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Proton pumping in cytochrome c oxidase: Energetic requirements and the role of two proton channels[☆]

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ABSTRACT

Cytochrome c oxidase is a superfamily of membrane bound enzymes catalyzing the exergonic reduction of molecular oxygen to water, producing an electrochemical gradient across the membrane. The gradient is formed both by the electrogenic chemistry, taking electrons and protons from opposite sides of the membrane, and by proton pumping across the entire membrane. In the most efficient subfamily, the A-family of oxidases, one proton is pumped in each reduction step, which is surprising considering the fact that two of the reduction steps most likely are only weakly exergonic. Based on a combination of quantum chemical calculations and experimental information, it is here shown that from both a thermodynamic and a kinetic point of view, it should be possible to pump one proton per electron also with such an uneven distribution of the free energy release over the reduction steps, at least up to half the maximum gradient. A previously suggested pumping mechanism is developed further to suggest a reason for the use of two proton transfer channels in the A-family. Since the rate of proton transfer to the binuclear center through the D-channel is redox dependent, it might become too slow for the steps with low exergonicity. Therefore, a second channel, the K-channel, where the rate is redox-independent is needed. A redox-dependent leakage possibility is also suggested, which might be important for efficient energy conservation at a high gradient. A mechanism for the variation in proton pumping stoichiometry over the different subfamilies of cytochrome oxidase is also suggested. This article is part of a Special Issue entitled: 18th European Bioenergetic Conference.

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1. Introduction

In aerobic respiration, molecular oxygen is reduced to water, a very exergonic reaction where a substantial part of the energy is conserved as an electrochemical gradient over the mitochondrial or bacterial membrane. The membrane gradient is used by ATP synthase to transform ADP to ATP, the energy currency of the cell. The terminal enzyme in the respiratory chain is often a member of the heme-copper oxidase family, also referred to as cytochrome c oxidase (CcO) since the electron donor is cytochrome c. The basic process for building up an electrochemical gradient is to transport charges from one side of the membrane to the other. In the heme-copper oxidases the charge transport occurs in two different ways. First, the chemistry of the O₂ reduction to water occurs by taking up the electrons and the protons from opposite sides of the membrane, giving a so called electrogenic reaction. The electron donor, cytochrome c is located on the P-side of the membrane (intermembrane space or periplasm), and by taking up the protons from the other side, the N-side (matrix or cytoplasm), each electron- and proton-pair used in the chemistry corresponds to the movement of one positive charge from the N-side to the P-side, see

Fig. 1. Secondly, coupled to the exergonic chemistry, an active transport of protons from the N-side of the membrane all the way to the P-side, increases the efficiency of the energy conservation. The second process is referred to as proton pumping. The heme-copper oxidases are the only oxygen reductases known to pump protons [1,2]. The reduction of one O₂ molecule can be written as follows, where *n* is the number of protons pumped per electron (see further below):



Both the electrogenic chemistry and the proton pumping require proton motion against the gradient. This implies the existence of a set of kinetic barriers, gating the protons to move in the right direction. In particular, due to the presence of proton pumping, some barrier heights must change during the reaction, to prevent proton uptake from the P-side at certain stages of the reaction, still allowing protons to be pumped to the P-side at other stages. A detailed description of how reaction (1), including gating, occurs is referred to as a proton pumping mechanism. To find those details is the goal of a large part of current research in bioenergetics, see for example ref. [3]. Significant knowledge and insight in these processes have already been gained [4–11], but some important unresolved questions still remain, which motivates the present study. The heme-copper oxidases have been divided into subfamilies [12], differing among other things in the exact form of the redox-active

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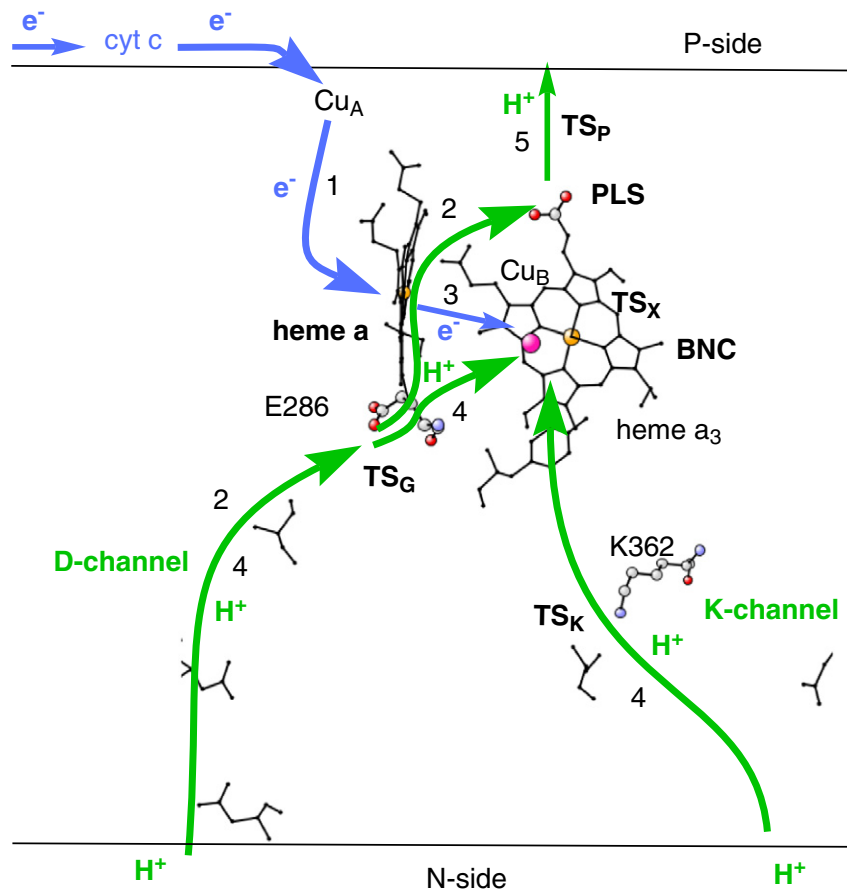


