S-Sulfhydration of the Catalytic Cysteine in the Rhodanese Domain of YgaP is Complex Dynamic Process

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S-sulphydration of the catalytic cysteine in the rhodanese domain of YgaP is complex dynamic process

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Abstract

The S-sulphydration of cysteine residues in proteins has emerged as a common modification that can modulate the activity of a protein. The ubiquity of the rhodanese domain and its occurrence in a wide variety of protein families indicates that it has diverse roles in physiology. Contrary to common expectations, previous structural studies of several rhodanese domains concluded that S-sulphydration does not induce a structural change in the protein. The presented x-ray structure of a thiosulfate-treated crystal of the rhodanese domain of the *E. coli* integral membrane protein YgaP reveals two important findings: (1) The S-sulhydrated catalytic cysteine C63 adopts an atypical conformation. (2) S-sulphydration leads to a destabilization of the N-terminal part of the helix adjacent to the catalytic loop. These findings assert that S-sulphydration is accompanied by a specific and complex dynamic process.

Objective

The main objective of this study is to characterize the structural changes, if any, that are induced in the YgaP rhodanese domain upon S-sulphydration.

Introduction

Hydrogen sulfide (H₂S), along with nitric oxide (NO) and carbon monoxide (CO), is an important gasotransmitter [1] [2] and plays an essential role in cell physiology by signaling through sulphydration of cysteine residues in proteins [3]. Rhodanese/sulfurtransferases form a group of enzymes widely distributed in prokaryotic and eukaryotic cells that via sulphydration are able to catalyze the transfer of sulfur from thiosulfate (S₂O₃²⁻) to cyanide (CN⁻) that is important for detoxification of cells [4]. The catalysis is a two-step reaction in which the thiol group of the cysteine first reacts with the thiosulfate anion to form an enzyme-persulfide intermediate (CYS-SH), which then reacts with the cyanide ion to produce the much less toxic thiocyanate (SCN⁻) [4]. The most well-studied rhodanese domain is that of bovine liver rhodanese [5] [6] [7] [8]. The active cysteine is located in the cradle-shaped catalytic loop formed by the backbone atoms of the loop residues in such a way that the reactive sulfur, most likely negatively charged, is pointing to the center of the cradle. It has been stipulated that the S-sulhydrated enzyme undergoes a significant conformational change. However, the crystal structures of the sulfur-free as well as S-sulhydrated enzyme are very similar [9]. Even more puzzling results came from the study that investigated in detail the impact of S-sulphydration on the structure and dynamics of the rhodanese domain of the *E. coli* integral membrane protein YgaP using solution NMR as the main technique [10]. The study revealed that the S-sulphydration of YgaP is a transient process: A titration with 1–4 mM sodium thiosulfate to the solution containing ¹³C,¹⁵N-labeled YgaP rhodanese domain revealed that rather than two distinct sets of cross-peaks corresponding to the S-bound and -unbound states, a single set of cross-peaks that shift upon titration is observed in two-dimensional [¹⁵N,¹H]-TROSY experiments indicating a fast exchange (i.e., in the micro- to millisecond range) between the S-bound and -unbound states [10]. These results have been independently confirmed in another study of YgaP [11]. Despite this peculiar finding, the crystal structure of the rhodanese domain of YgaP revealed that the active loop superimposes very well with the analogous bovine rhodanese loop. Furthermore, the structure also revealed that the protein overexpressed in *E. coli* is partially both S-sulhydrated and S-nitrosylated and that the S-sulphydration is likely present in two conformations [12]. It has been known that S-nitrosylation and S-sulphydration are mutually inhibitory processes,

which may play important roles in H_2S/NO signaling [13]. It has also been suspected that the ubiquity and frequent presence of the rhodanese domains in multidomain proteins implies physiological functions other than cell detoxification [14]. Therefore, it is important to understand the effects of these posttranslational modifications at atomic resolution. In this paper, we further investigated the impact of S-sulfhydration on the rhodanese domain of the *E. coli* integral membrane protein YgaP and found that S-sulfhydration triggers a dynamical process by destabilizing the α_4 helix in the domain.

