Application of Marcus theory for modeling proton transfer in cytochrome $c$ oxidase

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Abstract. The process of proton transport in cytochrome $c$ oxidase is studied in the framework of stochastic modeling. The activation energies are calculated using Marcus theory. This model allows to define the key amino acid residues and water molecules which form the main H$^+$ transduction pathway. According to the simulation results, Asn-207 and Asn-121 are not involved in direct proton translocation. The estimated rate of the proton transfer through the D-channel of cytochrome $c$ oxidase is $(1.43\pm0.18)\cdot10^4$ s$^{-1}$.

1. Introduction

Oxygen is necessary as an oxidizing agent for the process of cellular respiration of aerobic organisms. The reduction of molecular oxygen to water requiring electrons and protons to be taken up from the opposite sides of the inner mitochondrial or cytoplasmic membrane is carried out in cytochrome $c$ oxidase, which is the terminal complex of the respiratory chain. The number of its subunits differs for mammalian and bacterial forms, but the structure and amino acid sequence of three core subunits (I-III) are highly conserved [1]. This enzyme is also known to be a generator of the electrochemical proton gradient due to the fact that electron transfer is coupled with proton pumping across the membrane. The process of proton transfer in cytochrome $c$ oxidase is provided by the D-channel and K-channel, which names correspond to the entrance amino acid residues of aspartic acid (e.g. D) and lysine (e.g. K) respectively. Of particular interest is the D-channel, which is considered to transfer four pumped protons and at least two chemical protons required for the oxygen reduction [2,3]. Its orifice represented by Asp-132 (Rhodobacter sphaeroides nomenclature) can be probably protonated both directly from the matrix and by protein surface groups named proton-collecting antenna. They comprise of several carboxylates and histidine rings proceeding a rapid reprotonation of the entrance aspartate due to the fact that proton exchange between these residues is faster than proton diffusion in the bulk solution [4,5]. The final point of the D-channel is Glu-286, which is known to be a valve in the H$^+$ pumping. Since it is capable to switch conformations of the side chain from «up» to «down» and vice versa, Glu-286 provides proton transfer both to binuclear center and proton-loading site [6,7]. Different theoretical approaches, in particular, multistate empirical valence bond model, ab initio molecular dynamics and quantum mechanics/molecular mechanics hybrid method, are applied to studying the process of proton translocation in cytochrome $c$ oxidase [8,9,10]. It is a question of current interest, because proton transport dysfunction in this enzyme caused, for example, by mutations in the mDNA leads to some mitochondrial diseases [11]. In spite of intensive research, the mechanism of H$^+$ pumping is one of the key biophysical issues, because its atomistic details remain unexplored yet. In this work Marcus theory is considered as a tool of revealing the main transduction pathway of proton transport. Constructing a model defining key amino acid residues and...
water molecules allows us to study pH and pKₐ dependence of the proton transfer rate through the D-channel of cytochrome c oxidase.

2. Theoretical background
According to Marcus theory, the reaction of proton transport can be considered as a sequence of three explicit steps. The first one is mutual attraction of reagents due to change of free energy, which is followed by hydrogen bonding of acceptor and proton. Proton translocation from donor to acceptor occurring afterwards involves intramolecular reorganization and solvent reorganization required for formation of the transition state. The last step is mutual repulsion of products with breaking of a hydrogen bond between proton and donor [12].

In this theoretical approach proton is represented as a harmonic oscillator. The potential-energy profile of the proton transfer reaction consists of two parabolas. Their intersection corresponds to the transition state characterized by the activation energy \( \Delta G^* \), which is connected with the Gibbs free energy \( \Delta G_0 \) and the reorganization energy \( \lambda \):

\[
\Delta G^* = w_r + \frac{\lambda}{4} \left( 1 + \frac{\Delta G_0 + w_p - w_r}{\lambda} \right)^2
\]

where \( w_r \) and \( w_p \) are the electrical work of attraction of the reagents and repulsion of the products respectively. The reorganization energy consists of two terms: the intramolecular reorganization energy \( \lambda_i \) and the energy of solvent reorganization \( \lambda_o \). The first one is determined by the bond-length change between donor or acceptor and proton, while the second one is defined by the donor-acceptor distance and properties of the medium:

\[
\lambda = \lambda_i + \lambda_o = \frac{1}{2} \sum_k \left[ \frac{f_k^D f_k^A}{f_k^D + f_k^A} (\Delta d_k)^2 \right] + (\Delta e)^2 \left[ \frac{1}{\varepsilon_{op}} - \frac{1}{\varepsilon_{\nu}} \right] \left( \frac{1}{2R_D} + \frac{1}{2R_A} - \frac{1}{r_{DA}} \right),
\]

where \( f_k^D \) and \( f_k^A \) are the force constants of donor and acceptor respectively, \( \Delta d_k \) is the bond-length change, \( \Delta e \) is a transferred charge, \( \varepsilon_{op} \) and \( \varepsilon_{\nu} \) are the optical and static permittivities respectively, \( R_D \) and \( R_A \) are the radii of donor and acceptor respectively, \( r_{DA} \) is the donor-acceptor distance [13]. Obtaining the activation energy of the reaction allows to calculate the proton transfer rate using the Eyring equation, which describes the dependence between the energy barrier and the reaction rate:

\[
k = \kappa \frac{k_B T}{h} e^{-\frac{\Delta G^*}{RT}},
\]

where \( \kappa \) is a transmission coefficient, \( k_B \) is the Boltzmann constant, \( T \) is a temperature, \( h \) is the Planck constant and \( R \) is the gas constant.

