S11.P9

PSII manganese cluster: Protonation of W2, O5, O4 and His337 in the S1 state

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Photosystem II (PSII) is a membrane-bound protein complex that oxidizes water to produce energized protons, which are used to built up a proton gradient across the thylakoidal membrane in the leafs of plants. This light-driven reaction is catalyzed by withdrawing electrons from the Mn4CaO5-cluster (Mn-cluster) in four discrete oxidation steps [S1 — (S4/S0)] characterized in the Kok-cycle. In order to understand in detail the proton release events and the subsequent translocation of such energized protons, the protonation pattern of the Mn-cluster needs to be elucidated. The new high-resolution PSII crystal structure from Umena, Kawakami, Shen, and Kamiya is an excellent basis to make progress in solving this problem. Following our previous work on oxidation and protonation states of the Mn-cluster, in this work, quantum chemical/electrostatic calculations were performed in order to estimate the pKa of different protons of relevant groups and atoms of the Mn-cluster such as W2, O4, O5 and His337. In broad agreement with previous experimental and theoretical work, our data suggest that W2 and His337 are likely to be in hydroxyl and neutral form, respectively, O5 and O4 to be unprotonated.

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Reconstruction of the bc1 complex Qo and Qi sites of *Plasmodium falciparum* in the yeast enzyme

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The bc1 complex is central to mitochondrial bioenergetics and the target of antiprotozoal and antifungal drugs used to control human and plant pathogens. The inhibitor (and substrate quinol) binding sites, called Qo and Qi, are provided by the mitochondrially-encoded cytochrome b, the main subunit of the complex. Most of the anti-microbial drugs, such as the antimalarial compound atovaquone, target the Qo site. Unfortunately, the development of resistance, mainly caused by mutations in the inhibitor binding site, compromises the control of the pathogens. New drugs that could circumvent the resistance are needed. We use the yeast bc1 complex as a model to investigate the impact of reported mutations – acquired resistance mutations and polymosphisms – in the cytochrome b of *Plasmodium falciparum*, the malaria agent, and to study new therapeutic compounds. Cytochrome b sequences from yeast and *P. falciparum* are highly conserved, making yeast an attractive model to investigate sensitivity and resistance to drugs targeting the bc1 complex. In addition, genetic tools have been well developed to introduce designed mutations in yeast mitochondrially-encoded cytochrome b gene. In order to obtain a more accurate model for the study, we are modifying the inhibitor binding sites of the yeast to generate a bc1 complex that mimics the *P. falciparum* enzyme. We have constructed a series of yeast mutants harbouring variants of the Qo [1] or the Qi site where yeast residues have been replaced by *P. falciparum* equivalents. Their analysis provides information on the structural determinants of natural and acquired resistance.

Reference

[1] C. Vallières, N. Fisher, B. Meunier, Reconstructing the Qo site of Plasmodium falciparum bc1 complex in the yeast enzyme, PLoS One. 8 (2013) e71726.

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S11.P11

The Q-cycle tunes electron transfer chains using menaquinone

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Most isoprenoid quinones found in electron transfer chains (ETC) can be divided into only two main groups: the low potential menaquinone and the high potential ones such as ubiquinones and plastoquinones. The apparition of the latter p group possibly coinciding with increased oxygen pressures during evolution. The redox cofactors along the electron transfer chain have to adapt their redox potentials to the potential of the quinone. This in turn influences the driving forces available to translocate protons at each step along the chain. A large amount of data is available for ubiquinone and plastoquinone. On the other hand, data for menaquinone ETC are more scarce, especially concerning the Rieske/cytochrome b complexes. We report here results on our model *Geobacillus stearothermophilus*, a firmicute that grows by aerobic respiration but based on menaquinone. A supercomplex comprising the cytochrome b6c and the cytochrome c oxidase has been purified. The cytochrome b6c is a Rieske/cyt b that is more similar to the b6f than to the bc1 complex, due to the presence of a haem ci in the Qi site. We show that in the cytochrome b6c complex, the redox potentials are shifted to accommodate the downshift of the menaquinone redox midpoint potential relative to ubiquinone or plastoquinone. These results shine new light on the scarce and contradictory results in the literature concerning bc complexes using menaquinone, they stress the importance of basic principles that underlie the Q-cycle mechanism and influence whole ETC. Adaptation of the redox potential of the b6c complex to the one of the menaquinone imposes constraints downstream toward cytochrome c oxidase, but more importantly upstream toward dehydrogenases.

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bc1 complex mechanism

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Using a stochastic simulation without any other hypotheses, we demonstrated the natural emergence of the Mitchell Q-cycle in the functioning of the bc1 complex, with few short-circuits and a very low residence time of the reactive semiquinone species in the Qo site [1]. However, this simple model fails to explain both the inhibition by antimycin of the bc1 complex and the accompanying increase in