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# Fast conformational exchange between the sulfur-free and persulfide-bound rhodanese domain of *E. coli* YgaP

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ABSTRACT

Rhodanese domains are abundant structural modules that catalyze the transfer of a sulfur atom from thiolsulfates to cyanide via formation of a covalent persulfide intermediate that is bound to an essential conserved cysteine residue. In this study, the three-dimensional structure of the rhodanese domain of YgaP from *Escherichia coli* was determined using solution NMR. A typical rhodanese domain fold was observed, as expected from the high homology with the catalytic domain of other sulfur transferases. The initial sulfur-transfer step and formation of the rhodanese persulfide intermediate were monitored by addition of sodium thiosulfate using two-dimensional $^{1}H$–$^{15}N$ correlation spectroscopy. Discrete sharp signals were observed upon substrate addition, indicting fast exchange between sulfur-free and persulfide-intermediate forms. Residues exhibiting pronounced chemical shift changes were mapped to the structure, and included both substrate binding and surrounding residues.



## 1. Introduction

Rhodanese was first reported in 1933 as an enzyme capable of catalyzing the transfer of a sulfur atom from thiosulfate to the toxic cyanide, resulting in the non-toxic thiocyanate [1]. The cyanide detoxification activity of rhodanese has since been identified in all three major evolutionary phyla [2], and the enzyme has been given the full name thiosulfate:cyanide sulfurtransferase (TST, EC 2.8.1.1) [3]. Rhodanese domains are found as tandem repeats, single domain proteins or in combination with distinct protein domains [4].

The best-characterized are the mitochondrial bovine TST (Rhobov) [5,6] and the *Azotobacter vinelandii* rhodanese (RhdA) enzymes [7], both of which contain two tandem rhodanese domains and an essential conserved Cys residue in the C-terminal rhodanese domain that is critical for catalysis. It has been reported that lack of the Cys residue in the N-terminal rhodanese domain also results in an inactive enzyme [5,7]. Structurally, rhodanese domains are closely related to the human phosphatase Cdc25, both in tertiary structure and the location of the conserved Cys [8,9]. In *Escherichia coli*, eight proteins have been identified as containing rhodanese domains [10], and three (GlpE, PspE, and YgaP) belong to the single-domain rhodanese homology family and contain the essential Cys [11]. Among them, YgaP is the only membrane-associated rhodanese with the predicted two transmembrane helices near the C-terminus [12].

Currently, two separate sulfur-transfer steps are believed to occur during catalysis. In the first step, the sulfhydryl (−SH) group of the conserved Cys residue attacks the thiosulfate ($S_2O_3^{2-}$) anion, forming a covalent persulfide intermediate; in the second step, the persulfide intermediate is attacked by a cyanide ($CN^-$) ion to release the thiocyanate ($SCN^-$) product and regenerate the cysteine sulfhydryl group [3,7]. The three-dimensional structure of PspE was determined using solution NMR. It was reported that backbone dynamics of the persulfide intermediate of PspE was less stable and more conformationally flexible than the ligand-free form, despite of similar conformations between the two forms [13].

In this work, we determined the NMR solution structure of the rhodanese domain of YgaP from *E. coli*. The structure showed a typical fold reminiscent of GlpE [11] and PspE [13]. During preparation of this communication, Eichmann et al. [14] reported the solution NMR structure of full-length YgaP in the presence of detergent micelles, which formed a homo-dimer with strong hydrophobic interactions between the two transmembrane helices of each subunit, although no direct interactions between the N-terminal rhodanese domains were visible. The YgaP rhodanese domain structure determined in the present study was highly similar to full length YgaP. Formation of the persulfide intermediate resulting

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from the first sulfur-transfer step was monitored using two dimensional NMR following addition of sodium thiosulfate. Interestingly, significant chemical shift perturbations were observed in both substrate binding and surrounding residues. Line shape analysis indicated that a fast exchange takes place between the ligand-free and persulfide-bound forms.

## 2. Materials and methods

### 2.1. Protein preparation

The DNA sequence encoding the rhodanese domain of YgaP (residues 1–107) from *E. coli* was cloned into the expression vector p28 (modified from pET-28a, Novagen) to include an N-terminal 6× His tag. The recombinant protein $YgaP_{1\text{-}107}$ was expressed in *E. coli* host strain BL21-Gold(DE3) in M9 media and induced with 0.4 mM IPTG at 37 °C for 5 h. Isotopically enriched proteins were prepared using $^{15}NH_4Cl$ (1 g/L) and $^{13}$C-D-glucose (2.5 g/L) (Cambridge Isotope Laboratories) as the sole nitrogen and carbon sources, respectively. Expressed proteins were purified using Ni–NTA resin (Qiagen). The final NMR sample consisted of 0.5 mM $YgaP_{1\text{-}107}$ in 20 mM Tris–HCl pH 7.0, 20 mM NaCl in a 90% $H_2O$/10% $D_2O$ mixture, or in 100% $D_2O$.

#### 2.1.1. NMR spectra and structure determination

A standard set of multiple resonance NMR spectra including 2D $^1H/^{15}N$-HSQC, 3D CBCA(CO)NH, HNCACB, HNCO, C(CO)NH, HBHA(CO)NH, H(CCO)NH, HCCH-TOCSY, HCCH-COSY, $^{15}N$-edited NOESY (mixing time 80 ms) and $^{13}C$-edited NOESY (mixing time 150 ms) were recorded using a Varian 700 MHz spectrometer. NMR data were processed using NMRPipe [15] and analyzed with Sparky [16]. Backbone torsion angle restraints were predicted from assigned chemical shifts ($^{13}C\alpha$, $^{13}C\beta$, $^{13}CO$, $^1H\alpha$, $^{15}N$, $^1HN$) using the TALOS+ program [17]. The resulting dihedral angle and NOE restraints were applied in structure calculation of $YgaP_{1\text{-}107}$ using Xplor-NIH [18]. Finally, 10 structures with the lowest energy were selected from 100 calculated structures, and the quality was assessed using PROCHECK-NMR [19] and MOLMOL [20].