Figure Legend

The overall structure of the YgaP rhodanese domain with CYS-SH modification (A). The comparison of C63 modifications in the structure of the protein prepared without DTT in which CYS-SH in two conformations and CYS-NO are depicted (B) and in the structure of the crystal treated with sodium thiosulfate after which non-modified CYS and CYS-SH in a single atypical conformation are apparent (C). The comparison of the N-terminal part of the α_4 helix in the structure of the protein prepared with DTT (D), prepared without DTT (E), and in the crystal treated with sodium thiosulfate (F). The impact of sodium thiosulfate titration and the subsequent reduction of the active cysteine with KCN on the line width of the T69 cross-peak in the two-dimensional $[^{15}N,^1H]$ -TROSY spectra are shown (G).

Results & Discussion

The fold of the rhodanese domain of YgaP treated with thiosulfate is essentially the

same as that of unmodified protein (Figure 1A) [10]. Our previous structural studies of this domain showed that the catalytic cysteine C63 of the protein prepared with 1,4-dithiothreitol (DTT) is partially S-nitrosylated, while the same cysteine in the structure of the protein prepared without DTT is both S-nitrosylated and S-sulfhydrated. Furthermore, the S-sulfhydrated cysteine was found to adopt two conformations: one perpendicular to the plane of the catalytic loop and the second pointing toward T69 of the α_4 helix [12] (Figure 1B). Here, we show that when the crystals of the protein prepared without DTT are treated with sodium thiosulfate, the catalytic cysteine is primarily S-sulfhydrated and the additional sulfur is pointing towards T69 of the α_4 helix (Figure 1C). Even though the overall protein fold remains the same, the S-sulfhydration of C63 with SH pointing towards T69 appears to cause the destabilization of the N-terminal part of the α_4 helix in a progressive manner. This conclusion is based on the following observations: In the structure of the protein prepared with DTT, there is no S-sulfhydration and the α_4 helix is well-defined, as illustrated by an electron density that is similar throughout the structure (Figure 1A). However, when the protein was prepared without DTT, the S-sulfhydration with SH pointing towards T69 is present and the electron density is weaker for the first two turns of the α_4 helix (Figure 1B). Finally, the thiosulfate treatment of a protein crystal prepared without DTT leads to a higher occupancy of the SH pointing towards T69 and the electron density of the first two turns of the α_4 helix are even weaker (Figure 1C). The progressive destabilization of this part of the helix can also be illustrated quantitatively with B-factors. The average B-factors of the backbone atoms of the first two turns of helix α_4 (i.e., residues 67–72) in the structure of the protein prepared with DTT, the structure of the protein prepared without DTT, and the structure from the crystal treated with thiosulfate are progressively increasing with values of 22.69, 25.11, and 32.80, respectively, whereas the average B-factor of the last two turns of this helix (i.e., residues 74–79) remain similar with values of 13.93, 16.00, and 14.77, respectively. The induction of an S-sulfhydration-dependent dynamical destabilization of the α_4 helix is also supported by previous NMR titration studies [10]. The T69 cross-peak in $[^{15}\text{N}, ^1\text{H}]$ -TROSY spectra undergoes a significant broadening during the titration of the rhodanese domain with 1–4 mM sodium thiosulfate. S-sulfhydration-induced line broadening indicates conformational exchange dynamics of the N-terminal part of the α_4 helix (Figure 1G). In line with these interpretations and findings, the further treatment of the sample with 1–4 mM potassium cyanide, which causes the removal of the persulfide, shows a line narrowing of the T69 cross-peak in $[^{15}\text{N}, ^1\text{H}]$ -TROSY spectra (Figure 1G).

