ESRF	**Experiment title:** Substrate binding on A. vinelandii rhodanese	**Experiment number:** LS1372
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Shifts: 14 for BAG	**Local contact(s)** Dumon Laurence, Soichi Wakatsuki	*Received at ESRF:*

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Report:

The genome of *Azotobacter vinelandii* encodes for a protein, RhdA, which has been functionally characterized as a rhodanese (thiosulfate:cyanide sulfurtransferase, E.C. 2.8.1.1). The amino acid sequence of RhdA display weak but significant homology with the bovine rhodanese (22% identical residues), and include the conservation of the catalytic Cys residue which, in bovine rhodanese, hosts a persulfide bond during the catalytic process. The crystal structure of bovine rhodanese, solved about twenty years ago, displays a special conformation of the active site, which is formed by a five-residue loop. The catalytic process occurs via the formation of a stable rhodanese-sulfur intermediate. Interestingly, in spite of the conserved catalytic activity, the residues putatively building the active site loop in RhdA display extensive substitution with respect to the bovine enzyme.

The crystal structure of RhdA has been determined in our group using the multiple isomorphous replacement techniques to a resolution limit of 1.8Å. At ESRF we collected high resolution diffraction data (1.8 Å) of potential inhibitors, in order to study the formation of the sulfur-containing intermediate. The collected data showed that both KCN and sulfite are able to convert the sulfur-substituted enzyme into the sulfur-free form, and that the active site Cys residue, as well as two other neighboring residues, assume distinct conformation in the sulfur-free enzyme.

The crystals were grown at 390K from a solution containing 1.7M $MgSO_4$, 50mM MES pH 6.0, and 5% (v7v) eanediol. The crystals belong to the orthorhombic $P2_12_12$ space group, with unit cell a=40.3Å, b=150.3Å, c=53.4Å, and one molecule per asymmetric unit.

The structures of RhdA in soaking with KCN and SO3 were determined by using difference Fourier techniques, and refined using both REFMAC and CSN.

The following statistical values were obtained (data between 20 Å and 1.8 Å):

Rmerge: 7.5%

Completeness: 98.7%

R-factor: 19%

R-free: 22.0%

The data collected at ESRF are part of a work describing the enzyme and the catalytic activity of *A.vinelandii* rhodanese submitted to Structure.