Point mutants in the sulfite reductase hemoprotein

Kyle Smith and M. Elizabeth Stroupe
Florida State University, Department of Biological Science and the Institute for Molecular Biophysics

ABSTRACT: Sulfite reductase (SiR) is an enzyme found in plants and bacteria that performs the six electron reduction of sulfite to sulfide. SiR and a homologous protein, nitrite reductase, are the only known proteins that perform a single atom reduction of six electrons. This research is designed to analyze the structure function relationships of this unique redox protein by examining the roles of individual amino acids in its active site.

BACKGROUND: Two homologs of SiR catalyze the same chemical reaction but differ in their biological function. The assimilatory SiR (aSiR) is part of the pathway for reducing sulfur for incorporation into amino acids and cofactors. The dissimilatory SiR (dSiR) is the terminal electron acceptor in some forms of anaerobic respiration. They both have in their active sites a set of unique iron-rich cofactors: an iron-containing heme-like molecule called siroheme that is attached to an iron-sulfur cluster.

aSiR and dSiR also differ in some molecular details. For example, aSiR is a monomer with a single active site (Crane, *et al Science*). dSiR has two similar siroheme sites but one is hypothesized to be structural (Schiffer, *et al, JMB*). We want to explore substrate binding in aSiR through comparative analysis of the single aSiR active site with the structural site from dSiR.

We initially identified two residues significant to substrate binding because they are in the loop that surrounds the substrate binding pocket in the active site. Trp119 appears to be blocking substrate access in dSiR (Fig. 1a), whereas the spatially corresponding residue in aSiR, Asn149, does not (Fig. 1b).

Next, we looked at other amino acids in that loop. In aSiR, Arg153 binds the substrate in the active site (Fig. 2b). The dSiR counterpart, Ser129, lacks the charge and shape to perform this function (Fig. 2a). In aSiR, the loop containing Asn149 and Arg153 plays an important role in binding substrate. It is disordered when substrate is not bound but orders upon SO_3^{-2} binding (Crane, *et al Science*; Crane, *et al., Biochemistry*) In the structural site of dSiR, this loop is slightly longer and contains a large amino acid (Trp119) that projects into the active site, effectively blocking substrate access (Fig. 1b).

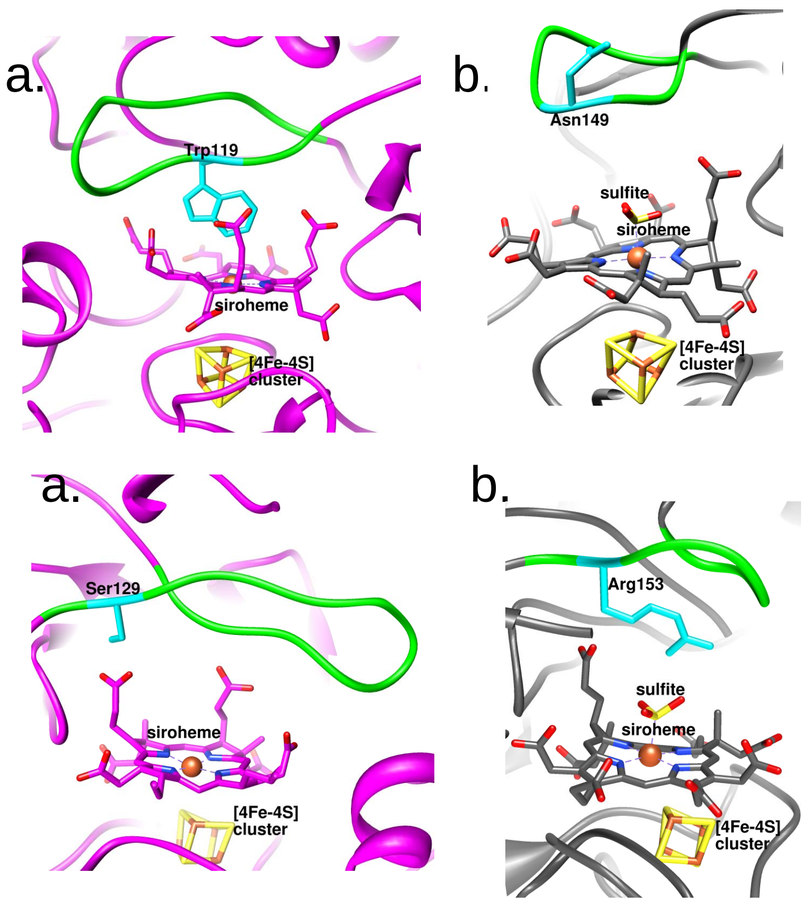

Figure 1*: Trp119 and Asn149*

a. The dSiR structural site's binding loop (green) contains Trp119 (cyan) that projects into the active site. (PDB code 3C7B, Schiffer et al.)

b. The aSiR active site, with bound sulfite, has Asn149 (cyan) in the equivalent position in the binding loop (green). (PDB code 1GEP, Crane et al.)

Figure 2: Ser129 and Arg153

a. dSiR structural site with Ser129 (cyan) in the binding loop (green). (PDB PDB code 3C7B, Schiffer et al.)

b. aSiR active site with bound sulfite that makes a hydrogen bond with Arg153 (cyan) from the binding loop (green). (PDB code 1GEP, Crane et al.)

