Published in final edited form as:

J Am Chem Soc. 2012 July 4; 134(26): 10745–10748. doi:10.1021/ja302186j.

Glutathione Complexed Fe-S Centers

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Abstract

Glutathione (γ -glutamyl-cysteinyl-glycine, GSH) is a major thiol-containing peptide with cellular levels of up to 10 mM.¹ Several recent reports have demonstrated glutaredoxins (Grx) to form $[\text{Fe}_2\text{S}_2]$ cluster-bridged dimers, where glutathione provides two exogenous thiol ligands, and have implicated such species in cellular iron sulfur cluster biosynthesis. We report the finding that glutathione alone can coordinate and stabilize an $[\text{Fe}_2\text{S}_2]$ cluster under physiological conditions, with optical, redox, Mössbauer and NMR characteristics that are consistent with a $[\text{Fe}_2\text{S}_2](\text{GS})_4$ composition. The Fe-S assembly protein ISU catalyzes formation of $[\text{Fe}_2\text{S}_2](\text{GS})_4$ from iron and sulfide ions in the presence of glutathione, and the $[\text{Fe}_2\text{S}_2]$ core undergoes reversible exchange between apo ISU and free glutathione.

Keywords

Glutathione; Iron; Sulfur; Cluster; ISU; Biogenesis

Glutathione serves an important cellular role as a redox buffering agent^{2–4} and in cellular defense mechanisms against reactive oxygen species (ROS), both as a direct scavenger and a cofactor of glutathione peroxidase.⁵ Posttranslational modification of many enzymes by GSH (protein S-glutathionylation)⁶ has been implicated in cell signaling pathways,⁷ regulation of redox homeostasis,⁸ ion channel activity,⁹ and protein folding.¹⁰ Glutaredoxins (Grx) have been reported to form $[\text{Fe}_2\text{S}_2]$ cluster-bridged dimers where glutathione provides two exogenous thiol ligands.^{11–15} While the physiological role for this cluster is not fully understood, Grx has been implicated in cellular iron sulfur cluster biosynthesis.^{16–18} There is, however, no precedent for cellular chemistry involving a non-protein-bound iron-sulfur cluster, and so the potential for involvement of an entirely glutathione-coordinated Fe-S cluster, $[\text{Fe}_2\text{S}_2](\text{GS})_4$, in pathways mediated by Grx and/or the iron-sulfur cluster scaffold protein ISU, which mediates iron-sulfur cluster assembly and delivery to target proteins, is of clear significance and interest. Herein we report the results of studies that demonstrate glutathione alone to coordinate and stabilize an $[\text{Fe}_2\text{S}_2]^{2+}$ cluster under physiological

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BRIEFS: An $[\text{Fe}_2\text{S}_2]$ iron-sulfur cluster complex coordinated by glutathione is stable under physiological conditions and demonstrates reversible cluster exchange with the iron-sulfur cluster scaffold protein ISU.

Supporting Information. Experimental details concerning the synthesis, characterization and reconstitution of the $[[\text{Fe}_2\text{S}_2](\text{GS})_4]^{2-}$ cluster complex, NMR experiments, Mössbauer experiments, and cluster transfer assays. This material is available free of charge via the Internet at <http://pubs.acs.org>.

conditions, with optical and NMR characteristics that are consistent with a $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ composition and strong antiferromagnetic coupling between the iron centers. The Fe-S assembly protein ISU mediates formation of $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ from iron and sulfide ions in the presence of glutathione. The $[\text{Fe}_2\text{S}_2]^{2+}$ core undergoes reversible exchange between apo ISU and free glutathione, suggesting a plausible role for this species as an active physiological component of cellular iron chemistry and iron-sulfur cluster biosynthesis.

There is no established precedent for formation of hydrolytically stable small-molecule ligated iron-sulfur clusters in water. An early study had documented the possibility of glutathione coordination stabilizing Fe-S centers in aqueous solution;¹⁹ however, the product was ill-defined with the characterization tools available at that time, and no subsequent work was pursued. We have found that mixing GSH with sulfide and ferric ion in aqueous solution yields a product with a UV/Vis spectrum that is characteristic of a $[\text{Fe}_2\text{S}_2]^{2+}$ iron-sulfur cluster,^{20,21} showing absorption peaks at 330 and 415 nm (Figure S1), while a control spectrum following addition of either ferrous or ferric ion to GSH (Figure S1) is distinct. The time-dependent formation of $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ is readily monitored when inorganic sulfide is provided enzymatically to limit the build-up of S^{2-} in solution (Figure 1). Satisfactory iron and sulfide analyses were obtained.

