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Energetics of the steps in proton pumping mechanism and preventing of backflow reactions in cytochrome *c* oxidase

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Cytochrome *c* oxidase (CcO) is the terminal enzyme of aerobic respiration, which is responsible for processing most of the biological oxygen and generating electrochemical proton gradient in aerobic cells [1]. The energy released from the reduction of molecular oxygen to water is used to pump protons across the mitochondrial or bacterial membrane. The structure of the enzyme has been solved for several organisms; however details of its molecular mechanism of proton pumping still remain elusive.

Recent time-resolved optical and electrometric experiments on the O→E transition have suggested a sequence of reaction steps for the proton-translocation mechanism of CcO [2]. The pump function introduces a mechanistic requirement of a valve that prevents protons from flowing backwards during the process. It was recently found that Glu242, a key amino acid in transferring protons to be pumped across the membrane and to the site of oxygen reduction, fulfills the function of such a valve by preventing simultaneous contact to the pump site and to the proton-conducting D-channel [3, 4]. Here we have included the conformational gating by Glu242 into the framework of the proposed His291 pumping model [5]. DFT/electrostatic calculations are employed to obtain energetics of proton and electron transfer reaction steps during the O→E transition, while transition state theory is used for estimating activation energies and kinetic barriers from the rate constant of transitions. The energy profile of the reaction mechanism is studied by exploring how the redox state of the adjacent metal centers, dielectric effects, and membrane potential gradient, affect the energy levels and the leaks of the Glu-valve.

Special emphasis is made on side-reactions that may short-circuit the pump, and the means by which these may be avoided. The state with the proton on the pump site (His291) is especially vulnerable to leak back to Glu^- instead of being released to the *P*-side of the membrane, what would result in a loss of proton-pumping.

Obviously, there are more different control mechanisms and gating situations employed by the enzyme to ensure the unidirectionality of the proton translocation and to prevent proton leak in the opposite direction.

References:

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