The stochastic modeling of proton pumping in cytochrome c oxidase: the contribution of the K-channel

V Yu Titova¹, S E Boronovskiy¹, J-P Mazat², S Ransac², Ya R Nartsissov¹

¹Institute of cytochemistry and molecular pharmacology, Moscow 115404, Russia
²Laboratoire de métabolisme énergétique cellulaire IBGC – CNRS and University of Bordeaux, Bordeaux, France

Abstract. The influence of pH on the activity of cytochrome c oxidase is investigated using the stochastic modelling based on Markov processes. The functioning of the K-channel is considered on four different schemes. A change of channel modes can be observed under high values of pH: the fraction of K-protons transferred through the membrane increases significantly while D-proton flow disappears. Moreover, the pumping efficiency at high pH values rises more than twice under the circumstances when at the beginning of the pumping there are three or four protons in the binuclear center under pumping both D- and K-protons.

1. Introduction
Cytochrome c oxidase (CcO, EC 1.9.3.1) is a terminal enzyme complex of the mitochondrial respiratory chain. It catalyzes oxygen reduction to water alongside with cytochrome c oxidation. It belongs to the protein superfamily of heme-copper oxidoreductases, which includes most of the currently known terminal oxidases that catalyze the four-electron reduction of oxygen molecule with the formation of water [1]. These reactions are coupled with proton pumping through the inner mitochondrial membrane from the N-side to the P-side and therefore the membrane potential is generated. Proton transfer through the biological membranes is the central reaction for a proton electrochemical gradient maintenance, involved in the energy transformation by the cell.

Cytochrome c oxidase consumes protons both as a substrate and for its translocation across the membrane. The results of X-ray crystallography and site-specific mutagenesis indicate the presence of two input proton channels – the D-channel and the K-channel [2]. It was supposed that mitochondrial oxidases have the additional H-channel and it plays a key role in the proton translocation through the membrane [3, 4]. However the matter of its presence stays questionable. Also the mechanisms of proton pumping and the functioning of the proton-loading site are still under consideration.

2. Theoretical background
In the present work CcO activity was estimated via the computer algorithm based on stochastic approach [5], where the catalytic cycle of enzyme is reported as a set of consecutive transitions between distinct states with the various numbers of metabolites.

Each transition is elementary and means the transfer of one proton, one electron or one molecule of oxygen to the binuclear center (BNC) or transmembrane proton transport [6]. The transition scheme is implemented on the basis of Markov stochastic processes. Transition probability from state i to state j depends on time linearly in this model, and the coefficient is the transition intensity that typifies the intensity of corresponding transfer process:
\[
\begin{align*}
P[S_i \rightarrow S_j] &= q_{ij} \cdot \Delta t + o(\Delta t) \\
P[S_i \rightarrow S_j] &= 1 - \sum_{i \neq j} q_{ij} \Delta t = 1 + q_{ii} \Delta t
\end{align*}
\]

(1)

Transition probability from state \(i\) to state \(j\) during time \(t\) as long as \(\tau\) is the transition time:

\[
P[S_i \rightarrow S_j \mid t \geq \tau] = \begin{cases} 
q_{ij}, & i \neq j \\
q_{ii}, & i = j 
\end{cases}
\]

(2)

The core of the simulation modeling of such systems is the search of the reaction pathway with following quantitative estimation of necessary biological characteristics. In general modeling algorithm may be divided into the four steps. The first one is the choice of the initial state of the system. Then the transition time for current state is calculated as a solution of the continuous random variable generation problem with given distribution law. Afterwards the modeling of transition direction is realized, in other words the determination of the next state taking into account selected transition time. The result is the solution of the discrete random variable generation problem on the base of the relative transition intensities. This completes the modeling of the transition from one state to another and the calculated last state becomes the new initial one, and the process is running on [7].

Since four protons, four electrons and one oxygen molecule are required for the synthesis of two water molecules, the scheme of all possible states and transitions for CcO includes 152 states. The initial state is the enzyme without any proton, electron or oxygen inside the BNC.