RESULTS:

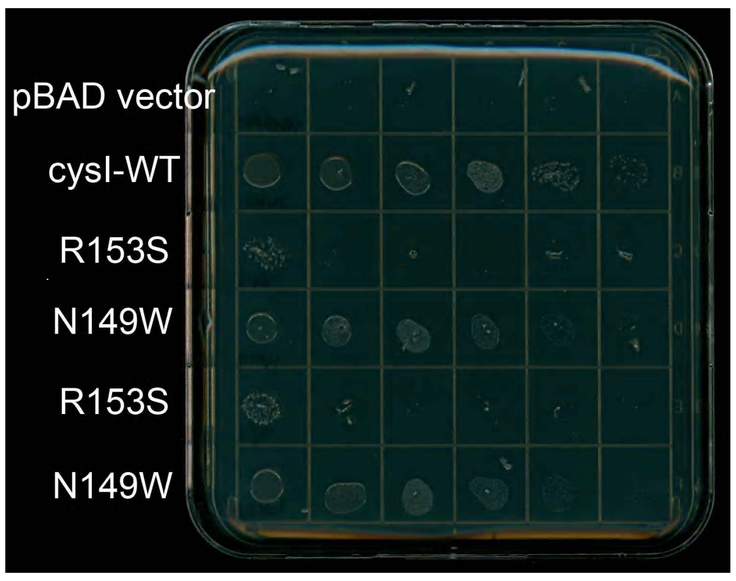

Figure 3: *Complementation of SiR minus E. coli*

SiR(-) E. coli were grown on agar plates made of M9 minimal media containing 0.05% arabinose, ampicilin, and kanamycin antibiotics. Cells were grown over two nights at 37° C. Plates were inoculated with $8.4x10^4$ cells from an overnight grown in Luria-Burtani media and subsequently washed in liquid M9 minimal media before plating. Negative control with empty pBAD vector shows no growth whereas complementation with wild-type (WT) SiR restores growth. R153S shows reduced viability and N149W shows no discernable phenotype from the wild type.

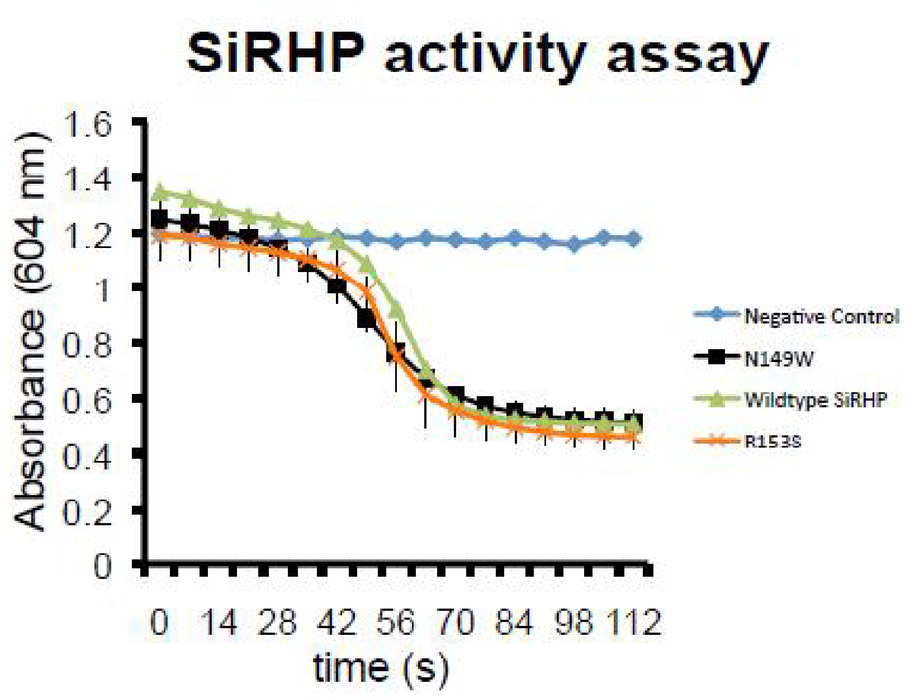

Figure 2: Graph of Spectroscopy Assay

Methyl viologen (0.07 mM), and sodium sulfite (5 mM) were introduced to a buffered solution KP_i pH 7.8 (100 mM). The solution was then degassed by bubbling in nitrogen to the solution stirring while venting for 1 hour. Sodium dithionite (1.5 mM) and SiR (7.7 ng/ml) were then introduced through a gas-tight syringe at the zero time point.

CONCLUSION: We hypothesized that this single amino acid is sufficient to inactive the enzyme so we replaced the equivalent amino acid in aSiR (Asn149) with a tryptophan. Our complementation assay shows that this single point mutation (N149W) is insufficient to inhibit enzyme activity (Fig. 3), suggesting that the active-site loop is sufficiently mobile to allow substrate binding.

However, the mutation of Arg153 to a serine residue appears to have partially inactivated the protein. The data suggests that the Arg153 residue was responsible for facilitating a second or third step within the mechanism for reduction. This would allow for partial reduction of sulfite, which would be indistinguishable from full reduction to sulfide in the spectroscopy assay. The partially reduced intermediate species can not be incorporated into biosynthesis, which fits our observations in the complementation assay. This evidence supports the hypothesized mechanism of a three step reduction.

$$H_2SO_3 + 2H^+ \rightarrow H_2SO_2 + H_2O + 2H^+ \rightarrow H_2SO + H_2O + 2H^+ \rightarrow H_2S + H_2O$$

Our data show that in aSiR, substrate binding residues are more important for activity than other amino acids in that loop. We can also conclude that the structural siroheme site in dSiR is partially inactivated by the serine at position 129 rather than solely by the steric block provided by the tryptophan on the mobile substrate-binding loop.

If you are interested in this or other projects in my laboratory, please contact me at mestroupe@bio.fsu.edu