The $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ complex lacks spectral features associated with cluster in the circular dichroism spectrum (Figure S1), consistent with the lack of the well-defined chiral environment associated with a protein-bound cluster. When a solution of the $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ complex in 10 mM GSH (pH 8.6) was analyzed by Mössbauer spectroscopy at 212 K, only the fully oxidized cluster species was evident (Figure 2), yielding parameters ($\delta = 0.393(1)$ mm/s; $\Delta E_Q = 0.676(2)$ mm/s) consistent with the proposed formulation.^{22–25} Cyclic voltammetric studies of a solution of the cluster complex show an irreversible reduction peak at ~ -340 mV (vs SHE), with no return oxidation peak observed (Figure 1), and so the cluster is reductively labile following isolation from the reaction mixture in which it is formed. The reduction potential is consistent with reported values for $[\text{Fe}_2\text{S}_2]$ cluster proteins.^{26–28}

^1H NMR spectra demonstrate coordination of glutathione in $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$, where both β cysteinyl protons are shifted downfield to a modest extent and splitting of the two pro-chiral β cysteinyl protons is evident (Figure 3). One of the β cysteinyl protons ($\text{C}_{\beta 1}$) shifts from 2.96 to 3.32 ppm while $\text{C}_{\beta 2}$ shifts from 2.89 to 2.99 ppm (Figure 3). The α cysteinyl proton (C_α) shifted from 3.72 into the $^1\text{HO}^2\text{D}$ peak at 4.70 ppm, which was confirmed by a proton homonuclear decoupling experiment (Figure S2). Control spectra obtained with GSH and Fe^{3+} verified that the change in ^1H chemical shift observed with the cluster-bound glutathione did not arise from either free Fe^{3+} or the complex of GSH and Fe^{3+} (Figure S3). A protein-bound iron-sulfur cluster is normally considered a paramagnetic center, which results in resonance broadening and a hyperfine shift of the cysteinyl protons.²⁹ For $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$, the $\text{C}_{\beta 1}$, $\text{C}_{\beta 2}$, E_β and E_γ protons on the cluster are found to shift downfield, but still remain within the diamagnetic window and paramagnetic broadening is not significant, with splitting patterns clearly observable. Apparently there is strong antiferromagnetic coupling between the pair of ferric centers in the relatively symmetric coordination environment, resulting in a negligible paramagnetic influence. Consistent with this, variable temperature studies show the protons on cluster-bound glutathiones to display a negligible temperature dependence, relative to free glutathione.

T_1 relaxation studies show that after forming the GS^- -coordinated $[\text{Fe}_2\text{S}_2]^{2+}$ complex, the spin-lattice relaxation rates of the $\text{C}_{\beta 1}$, $\text{C}_{\beta 2}$, E_β and E_γ protons (Figure S4) increase almost twofold. T_2 relaxation studies show an increase in spin-spin relaxation rates for $\text{C}_{\beta 1}$, $\text{C}_{\beta 2}$, E_β

and E_γ protons on the order of two- to six-fold (Figure S4, Table S1). The increased relaxation rates for cysteine protons is consistent with cluster ligation by GS⁻, and the observed increase in relaxation rates for glutamate protons suggests the glutamate side chains to wrap around the cluster core (most likely stabilized through salt bridge formation with protonated amines), preventing solvent access and cluster degradation.

Glutathione-coordinated cluster can also form in solutions containing GSH incubated with Fe³⁺, cysteine, and a NifS/IscS-type sulfide donor (Figure 1). Additional studies were carried out to determine the relationship between $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ and ISU-bound cluster, and these demonstrated the glutathione-stabilized $[\text{Fe}_2\text{S}_2]^{2+}$ core to be exchangeable with iron sulfur cluster scaffold protein ISU, in which the cluster forms and then is delivered to target iron sulfur cluster proteins.

When holo human ISU was incubated with GSH, the absorbance at 330 nm was observed to decrease until it reached a plateau (Figure 4), with a change in absorbance consistent with the difference in extinction coefficient for ISU-bound and glutathione-coordinated cluster (holo ISU displays a higher extinction coefficient for cluster relative to $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$, 15,600 versus 7,600 M⁻¹cm⁻¹, respectively). A significantly smaller change in absorbance was noted in the absence of GSH, reflecting the hydrolytic instability of ISU-bound cluster. While free glutathione has a pK_A ~ 8.6, this is lowered when complexed to cluster ferric ion and the GS⁻-coordinated cluster is found to be stable at physiological pH, as detailed below.

In the reverse direction, reconstitution of holo ISU resulted from incubation of apo ISU with $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ with an increase in absorbance at 330 nm following cluster transfer to ISU (Figure 4) that is again consistent with the higher extinction coefficient for holo ISU, relative to $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$. Cluster transfer from $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ to apo ISU was also confirmed by absorbance measurements of the isolated holo ISU following cluster transfer (Figure S5). A cluster reconstitution yield of almost 90% was achieved with native ISU, and up to 50% with the D46A ISU derivative, where the conserved carboxylate in the cluster binding pocket is replaced with alanine.^{20,30} The latter derivative typically binds cluster in a more stable manner, but may not receive cluster as efficiently as native.