The catalytic loop of the YgaP rhodanese domain (residues 63–68) is characteristic of rhodaneses and overlaps very well with those from other rhodaneses. The N-H moieties of the loop are pointing to the center of the loop, where the SH sulfur atom of the S-sulfhydrated cysteine is expected to be based on the x-ray structures of bovine liver rhodanese and GlpE [5] [6] [7] [8] [15]. The N-H dipoles create thereby a positively charged pocket in which the reactive negatively charged sulfur can be stabilized. In line with this interpretation, the crystal structure of the YgaP rhodanese domain prepared without DTT showed an electron density in this positively charged pocket at C63, which could be modeled as SH. Moreover, there is an additional electron density, which we attributed to NO from S-nitrosylation, since this electron density is strengthened when the crystals are soaked with the NO donor, S-nitrosocysteine (SNOC). Furthermore, there is yet an additional electron density pointing towards T69 of the α_4 helix, suggesting yet another modification of C63 (Figure 1B). Initially we thought that this is an alternative configuration of NO from S-nitrosylation. However, the current study of the structure of the crystal treated with thiosulfate shows that as the result of this treatment the electron density at C63 pointing towards T69 is indeed significantly more pronounced and accompanied with a corresponding loss of the electron density of helix α_4 (Figure 1F). This result, on the other hand, is against the expectation that the sulfur introduced by S-sulfhydration should be perpendicular to the plane of the catalytic loop as observed in other rhodaneses. Also peculiar is the transient nature of S-sulfhydration of YgaP. In an attempt to correlate the two unusual properties of YgaP, it can be speculated that this atypical configuration of S-sulfhydration pointing to the N-terminus of the helix α_4 destabilizes the helix by weakening its dipole, inducing the

dynamical interplay between the helix α_4 and S-sulphydration that makes the CYS-SH bond transient. Unfortunately, without knowing the physiological role of YgaP, it is not possible to annotate the biochemical processes that accompany the transient nature of S-sulphydration and the interplay between S-sulphydration and the helical dynamics.

Additional Information

Methods and Supplementary Material

Please see <https://sciencematters.io/articles/201602000004>.

