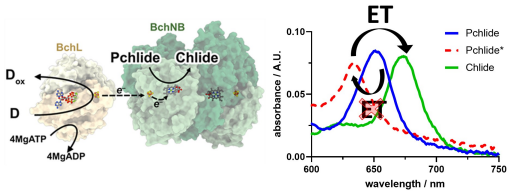
Spectroelectrochemical Investigation of the Nitrogenase-like Dark Operative Protochlorophyllide Oxidoreductase (DPOR)

G. Bedendi[1], A. Kulkarni[1], P. Maroni[1], R. D. Milton[1]*

[1]Department of Inorganic and Analytical Chemistry, University of Geneva, Sciences II, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland

Dark-Operative Protochlorophyllide Oxidoreductase (DPOR) is an enzyme made of a homodimer called BchL and an $\alpha_2\beta_2$ heterotetramer (two catalytic αβ halves) called BchNB. BchL has two ATP binding sites and contains an iron-sulfur cluster, while BchNB has an iron-sulfur cluster and a substrate binding site in each αβ half. This enzyme participates in the biosynthetic pathway of bacteriochlorophyll, enabling photosynthesis by photosynthetic bacteria: the transfer of electrons (which happens through the iron-sulfur clusters) by DPOR's transiently associating proteins is coupled to the hydrolysis of ATP, resulting in the stereoselective $2e^-$ reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide). The substrate reduction can be observed *in situ* by using UV/visible spectroscopy. DPOR has structural and mechanistic similarities with the N_2-fixing enzyme nitrogenase and, as is the case for nitrogenases, neither the dependence of this enzyme on ATP hydrolysis nor the order of events are fully understood. Further, the reduction potentials of DPOR's iron-sulfur clusters have not been reported yet. Thus, spectroelectrochemistry is proposed to be a useful tool to (i) determine these reduction potentials and (ii) study the reduction of Pchlide by DPOR.

In our previous work[1], we proposed three alternative electron donors that support Pchlide reduction in the DPOR system, which can also be used as mediators for electrochemical studies. Now, we use mediated electron transfer to control the electron delivery and thus, the reduction of Pchlide. We investigated the properties of the iron-sulfur clusters in DPOR using spectroelectrochemistry. Moreover, we are able to isolate the ES complex formed when Pchlide is bound to BchNB, (Pchlide*). The study of the binding event enables us to determinate the rate determining step of the reaction and to confirm that ATP hydrolysis does not have a role in the substrate binding step. We propose that the rate-limiting step is the formation of Pchlide* prior to subsequent interactions with BchL. Our research on DPOR is expected to be informative to related metalloenzymes, such as nitrogenase



[1] G. Bedendi, A. Kulkarni, P. Maroni, R. D. Milton, ChemElectroChem 2022, 9, e202200774.