Determining the Structure of the N-Terminus of the Sulfite Reductase Flavoprotein

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Abstract:

Sulfite Reductase (SiR) is a unique protein which is responsible for turning sulfur into biomass. SiR has two types of subunits: a flavoproteins (SiRFP) and a hemoprotein (SiRFP). The N-terminus of SiRFP allows the eight SiRFP subunits to combine and form an octamer. To better understand the structure of the SiRFP octamer and SiR in general, the N-terminus's structure must first be determined. This is difficult because the whole octamer is a large protein complex. To simplify the process, we have fused just the N-terminus of SiRFP to a smaller protein called SUMO (NFPSh). To get the N-Terminus we must first grow a culture of cells which carry the gene that express NFPSh, lyse the cells, and send the protein through various columns to purify it. Once we have the protein, we use a robotic machine called the Gryphon to test our protein under many different chemical conditions to identify the conditions in which NFPSh is inclined to crystallize. From there, we can set up manual trays with similar conditions to attempt to get the protein to form large crystals. Once the protein has been successfully crystallized, a technique called X-ray crystallography will be used to get a diffraction pattern. We can use the diffraction pattern to find the electron density and determine the structure of the protein. Once the structure has been determined, it will give insight into how the N-terminus functions to octomerize SiRFP, which will help us better understand the structure and function of the molecule, SiR.

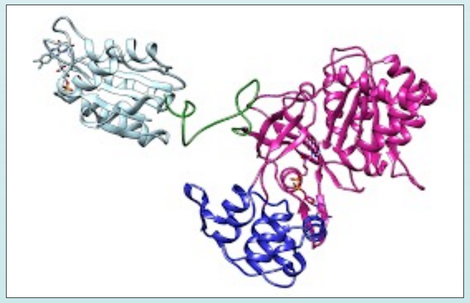

Figure 1: Quaternary Structure of a Sulfur Reductase Flavoprotein without the N-terminus (Tavoulieri et al., 2019). This shows the protein after the N-terminus is removed, so the structure of a single monomer can be determined. Determining the structure of the full octamer would be much mor difficult and complex.

Introduction:

Sulfite Reductase (SiR) is a protein that is responsible for breaking down sulfur for biomass incorporation. SiR breaks down sulfite (SO_3^{2-}) to sulfide (S^{2-}), which is a six-electron reduction. SiR is a dodecamer which means it is composed of 12 different monomers (Siegel et al., 1974) SiR contains eight sulfite reductase flavoprotein subunits (SiRFP) which form an octamer, and four sulfite reductase hemoprotein subunits (SiRHP). The names come from the flavin or iron cofactors that bind the SiRFP or SiRHP subunits, respectively. SiRFP's N-terminus allows it to octomerize or combine eight monomers into one octamer (Zeghouf et al., 1998). When the N-terminus of SiRFP is removed from the protein, the structure of a single monomer can be determined (Tavoulieri et al., 2019). However, it is still unknown how the N-terminus allows the subunits to octomerize.

The N-terminus separated from SiRFP was fused in a recombinant gene to a hexahistidine-SUMO tag that we can use it to generate the NFPSh protein (N-terminus of flavoprotein with Sumo histamine tag). This is a smaller, simpler octamer than SiRFP. Once the structure of NFPSh is determined, it will help us understand how the N-terminus octomerizes the single monomers of SiRFP. From there, the overall structure of SiR can be better understood. Once the structure of SiR is understood, it can be determined how the protein moves six electrons from the cofactors on SiRFP to those on SiRHP and, finally, SO_3^{2-}.

Methods:

Protein Acquisition

- First *E. coli* cells must be grown in culture.
- Then the cells are lysed (broken apart) to extract the protein
- The protein extracted from the cells is run through 3 different columns. The protein is purified more after each column run.
- The columns separate the protein through different methods, such as using charge and size exclusion.
- To test that the correct protein is acquired, the protein is put through gel electrophoresis, since the approximate mass in Kilodaltons of NFPSh is known.

Protein Crystallization

- The protein is tested in a 92 wells robot set up. Each well tests a different condition for crystallization. Theis is known as the sitting drop method
- When the protein shows signs of crystallization in one of the robot wells, manual trays can be set up.
- The manual trays use a method called hanging drop method.
- Both method are considered vapor diffusion methods
- The manual trays will use similar conditions to the well that already showed signs of crystallization, but with small changes in pH and concentration.

X-Ray Crystallography

- After a crystal has been grown successfully, X-Ray beams are sent through the crystal.
- Based on the structure of the protein, the beam will be refracted by electrons in a certain pattern.
- The electron diffraction pattern can be used to determine the overall structure of the molecule. This is a similar method to the one Rosalind Franklin used to determine the structure of DNA, (Fig.2).

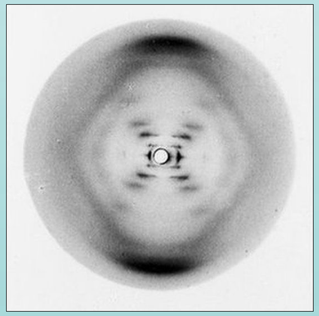

Figure 2: The picture to the left is known as Photo 51 taken by Rosalind Franklin and Ray Gosling in 1952. It depicts an x-ray diffraction pattern taken from DNA. This photo is what revealed the double-helix structure of DNA (Franklin & Gosling, 1953). The methods used to take this picture are similar to the methods of x-ray crystallography used in this lab to determine the structure of NFPS.

Figure 3: Both image shown below are of the same well in a crystal screening, but they are taken at different times. The image on the left shows the well taken on day 0. The image on the right was taken on day 10 and shows the beginning of crystal growth, referred to as "needles".

Results:

After testing different growth condition using the sitting drop method, it was found that the NFPSh protein shows early signs of crystallization, with 0.2 M ammonium acetate as the salt, 0.1 M sodium acetate trihydrate pH 4.6 as the buffer, and 30% w/v Polyethylene glycol 4,000 as the precipitant (Fig.3). Similar conditions are currently being tested using the hanging drop method but changing things slightly in each tray. For example, in one tray, different pH levels of the buffer are used and in another tray the pH remains constant, but the concentration is changed. Once the best conditions are found for crystallization, a large crystal will be used to preform x-ray crystallography to determine the structure of NFPSh.

Conclusion:

Once the electron diffraction pattern is determined it can be used to create an electron density map and then an atomic model like (Fig. 1) can be created for NFPSh. Understanding the six-electron transfer that sulfite reductase performs. (Fig.4) is the ultimate goal of studying this protein. Once the structure of NFPSh is determined, it can be better understood how the octamerization of SiRFP occurs. Further studies can also attempt to understand how the octermization of SiFRP contributes to the six-electron transfer and how the electrons are moved from SiRFP to the other part of SiR, SiRHP.

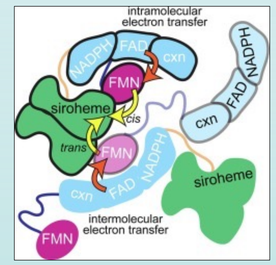

Figure 4: Diagram of the six-electron transfer that occurs in sulfite reductase (Murray et al., 2021).

References:

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