Ethics Statement

Not applicable

Citations

- [1] Hideo Kimura. "Hydrogen Sulfide and Polysulfides as Biological Mediators". In: *Molecules* 19.10 (Oct. 2014), pp. 16146–16157. DOI: 10.3390/molecules191016146. URL: <http://dx.doi.org/10.3390/molecules191016146>.
- [2] Jerzy Beltowski. "Hydrogen sulfide in pharmacology and medicine – An update". In: *Pharmacological Reports* 67.3 (June 2015), pp. 647–658. DOI: 10.1016/j.pharep.2015.01.005. URL: <http://dx.doi.org/10.1016/j.pharep.2015.01.005>.
- [3] A. K. Mustafa et al. "H₂S Signals Through Protein S-Sulphydration". In: *Science Signaling* 2.96 (Nov. 2009), ra72–ra72. DOI: 10.1126/scisignal.2000464. URL: <http://dx.doi.org/10.1126/scisignal.2000464>.
- [4] D. Bordo. "The rhodanese/Cdc25 phosphatase superfamily: Sequence-structure-function relations". In: *EMBO Reports* 3.8 (Aug. 2002), pp. 741–746. DOI: 10.1093/embo-reports/kvf150. URL: <http://dx.doi.org/10.1093/embo-reports/kvf150>.
- [5] Jan H. Ploegman et al. "The covalent and tertiary structure of bovine liver rhodanese". In: *Nature* 273.5658 (May 1978), pp. 124–129. DOI: 10.1038/273124a0. URL: <http://dx.doi.org/10.1038/273124a0>.
- [6] J.H. Ploegman et al. "Structure of bovine liver rhodanese". In: *Journal of Molecular Biology* 123.4 (Aug. 1978), pp. 557–594. DOI: 10.1016/0022-2836(78)90207-3. URL: [http://dx.doi.org/10.1016/0022-2836\(78\)90207-3](http://dx.doi.org/10.1016/0022-2836(78)90207-3).
- [7] Jan H. Ploegman et al. "The structure of bovine liver rhodanese". In: *Journal of Molecular Biology* 127.2 (Jan. 1979), pp. 149–162. DOI: 10.1016/0022-2836(79)90236-5. URL: [http://dx.doi.org/10.1016/0022-2836\(79\)90236-5](http://dx.doi.org/10.1016/0022-2836(79)90236-5).
- [8] R. J. Trevino et al. "NH₂-terminal Sequence Truncation Decreases the Stability of Bovine Rhodanese, Minimally Perturbs Its Crystal Structure, and Enhances Interaction with GroEL under Native Conditions". In: *Journal of Biological Chemistry* 274.20 (May 1999), pp. 13938–13947. DOI: 10.1074/jbc.274.20.13938. URL: <http://dx.doi.org/10.1074/jbc.274.20.13938>.
- [9] F. Gliubich et al. "ACTIVE SITE STRUCTURAL FEATURES FOR CHEMICALLY MODIFIED FORMS OF RHODANESE". In: (Oct. 1995). DOI: 10.2210/pdb1ora/pdb. URL: <http://dx.doi.org/10.2210/pdb1ora/pdb>.
- [10] C. Eichmann et al. "Solution NMR Structure and Functional Analysis of the Integral Membrane Protein YgaP from *Escherichia coli*". In: *Journal of Biological Chemistry* 289.34 (June 2014), pp. 23482–23503. DOI: 10.1074/jbc.M114.571935. URL: <http://dx.doi.org/10.1074/jbc.M114.571935>.
- [11] Wei Wang et al. "Fast conformational exchange between the sulfur-free and persulfide-bound rhodanese domain of *E. coli* YgaP". In: *Biochemical and Biophysical Research Communications* 452.3 (Sept. 2014), pp. 817–821. DOI: 10.1016/j.bbrc.2014.09.002. URL: <http://dx.doi.org/10.1016/j.bbrc.2014.09.002>.
- [12] Our work. "Environmentally modulated S-Nitrosylation Induces Structural and Dynamical Changes in a Rhodanese Family Protein". In: *Submitted elsewhere* (2016).
- [13] Inga Kwiecień et al. "Inhibition of the catalytic activity of rhodanese by S-nitrosylation using nitric oxide donors". In: *The International Journal of Biochemistry and Cell Biology* 35.12 (Dec. 2003), pp. 1645–1657. DOI: 10.1016/S1357-2725(03)00005-0. URL: [http://dx.doi.org/10.1016/S1357-2725\(03\)00005-0](http://dx.doi.org/10.1016/S1357-2725(03)00005-0).
- [14] Jutta Papenbrock, Sebastian Guretzki, and Melina Henne. "Latest news about the sulfurtransferase protein family of higher plants". In: *Amino Acids* 41.1 (Feb. 2010), pp. 43–57. DOI: 10.1007/s00726-010-0478-6. URL: <http://dx.doi.org/10.1007/s00726-010-0478-6>.
- [15] Andrea Spallarossa et al. "Escherichia coli GlpE Is a Prototype Sulfurtransferase for the Single-Domain Rhodanese Homology Superfamily". In: *Structure* 9.11 (Nov. 2001), pp. 1117–1125. DOI: 10.1016/S0969-2126(01)00666-9. URL: [http://dx.doi.org/10.1016/S0969-2126\(01\)00666-9](http://dx.doi.org/10.1016/S0969-2126(01)00666-9).
- [16] Christos Tzitzilonis et al. "Detergent/Nanodisc Screening for High-Resolution NMR Studies of an Integral Membrane Protein Containing a Cytoplasmic Domain". In: *PLoS ONE* 8.1 (Jan. 2013), e54378. DOI: 10.1371/journal.pone.0054378. URL: <http://dx.doi.org/10.1371/journal.pone.0054378>.
- [17] Zbyszek Otwinowski and Wladek Minor. "[2 θ] Processing of X-ray diffraction data collected in oscillation mode". In: (1997), pp. 307–326. DOI: 10.1016/S0076-6879(97)76066-X. URL: [http://dx.doi.org/10.1016/S0076-6879\(97\)76066-X](http://dx.doi.org/10.1016/S0076-6879(97)76066-X).
- [18] P. Emsley et al. "Features and development of Coot". In: *Acta Crystallogr D Biol Cryst* 66.4 (Mar. 2010), pp. 486–501. DOI: 10.1107/S0907444910007493. URL: <http://dx.doi.org/10.1107/S0907444910007493>.
- [19] Garib N. Murshudov et al. "REFMAC 5 for the refinement of macromolecular crystal structures". In: *Acta Crystallogr D Biol Cryst* 67.4 (Mar. 2011), pp. 355–367. DOI: 10.1107/S0907444911001314. URL: <http://dx.doi.org/10.1107/S0907444911001314>.

- [20] P. J. Kraulis. "MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures". In: *J Appl Cryst* 24.5 (Oct. 1991), pp. 946–950. DOI:

10.1107/S0021889891004399. URL: <http://dx.doi.org/10.1107/S0021889891004399>.