The structure of the D-channel involves several highly conserved polar amino acid residues and water molecules, which can play a direct role in the proton transfer. Since the proton pathway is represented as a set of donors and acceptors shown in Figure 1, it is reasonable to apply the stochastic approach based on the continuous-time Markov chain for the system with discrete states. Transition probability \( P[i \rightarrow j \mid \Delta t] \) is supposed to be directly proportional to transition time \( \Delta t \):

\[
\begin{align*}
P[i \rightarrow j \mid \Delta t] &= q_{ij} \Delta t, \\
P[i \rightarrow i \mid \Delta t] &= 1 - \sum_{ij} q_{ij} \Delta t,
\end{align*}
\]

where \( q_{ij} \) is the transition intensity from state \( i \) to state \( j \) and \( P[i \rightarrow i \mid \Delta t] \) is the probability to stay in state \( i \). Simulation modeling of the process of proton transfer is performed by generation of continuous and discrete random variables. The former is used for obtaining sojourn time in state \( i \), which is exponentially distributed, while the latter defines what state \( j \) is chosen for the transition from state \( i \).
3. Results and discussion

The main advantage of our constructed model is that it requires only structural data and pK_a values to reveal important details concerning the process of proton transport. The scheme of the spatial arrangement of the D-channel was based on the X-ray crystal structure of cytochrome c oxidase from *Rhodobacter sphaeroides*, which was solved at 2.3/2.8 Å (PDB ID: 1M56). Markov-like stochastic model was used for analyzing of the D-channel structural elements, involved in the process of proton translocation in cytochrome c oxidase.

According to the simulation results, the states Asp-132, Asn-139, Tyr-33, Glu-286 and the water molecules sequence extending from Asn-139 to the terminal residue of the D-channel form the main transduction pathway (Figure 2). Thus, under normal physiological conditions Asn-207, Asn-121 and the water molecules 2052, 2083 and 2037 do not participate in direct proton transfer. It is consistent with the fact that the asparagine residues form a flexible gate, which influences a spatial organization of hydrogen network, instead of being directly protonated. MS-RMD results reported the asparagine side chains to undergo conformational changes, which control the hydration state of the central region of the D-channel [14]. The proton transport rate through the D-channel calculated during our simulation is \( (1.43 \pm 0.18) \times 10^4 \) s\(^{-1}\), which is confirmed by the experimental data [15,16].

Since the key amino acid residues located in the core subunits of cytochrome c oxidase are highly conserved both for mammalian and bacterial forms of the enzyme, one can talk about the protonation of Asp-132 from the mitochondrial matrix. Its actual mechanism still remains unclear, therefore in our study we consider the entrance aspartate to be protonated only from the bulk solution directly. On this point, the dependence of the proton transfer rate on the matrix pH varying from 4 to 10 was obtained. Figure 3 shows that at physiological pH the rate values range from \( 1.5 \times 10^3 \) s\(^{-1}\) to \( 7.7 \times 10^3 \) s\(^{-1}\). The upper limit of \( 1.5 \times 10^4 \) s\(^{-1}\) is observed at pH values below 5.
Figure 3. The dependence of the proton transfer rate on the mitochondrial matrix pH. The points are the means±SD of 10 independent computer simulations for each pH value.

Due to the fact that the task of the experimental estimation of pK$_a$ corresponded to the particular amino acid residues buried in protein is not trivial, there is a wide range of their values estimated computationally. Experiments based on Fourier transform infrared (FTIR) spectroscopy showed that the apparent pK$_a$ of Glu-286 is 9.4 [17], while its continuum electrostatic evaluation is from approximately 8.0 to 10.6 according to the catalytic state of cytochrome $c$ oxidase [18]. Similar calculations done for Asp-132 produced a range from 3.2 to 6.2 [19,20], besides, its minimum value is close to pK$_a$ of aspartate in solution. In case of Tyr-33, the pK$_a$ range 12.5–21.7 was achieved [19]. Based on the above data, it is appropriate to speak about pK$_a$ shift for the key amino acid residues of the D-channel. The dependence of the proton transfer rate on the pK$_a$ shift obtained for Tyr-33 is represented in Figure 4. This residue is known to be hydrogen-bonded with one of the water molecules located within the pathway [21]. From this result, one can conclude that Tyr-33 is not robust and may play an important role in the process that triggers direction of proton transitions.

Figure 4. The dependence of the proton transfer rate on the pK$_a$ shift for Tyr-33 with pK$_a$ = 9.8. The points indicate the mean±SD of 10 independent computer simulations for each ΔpK value.

Similar plots relating to Asp-132 and Glu-286 show that these residues do not affect the total k$_D$ value (Figure 5). Thus, the proton-pumping stoichiometry depends to a great extent on changes from “up” conformation of the side chain of Glu-286 to “down”.

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Figure 5. The dependence of the proton transfer rate on the pK\textsubscript{a} shift for a) Asp-132 with pK\textsubscript{a} = 3.2 and b) Glu-286 with pK\textsubscript{a} = 9.4. The points are the means±SD of 10 independent computer simulations for each ∆pK value.

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