PhD thesis title : Electron-bifurcation: mechanistic and structural aspects

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Study background

Some enzymes (biological catalysts) use the mechanism of electron bifurcation during catalysis. The electron bifurcation mechanism requires a center that can exchange 2 electrons (quinone or flavine). This center transfers its 2 electrons asymmetrically to two acceptors, one of which has a more positive electrochemical potential (exergonic reaction) and the other more negative (endergonic reaction). The endergonic reaction is made possible by the strict coupling of the two redox events and by a free energy for the overall reaction which remains negative, therefore favorable. The molecular bases of the electron bifurcation are the subject of controversy [1]. Indeed, there is the question of the almost perfect energy coupling between the 2 reactions (endergonic and exergonic) and the probability of reverse reaction adapted to physiological needs (the reaction must be able to take place in both directions, bifurcation and confurcation). Two non-exclusive hypotheses are currently proposed: i) the electrochemical model in which the potentials of the 2 redox transitions of flavin (oxidized/semi-reduced and semi-reduced/reduced) are the determining factors for the bifurcation mechanism to take place; ii) the electron-gating model by conformational change in which the electron transfer is controlled by structural changes (Marcus theory [2]).

PhD project description

The thesis project focuses on the determination of key parameters of the electron bifurcation mechanism by studying several enzymatic models and in particular the tetrameric Hnd hydrogenase of the bacterium *Desulfovibrio fructosovorans*. We have shown that it can be aerobically purified while maintaining its activity, which is unusual for an FeFe hydrogenase. Through biochemical studies on this enzyme, we have shown that it performs electron bifurcation [3]. The aim of the thesis is to determine whether one of the 2 hypotheses on the molecular basis of electron bifurcation presented above is true or whether electron bifurcation is a combination of these 2 models. For this, the potentials of the different redox centers of the enzyme will be determined, in particular the electron bifurcation center (flavin), using titrations followed by optical spectroscopy or EPR and/or electrochemistry. The influence of the pH of the medium or protein environment of these centers will be determined and may be modified by mutagenesis. Structural studies are also envisaged in collaboration with a partner laboratory.

Bibliography

[1] F. Baymann et al. Review, Front. Microbiol. 9 (2018) 1357; J. Peters et al. Chem. Commun., 54 (2018) 4091–4099.
[2] R. A. Marcus. J. of Chem. Physics, 24(5) (1956) 966–978.
[3] A. Kpebe et al. BBA-Bioenergetics, 1859(12) (2018)1302–1312.