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Review

The mechanism for proton pumping in cytochrome c oxidase from an electrostatic and quantum chemical perspective ☆

Margareta R.A. Blomberg *, Per E.M. Siegbahn

Department of Physics, AlbaNova University Center, Stockholm University, SE-106 91, Stockholm, Sweden
Department of Biochemistry and Biophysics, Arrhenius Laboratory, Stockholm University, SE-106 91, Stockholm, Sweden

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ABSTRACT

The mechanism for proton pumping in cytochrome c oxidase in the respiratory chain, has for decades been one of the main unsolved problems in biochemistry. However, even though several different suggested mechanisms exist, many of the steps in these mechanisms are quite similar and constitute a general consensus framework for discussing proton pumping. When these steps are analyzed, at least three critical gating situations are found, and these points are where the suggested mechanisms in general differ. The requirements for gating are reviewed and analyzed in detail, and a mechanism is suggested, where solutions for all the gating situations are formulated. This mechanism is based on an electrostatic analysis of a kinetic experiment fior the O to E transition. The key component of the mechanism is a positively charged transition state. An electron on heme *a* opens the gate for proton transfer from the N-side to a pump loading site (PLS). When the negative charge of the electron is compensated by a chemical proton, the positive transition state prevents backflow from the PLS to the N-side at the most critical stage of the pumping process. The mechanism has now been tested by large model DFT calculations, and these calculations give strong support for the suggested mechanism. This article is part of a Special Issue entitled: Respiratory Oxidases.

1. Introduction

Cytochrome c oxidase (CcO) is the terminal enzyme in the respiratory chain, located in the inner mitochondrial or bacterial membrane. In this enzyme molecular oxygen is reduced to water by electrons delivered from cytochrome c in the outer cytosol, the P-side of the membrane, and protons delivered from the inside, the N-side of the membrane. The fact that the electrons and protons are taken from different sides results in the generation of an electrochemical gradient across the membrane, storing some of the energy from the exergonic oxygen reduction. This energy, in the form of the gradient, is used by ATP-synthase to make ATP, the energy currency of the cells. The cofactors responsible for electron transfer and the oxygen chemistry are shown in Fig. 1 for cytochrome c oxidase belonging to class A. The electrons are transferred from cytochrome c via a dinuclear copper complex, Cu_A, and a heme group, heme *a*, to the binuclear center (BNC) consisting of another heme group, heme a_3 and a mononuclear copper complex, Cu_B, where the redox chemistry occurs, forming two water molecules for each O_2 molecule consumed.

Already in 1977, Wikström discovered that the redox chemistry in CcO is coupled to a proton pump. For each O_2 molecule consumed, an additional four protons are translocated across the entire membrane against the electrochemical gradient [1]. This proton translocation further contributes to the gradient buildup and thus to the efficiency of the energy storage. The molecular mechanism for the proton pumping against the gradient is far from obvious, and still remains controversial [2]. Many different mechanisms have been suggested, but the nature of the gates that separate the protons consumed in water formation from the protons being pumped has not been understood [3]. Progress in elucidating the pumping mechanism was for several years partly hampered by the belief that only two of the four reduction steps were coupled to proton pumping [4], implying that for those steps, two protons should be pumped per electron (apart from the proton taken up for the chemistry). A mechanism of that type is clearly extremely difficult to realize, and it was never successful. Still, it was rather early possible to formulate some general elements of a pumping mechanism, which are included in most of the mechanisms that have been suggested. One such element is the assumption that the electron transfer into CcO is coupled to a proton transfer into a pump loading site (PLS), where the protons to be pumped are temporarily stored, and which is separated from the site where the oxygen chemistry occurs [5,6]. The driving force for the proton transfer is assumed to be an electrostatic interaction with the incoming electron. After the transfer of a proton to the PLS, another proton is taken up for the chemistry in the BNC. The second general element of the pump mechanism is the assumption that the electrostatic repulsion from this second proton will expel the proton at the PLS out of the enzyme to the P-side [5,6]. These two assumptions,

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* Corresponding author. Tel.: +46 8 16 12 64.
E-mail address: mb@fysik.su.se (M.R.A. Blomberg).

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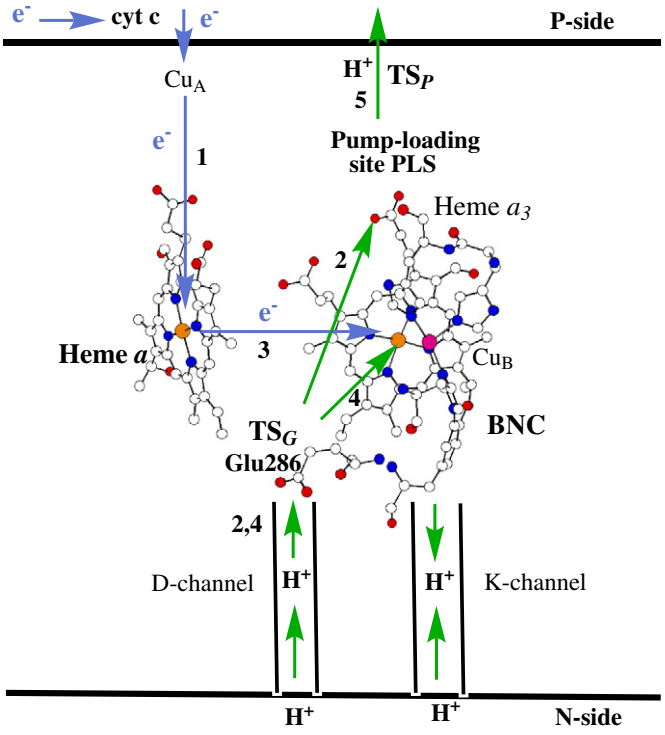