#### 2.1.2. Monitoring persulfide intermediate formation by solution NMR

$^{15}N$-labeled $YgaP_{1\text{-}107}$ (20 mM Tris pH 7.0, 50 mM NaCl, 0.5 mM $YgaP_{1\text{-}107}$) was used in the NMR titration experiment. A 50 mM stock solution of $Na_2S_2O_3$ and NaSCN were also prepared in the same buffer as $YgaP_{1\text{-}107}$. Several $^1H/^{15}N$-HSQC spectra were recorded using a Varian 700 MHz spectrometer, with the following $Na_2S_2O_3/YgaP_{1\text{-}107}$ (or NaSCN/$YgaP_{1\text{-}107}$) molar ratios: 0:1, 0.2:1, 0.5:2, 2:1, 5:1, and 10:1. Potential binding residues were determined from the chemical shift perturbation (CSP) data calculated using formula (1),

$$\mathrm{CSP} = \sqrt{\Delta H^2 + \left(\frac{\Delta N}{5}\right)^2} \quad (1)$$

where $\Delta H$ and $\Delta N$ are the chemical shift differences between the backbone H and N atoms of the ligand-free and ligand-bound form.

The dissociation constant (Kd) of the interaction for the fast exchange binding process was estimated by curve fitting using the one-site-binding model as in formula (2),

$$Y = \frac{B_{\max} \times X}{K + X} \quad (2)$$

where $X$ is the concentration of the substrate ($Na_2S_2O_3$), $B_{\max}$ is the maximum CSP when substrate concentration is $\infty$, $K$ is the dissociation constant, and $Y$ is the CSP.

## 3. Results and discussion

### 3.1. Overall structure of the YgaP rhodanese domain

Approximately 98% of backbone resonances ($^{13}C\alpha$, $^{13}CO$, $^1HN$, and $^{15}N$) and 98% of side chain resonances ($^1H$) were assigned, excluding those of the 6× His-tag. A $^1H/^{15}N$-HSQC spectra with peaks assigned is shown in Supplementary Fig. S1. The structure of $YgaP_{1\text{-}107}$ was determined on the basis of 1998 restraints, including 1872 inter-proton distance restraints, and 126 dihedral angle restraints. The 10 lowest energy structures were selected out of 100 calculated structures, and the statistics on model quality are summarized in Table 1. The backbone superimposition of the final 10 conformers and representative structures are presented in Fig. 1A and B.

The overall structure of the rhodanese domain of $YgaP_{1\text{-}107}$ adopted the α–β–α sandwich fold typical of a rhodanese domain, with a central five-stranded parallel β-sheet (β1, residues 4–6; β2, residues 20–22; β3, residues 39–40; β4, residues 59–63; β5, residues 86–89) surrounded by five α-helices (α1, residues 8–16; α2, residues 27–32; α3, residues 43–48; α4, residues 68–80; α5, residues 93–98) and a $3_{10}$ helix (residues 53–55) on both sides (Fig. 1B). The structural topology was generated by PDBsum [21] and is shown in Supplementary Fig. S2.

The catalytic residue Cys64 is located at the edge of β4 of the central five-stranded parallel β-sheet, and is surrounded by four α-helices (α2–α4) (Fig. 1B) that form a cavity in which substrate binds and sulfur-transfer takes place. Cys64 is the first residue of the 6-residue loop (Cys64–Arg69) between β4 and α4 that forms a cradle-like semi-circular structure, with the catalytic Sγ atom located at the geometrical center of the substrate binding pocket. Backbone amide protons of residues Gln65-Arg69 all point towards the Cys64-Sγ (Fig. 1C). The electrostatic potential surface of $YgaP_{1\text{-}107}$ showed that the active site pocket is guarded by a positively charged bulb (Lys68 and Arg69) and a negatively charged bulb (Asp26, Asp28 and Glu29) (Supplementary Fig. S3).

### 3.2. Structural comparison of rhodanese domains

A total of eight rhodanese domain-containing proteins are encoded in the *E. coli* genome [11], and three-dimensional structures of three of these (GlpE, PDB code 1GMX; PspE, PDB code 2JTQ; YgaP, PDB code 2MRM) contain a single rhodanese domain

**Table 1**
Structural statistics for the final 10 conformers of the rhodanese domain of YgaP from *E. coli*.

| | |
|---|---|
| Number of distance constrains | |
| NOE distance constraints | 1872 |
| Intraresidue ($i - j = 0$) | 345 |
| Sequential ($i - j = 1$) | 552 |
| Medium-range ($2 \leqslant i - j \leqslant 4$) | 456 |
| Long-range ($i - j \geqslant 5$) | 519 |
| Number of dihedral angle constraints | 126 |
| Rmsd for experimental restraints | |
| NOE distance constraints | 0.009 ± 0.001 |
| Dihedral angle constraints | 0.222 ± 0.042 |
| Rmsd from idealized covalent geometry | |
| Bonds (Å) | 0.001 ± 0.000 |
| Angles (°) | 0.395 ± 0.004 |
| Impropers (°) | 0.237 ± 0.006 |
| Average RMSD of atomic coordinates (Å) | |
| Backbone atoms (secondary structure region) | 0.435 |
| All heavy atoms (secondary structure region) | 0.965 |
| Ramachandran plot analysis (%) | |
| Most favorable region | 80 |
| Additional allowed regions | 16.1 |
| Generously allowed regions | 2.9 |
| Disallowed regions | 1.0 |