Consistent with a cellular presence and functional role, the glutathione-complexed cluster is observed to be stable to the presence of dioxygen in the presence of physiological glutathione concentrations. In the absence of excess glutathione a relatively slow degradation of cluster is observed (t_{1/2} = 19 ± 2 min) which is attributed to oxidation of glutathione following dissociation from the cluster as represented in Scheme 1. Indeed, the complex is stable indefinitely under anaerobic conditions.

To conclude, we have synthesized and characterized an iron sulfur cluster complex coordinated by GS⁻ that is stable under physiological conditions and demonstrated reversible cluster exchange with the iron sulfur cluster scaffold protein ISU. Given the inherent lability of the $[\text{Fe}_2\text{S}_2]^{2+}$ center when bound to the surface accessible site of the holo ISU protein, we propose that glutathione exchange of the ISU-bound cluster provides a mechanism to store cluster in a cellular form that is readily accessible, but stable. The exchange of cluster between ISU and glutathione suggests catalytic formation of $[\text{Fe}_2\text{S}_2]^{2+}$ cluster promoted by the ISU scaffold, with subsequent glutathione extraction to prevent hydrolytic degradation, but return of the cluster to ISU when ISU-promoted delivery of cluster to target protein is required. The inability of the $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ complex to reconstitute apo Fd (a reaction readily mediated by holo ISU³¹), supports a requirement for protein-promoted cluster delivery to target proteins in cases where the apo target protein lacks sufficient structure to immediately define a cluster binding pocket.

These results have important implications for understanding the molecular mechanism of cellular iron-sulfur cluster biosynthesis pathways. In particular, glutathione has been implicated in cytosolic Fe-S cluster maturation by an ill-defined mechanism,³² and it has been proposed that GSH is involved in Fe-S cluster mitochondrial export pathways.^{32–34} A stable $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ complex is certainly a viable substrate candidate for the mitochondrial ABC7-type export protein. Recent work has also suggested that glutathione is not required in thiol-redox maintenance, where it only serves a backup role; but rather is essential for iron sulfur cluster assembly.³⁵ The possible cellular presence of a glutathione cluster complex, which could transiently store Fe-S cluster, facilitate cluster exchange with the cellular Fe-S cluster biosynthesis machineries, and regulate the biosynthesis of Fe-S cluster, would underscore an essential physiological role. Studies to elaborate the cellular chemistry of this species are the focus of ongoing studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by a grant from the National Institutes of Health [AI072443].

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Figure 1.

(Top) A solution of 10 mM GSH, pH 8.6 was mixed with 1 mM FeCl_3 , 10 μM of the NifS sulfur-donor protein from *T. maritima* and 1 mM Cysteine under anaerobic conditions. Following the addition of cysteine, cluster formation was observed by absorbance spectroscopy. (inset: the absorbance change at 415 nm reflecting the formation of the GS^- -coordinated $[\text{Fe}_2\text{S}_2]^{2+}$ cluster). **(Bottom)** Cyclic voltammetric experiments display an irreversible reduction wave around -340 mV (vs. SHE).

Figure 2.

Mössbauer spectrum (taken at 212 K) from a 9.3 mM cluster solution in GSH 10 mM (pH 8.6). The solid line corresponds to a quadrupolar interaction characterized by $\delta = 0.393(1)$ mm/s and $\Delta EQ = 0.676(2)$ mm/s.

Figure 3.

Schematic representation of the glutathione complex of $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ showing cysteine α and β protons (C_α , $C_{\beta 1,2}$) and glutamate β protons ($E_{\beta 1,2}$), as well as ^1H NMR spectra of glutathione (top) and the $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ complex (bottom). For the latter the two cysteine β protons are observed to shift from 2.96 ppm to 3.32 ppm, and from 2.89 ppm to 2.99 ppm, respectively. The cysteine α -proton shift from 3.72 ppm into the water peak at 4.70 ppm. Spectra were obtained from a 1 mM $[[\text{Fe}_2\text{S}_2]^{2+}(\text{GS}^-)_4]^{2-}$ solution in D_2O at 300.1 K, using a Bruker DRX 500 MHz spectrometer.

Figure 4.

(Left) The absorbance at 330 nm is observed to decrease when $50 \mu\text{M}$ holo ISU is incubated with 10 mM GSH in 50 mM Hepes, 100mM NaCl, pH 8.6. Red: holo ISU in the absence of GSH; Blue: holo ISU with 10 mM GSH added; **(Right)** The absorbance at 330 nm is found to increase when $15 \mu\text{M}$ apo ISU is incubated with $100 \mu\text{M}$ $[\text{Fe}_2\text{S}_2](\text{GS})_4$ in 50 mM Hepes, 100 mM NaCl, 1 mM DTT, pH 8.6 as a result of cluster transfer to the apo protein.

Scheme 1.

Degradation of cluster in the absence of excess glutathione