Cryo-EM Reveals the Structural Origins of Asymmetric Electron Transfer in Nitrogenase-Like Enzymes

Dr Rajnandani Kashyap[1], Dr Jaigeeth Deveryshetty[1], Dr Edwin Antony[1]
[1]*Saint Louis University*
rajnandani.kashyap@health.slu.edu

Anoxygenic bacteria undergo bacteriochlorophyll biosynthesis in the absence of oxygen and utilize a unique series of enzymes to convert the tetrapyrrole substrate to the chlorophyll.
One key enzyme in the biosynthesis pathway is the dark-operative protochlorophyllide oxidoreductase (DPOR). It's a nitrogenase-like enzyme that catalyzes the stereo-specific reduction of the C17=C18 double bond of protochlorophyllide (Pchlide) to chlorophyllide (Chlide). DPOR is widely through to function as a structurally symmetric octameric complex that consists of an electron donor (BchL) and an electron acceptor/substrate binding (BchN- BchB) component protein. Both contain Fe-S clusters and form a transient complex in the presence of ATP. We recently showed that electron transfer from BchL to BchN-BchB is both sequential and asymmetric between the two identical halves of DPOR. This mechanism is similar to what we uncovered in nitrogenase (Danyal et. al. PNAS 2016).
These findings raise several interesting questions about the structure-function relationships in such electron transfer enzymes that function as higher order complexes. This higher order complex functions with a cascade of events like ATP hydrolysis, protein-protein interactions, and catalysis.
DPOR, one such electron transfer enzymes, also has an intricate structure assembly of two identical functional halves with a twofold symmetry featuring homodimer of BchL and heterotetramer of BchN-BchB. Our overall objective is to understand how does this electron transfer protein complex relays information over long range distances (52 Å for DPOR) and provide exquisite allosteric control over the other half of the complex.
Cryo-EM will help us in understanding dynamics and the transition states involved in such electron transfer enzyme.
For the very first time, here we report the challenges involved in sample preparation due to anaerobic nature of protein and the cryo-EM structures of DPOR complex, the individual components (BchL and BchN-B) and mutant protein complex. We have trapped the complex in different transient states in the presence/ absence of ATP and substrate Pchlide, interacting protein components. Our structures enable us to understand the catalytic events in a more profound manner from the relevant conformational changes observed. This study is a pioneer to understand the structural origins of asymmetrical electron transfer in a more dynamic environment via cryo-EM as compared to the reported static X- ray crystal structures.

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