Fig. 1. Overview of electron and proton transfer in CcO (A-family). The numbers refer to the elementary steps in the proton pumping mechanism discussed in Section 3.2. The D-channel is known to be used for all protons pumped and for two of the protons to the BNC. The K-channel is only used for the remaining two protons to the BNC, see further in the text.

cofactors. Most knowledge has been gained for cytochrome c oxidases belonging to the largest family, the so called A-family, which is also the main target for the present investigation. One difference between the A-family and the other oxidase families (B and C) is the stoichiometry of the proton pumping, i.e. the value of n in Eq. (1). A-family oxidases have been found to pump four protons per oxygen molecule, i.e. n is equal to one (one proton pumped per electron), while oxidases from the B- and C-families have a lower value of n (probably close to 0.5) [2]. Another difference is that the A-family oxidases have two channels for proton uptake, the D- and the K-channels, see Fig. 1, while the B- and C-families only have one, often referred to as the K-channel analog since it is located in the same place as the K-channel in the A-family [2].

One of the most important unresolved questions for the cytochrome c oxidases concerns the uneven distribution of the free energy released in the four reduction steps in Eq. (1) [10,13,14]. The active site where the chemistry of the transformation of molecular oxygen to water occurs is a binuclear center (BNC) consisting of a high spin heme group (heme a_3 in A-family oxidases) and a copper complex (Cu_B) with a tyrosine residue cross-linked to one of its histidine ligands. The O_2 molecule binds reversibly to the reduced BNC, **R** (Fe(II) and Cu(I)), forming intermediate **A**, and in the next step the O–O bond is cleaved, forming intermediate **P_M**. In the **R** to **P_M** reaction steps, the active site redox centers provide all four electrons to the oxygens: Fe(II) is oxidized to Fe(IV),

Cu(I) is oxidized to Cu(II) and the tyrosine is oxidized to a neutral radical, see the scheme in Fig. 2. To complete the catalytic cycle of Eq. (1) and return to the reduced BNC, **R**, four electrons and four protons (to complete the water formation) has to be taken up by the enzyme, which occurs in four separate reduction steps. Based on experimental mid-point potentials it was early noted that the free energy release seems to be much larger in the first two reduction steps after the O–O bond cleavage, **P_M** to **O** (often referred to as the oxidative part of the catalytic cycle) than in the reduction steps **O** to **R** (referred to as the reductive part) [13]. Later, quantum chemical calculations on the catalytic cycle have given the same picture, with large exergonicities in the first two reduction steps after the O–O bond cleavage, and very small exergonicities in the other two reduction steps [15–17]. It has therefore been a point of major concern, that even if the total free energy released in the O_2 reduction is more than enough for pumping four protons across the membrane, the free energies for two of the steps, **O** to **E**, and **E** to **R**, do not seem to be large enough to afford proton pumping, at least not with a significant gradient across the membrane [10,14,18,19]. At an early stage this situation even led to the suggestion that all four protons were pumped in the first two reduction steps after O–O bond cleavage, i.e. two protons pumped per electron in these steps, and no proton pumping in the other two reduction steps [20]. It was later shown experimentally that there is actually one proton

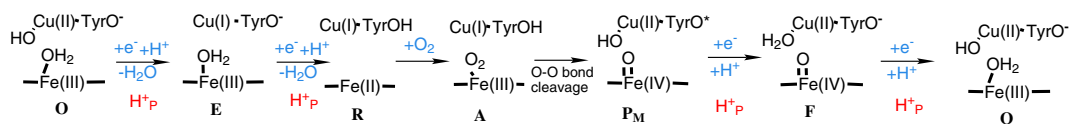


Fig. 2. Catalytic cycle of CcO starting from the oxidized state **O**. The notation H_p^+ corresponds to pumped protons.

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