The Rate-Limiting Step of Nitrogenase-like Dark Operative Protochlorophyllide Oxidoreductase (DPOR)

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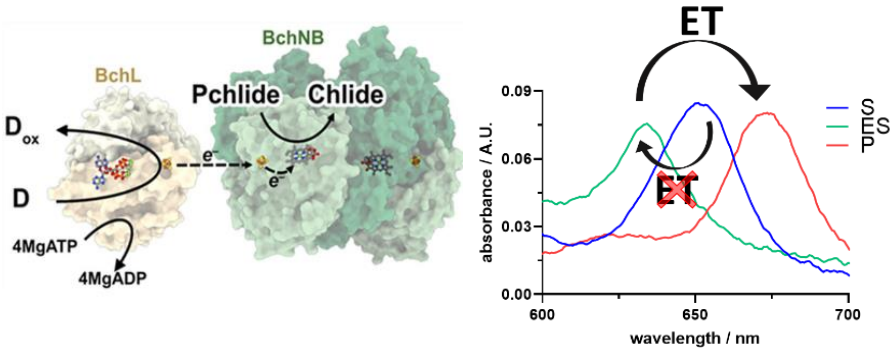
Dark-operative protochlorophyllide oxidoreductase (DPOR) is a two-component metalloenzyme: BchL, a homodimer containing an iron-sulfur cluster and two ATP binding sites, and BchNB, an $\alpha_2\beta_2$ heterotetramer that also has an iron-sulfur cluster and a substrate binding site on each αβ half. In bacteriochlorophyllide-producing organisms, the transfer of electrons by DPOR's transiently associating proteins coupled to the hydrolysis of ATP catalyzes the stereoselective $2e^-$ reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide).[1] The importance of ATP hydrolysis is not fully understood. Further, the thermodynamic properties of the iron-sulfur clusters of DPOR (i.e., their reduction potentials) have not been reported. DPOR has structural and mechanistic similarities with the N_2-fixing enzyme nitrogenase. Nitrogenase is known for its ability to reduce dinitrogen under mild conditions, although its mechanism is not well-understood. In comparison, DPOR's catalytic mechanism is comparatively simpler to observe considering that it is possible to follow substrate reduction *in situ* by UV/visible spectroscopy; for this reason, our research on DPOR is expected to be informative to related metalloenzymes such as nitrogenase.

We proposed three alternative electron donors that support Pchlide reduction in the DPOR system, which can also be used as mediators for electrochemical studies.[3] We aim to use mediated electron transfer to investigate the properties of iron-sulfur clusters in DPOR; moreover, we are able to isolate the Enzyme–Substrate (ES) complex. The study of the substrate-binding event enables us to determinate the rate determining step of the reaction and to study the role of ATP hydrolysis in the substrate binding step. We propose that the rate-limiting step is the formation of ES prior to subsequent interactions with BchL, including electron transfer and ATP hydrolysis.



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