (2010) 4028–4033.
[26] L. Wang, M. Elliott, T. Elliott, Conditional stability of the HemA protein (glutamyl-tRNA reductase) regulates heme biosynthesis in *Salmonella typhimurium*, J. Bacteriol. 181 (1999) 1211–1219.
[27] V.D. Rodrigues, P.F. Martins, S.A. Gaziola, et al., Antioxidant enzyme activity in *Acidithiobacillus ferrooxidans* LR maintained in contact with chalcopyrite, Process Biochem. 45 (2010) 914–918.
[28] D.E. Rawlings, H. Tributsch, G.S. Hansford, 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 145 (1999) 5–13.
[29] D. Jahn, Complex formation between glutamyl-tRNA synthetase and glutamyl-tRNA reductase during the tRNA-dependent synthesis of 5-aminolevulinic acid in *Chlamydomonas reinhardtii*, FEBS Lett. 314 (1992) 77–80.
[30] S. Paravisi, G. Fumagalli, M. Riva, et al., Kinetic and mechanistic characterization of *Mycobacterium tuberculosis* glutamyl-tRNA synthetase and determination of its oligomeric structure in solution, FEBS J. 276 (2009) 1398–1417.