In the present study the proton channels activity was explored under different pH conditions. The rest of substrates such as oxygen and electrons from cytochrome c are not the limiting factors of the catalysis in the model. In general the pumping protons could be uptaken not only via the D-channel, but also via the K-channel, and the possibility of such pumping was implemented in the distinct schemes. To explore the contribution of the K-channel in the functioning of cytochrome c oxidase there were investigated four different schemes, providing various requirements on proton pumping process initiation (Figure 1). The scheme 1 implicates that only protons consumed via the D-channel (D-protons) can be transferred through the membrane. The pumping process in this scheme is carried out when the binuclear center is fulfilled with protons. The possibility of protons consumed via the K-channel (K-protons) to be pumped is added in the scheme 2. Herewith the conditions of pumping initiation are the same as in the scheme 1. The scheme 3 also allows the pumping of both D- and K-protons, while it begins when the BNC is filled by three or four protons. The last scheme (scheme 4) describes the situation when only D-protons are transferred through the membrane and there are three or four protons in the BNC.

Figure 1. The example of possible states of the BNC necessary for the initiation of the proton pumping for: a) scheme 1, b) scheme 2, c) scheme 3, d) scheme 4.

3. Results and discussion

As the result of single experiment we can obtain the sequence of the states, which the system has passed and the time values for these transitions (Figure 2a). Thus all the metabolic flows can be calculated (Figure 2b).

The results for schemes 1 and 4 appeared to be statistically indistinguishable, so the figures for the last are not given. The differences between these schemes are the conditions for proton pumping initiating, thus we can assume that the process of pumping starts when there are four protons in the BNC.
The pH dependences of oxygen and water flows are almost coinciding when the pumping process is starting under the binuclear center fulfilled with protons (Figure 3a,b), which is explained by the transmembrane transfer of the protons consumed in addition giving the chance of pumping of K-protons. The synthesized water flow decreases by approximately 14% under conditions described by scheme 3.

Figure 2. a) Time dependence of number of the state. The transition from state 151 (four protons, four electrons and molecule of oxygen are in the BNC) to state 0 (the enzyme without protons, electrons and oxygen) corresponds to the completion of a single enzyme cycle. b) Time dependence of the number of the particles for a single experiment.

Figure 3. Oxygen and water flows for the different investigated schemes: a) scheme 1, b) scheme 2, c) scheme 3.

Figure 4a-c shows the monotonous decrease of the pumped D-proton flow, whereas the dependence of pumped K-protons appears to be less obvious. This dependence has a peak under pH from 8.4 to 8.6 and further lowering up to pH 10 when the pumping of K-protons is possible (Figure 4b,c). It is interesting that the fraction of K-protons transferred through the membrane was ca. 11% at pH 4 and ca. 94% so far at pH 10 in both schemes (schemes 2 and 3). Furthermore the numbers of pumped D- and K-protons were equal under pH 8.6, and significant predominance of K-proton flow was observed under pH from 8.6 to 10. This fact shows that one can observe the change of channel modes due to the alkalization of the enzyme environment: the D-channel activity decreases significantly in favor of the K-channel. Moreover this provides enzyme activity at the extremely high values of pH.
Figure 4. The flows of pumped protons for different investigated schemes: a) scheme 1, b) scheme 2, c) scheme 3.

The pumping efficiency \((H^+/e^-)\) for D-protons decreases in the same way as the flow of pumped D-protons (Figure 5a-c). However the local maximum of the pumping efficiency for K-protons at pH 8.4 was obtained when pumping of K-protons occurs under the fully occupied binuclear center (Figure 5b). In the same time the pumping efficiency for K-protons monotonically increases when three or four protons are present in the binuclear center (Figure 5c). Besides pumping efficiency at high pH values rises more than twice in scheme 3 in comparison with the results of scheme 2, that follows from the simultaneous 14% increase of consumed proton flow and 14% decrease of consumed electron flow. Thus, this work reveals the abilities of stochastic modeling in studying the role of the cytochrome c oxidase proton channels according to the different pH.

Figure 5. Pumping efficiency \((H^+/e^-)\) for different investigated schemes: a) scheme 1, b) scheme 2, c) scheme 3.

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