Fig. 1. Overview of electron and proton transfer in cytochrome c oxidase (class A).

the initial uptake of the pump-proton into a pump loading site, and the removal of this proton by electrostatic repulsion from the chemical proton (i.e. the proton that takes part in the chemistry), can be considered as necessary components of a pump mechanism, but they give no information of the nature of the gates, that govern the protons in different situations to go in the right direction, in particular, to move against the electrochemical gradient. However, it can be noted that already these rather general elements of a pumping mechanism point out the three most important gating situations [5,6]. The first gate is needed to guide the first proton to the PLS and not to the water production site at the BNC. Since proton pumping corresponds to a translocation of a proton from the N-side to the P-side, there is also a gate needed that prevents the PLS proton to be taken up from the P-side of the membrane. Furthermore, a gate is needed that prevents the PLS proton to move back to the N-side of the membrane when it is destabilized by the chemical proton.

An important step forward was taken in 1998 when Michel challenged the idea of only two pumping steps [7], which initiated further investigations and finally led to a new interpretation of experimental data suggesting that one proton is pumped in each of the four reduction steps [8]. With one proton being pumped for each electron transferred, it is natural to assume that the general mechanisms for proton consumption and proton pumping are similar for all four reduction steps. This should make it possible to construct a general pumping mechanism in more detail. Several mechanisms have been suggested, and most of them contain the two main electrostatic elements mentioned above. However, the mechanisms differ in several details concerning both the actual order of elementary proton and electron transfer steps, and in the nature of the different gates suggested.

Essentially all different pumping mechanisms suggested start with the transfer of an electron from cytochrome c into the CcO enzyme. In the earliest suggested mechanisms, e.g. the histidine cycle by Wikström and coworkers in 1994 [5], and the mechanism described by Rich et al. in 1996 [6], it was assumed that the electron was transferred all the way to the BNC before the pump-proton was transferred to the PLS. However, in 1998 Michel presented an explicit mechanism where the pump-proton was taken up already when the electron is at heme *a*, i.e. before it arrives at the BNC [7]. Coupling between reduction of heme *a* and proton pumping had in more rudimentary forms been suggested before, e.g. by Babcock et al. in 1983 [9]. The idea that it is the electron transfer to heme *a* that triggers the next step, the uptake of the pump-proton, has been adopted as an important ingredient in several more recently suggested mechanisms [8,10–13], and the analysis of the recent kinetic experiments for one reduction step (O to E) by Wikström and coworkers supports this mechanism [14]. That idea actually makes it much easier to construct a gating mechanism that prevents this first proton to go to the BNC, which would waste the energy to be conserved by the proton pumping. One suggestion is the water-gated mechanism, where it is assumed that the position of the electron at heme *a* governs the hydrogen bonding between a chain of water molecules so that the first proton is prevented to go to the BNC [8]. Another suggestion is that the endergonicity of proton transfer to the oxidized BNC results in a too high barrier for completing the chemistry at the BNC. Instead, there first has to be an uptake of a proton to the PLS [11,12], to allow the electron to go to the BNC. Only at that stage can the chemistry be completed. Surprisingly, some quite recently suggested pumping mechanisms still pursue the idea that the first step is electron transfer to the BNC [2,15]. In fact, to our knowledge, no specific gating mechanism has in that case been suggested that prevents the first proton to perform the very exergonic transfer to the reduced BNC, but it has still been assumed that there exists such a kinetic gate [15]. In a fundamentally different mechanism, suggested by Brzezinski and coworkers, the chemical proton is actually assumed to go to the reduced BNC before the pump-proton is taken up [16,17].

The second step, the protonation of the PLS can occur either via the transfer of an "extra" proton [11,12,18,19], or by a charge separation process where a conserved glutamic acid (Glu286 in *Rhodobacter sphaeroides* notation), situated in the proton transfer D-channel (see Fig. 1), delivers its acidic proton to the PLS as suggested in several mechanisms [2,13,15,20,21]. The extra proton might be available in the D-channel [22] or it can be taken up all the way from the N-side of the membrane. In the charge separation mechanism, the Glu286 is suggested to be reprotonated in a following step. Clearly the two mechanisms can be considered as two extremes of a similar process, where the Glu286 is first deprotonated and then becomes reprotonated either more or less concertedly, or more slowly, by another proton from the N-side [12]. The assumption of the character of this proton transfer step will have implications for the possibility to prevent back leakage of the pump-proton at a later stage, which will be discussed below [11,12]. An important observation from the kinetic experiment [14] mentioned above, is that the barrier for this proton transfer step is quite high, about 11 kcal/mol using transition state theory [12]. This rather high barrier actually turns out to be necessary for making a gating mechanism possible. The specific location of the PLS is not known, but there are different suggestions, most of them locate the PLS in the vicinity of the propionate groups of the two hemes. A general requirement is that this position has to be close enough to electrostatically interact with the electron in heme *a* and/or the BNC. To make the interaction large enough, the dielectric constant must be rather small ($\epsilon = 3$–4) [11,12].

If the electron is at heme *a* when the PLS becomes loaded with a proton, the next step should be transfer of the electron to the BNC. A commonly adopted requirement for the PLS is that it is located closer to the BNC than to heme *a*, and therefore it is the PLS proton that triggers this electron transfer. It is also possible that the pump-proton moves from a location close to heme *a* to one close to the BNC, in concert with the electron transfer. When the BNC is reduced, it is favorable for a second proton to be taken up from the N-side of the membrane and to move into the BNC to perform the chemistry. This exergonic step is made even more exergonic when it is followed by the removal of the pump-proton from the PLS. However, from a thermodynamic point of view there is no advantage for the pump-