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Proton transport through D- and H-channels in the bovine heart cytochrome *c* oxidase

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Abstract
We study proton transport in the cytochrome *c* oxidase complex of bovine heart. A simple model of proton motion based on Langevin equation is combined with the determination of the electrostatic potential using molecular dynamics software packages. We compare the proton transfer rates in the fully oxidized and fully reduced forms of the enzyme and conclude that the H-channel becomes more open in the reduced form, but the D-channel does not, in agreement with experimental results.

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(Some figures may appear in color only in the online journal)

1. Introduction

To describe a physical system adequately, it is necessary to maintain a proper balance between the complexity of a proposed model and its simplicity. It is appealing to account for all possible factors affecting the system behavior, but the obtained set of equations might not be possible to solve. On the other hand, models allowing simple solutions can overlook important components. It is especially true for biological systems where the physical properties can be dominated by single electron or proton transfer events, but these processes occur in extremely complex protein surroundings. Since it is impossible to examine such systems analytically, vastly simplified models have to be proposed, or numerical methods, such as molecular dynamics [1, 2], should be used. Initially, the latter approach was successfully applied to liquids [3, 4] and after the first simulation of proteins [5], it became the common tool for researchers. In the present time, one can find molecular dynamics simulations of solvated proteins, protein–DNA complexes, lipid systems and many other structures. However, the time scales of simulations are limited up to nanoseconds and cumulative errors in numerical integrations can be minimized using proper algorithms but not eliminated completely.

In the present paper, we attempt to combine these two methods using a simple transport model based on the Langevin equation with external fields provided by molecular dynamic simulations. In particular, we use visual molecular dynamics (VMD) [6] and adaptive Poisson–Boltzmann solver (APBS) [7] software packages to determine the electrostatic potential. As a test, we apply this approach to proton transport through D- and H-channels in the cytochrome *c* oxidase complex of the bovine heart. In particular, we verify the results of [8, 9] where it was demonstrated that in this animal enzyme the proton pumping occurs via the H-channel, not via D-channel, as in the bacterial complexes. We show that indeed the D-channel does not become more open when electrons are added to metallic sites, but the H-channel does.

2. Functionality of the cytochrome *c* oxidase

Cytochrome *c* oxidase [10] is the last enzyme in the respiratory electron transport chain of the mitochondria, and one of its functions is to convert electron energy into a proton gradient across the inner mitochondria membrane [10–15]. The main steps of its operation are shown in figure 1.

The process of energy conversion starts when high-energy electrons are delivered, one by one, to a dinuclear copper center, Cu_a, located near a positive side of the inner mitochondrial membrane. Subsequently, electrons are transferred from the Cu_a center to the low-spin heme

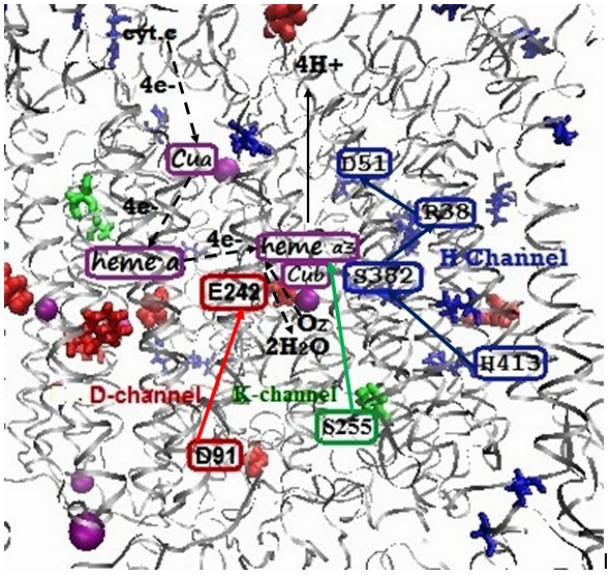

Figure 1. Schematics and operations of the cytochrome *c* oxidase complex of bovine heart. Shown: electron pathway via Cu_a center, heme *a*, heme a_3, and Cu_b; proton D-channel (from *D*91 residue to *E*242), K-channel (from *S*255 to Cu_b) and H-channel (from *H*413 to *S*382, to *R*38 and to *D*51). The figure was created using VMD software package [6].

a (Fe-*a*) and to heme a_3 (Fe-a_3). Heme a_3, jointly with the next electron acceptor in line, a copper ion Cu_b, form a binuclear center, serving as an active catalytic site for dioxygen reduction to water.

Per one electron, two protons are taken from the negative side of the membrane, with one proton pumped to the positive side. The second proton and the electron are consumed at the catalytic site to finally produce a water molecule around the binuclear center. For the bacterial cytochrome *c* oxidase complexes, the proton path from the negative side of the membrane toward the positive side (for 'pumped' protons) and toward the binuclear center (for 'substrate' protons for the water creation) goes through the residue *E*278 (for the *Paracoccus denitrificans* enzyme [15], for bovine heart, it is *E*242). This residue is located at the end of the so-called D-pathway (figure 1). A fraction of the substrate protons can also be delivered directly to the binuclear center via an additional K-pathway. It was argued [8, 9], however, that for the animal enzymes, like that of the bovine heart, the D-channel supplies the substrate protons only, but the proton pumping occurs via the H-channel. It was shown experimentally using various mutations that the reduction of the metallic sites opens the H-channel for proton pumping but the D-channel remain closed. Naively, one can assume that the addition of the negative charge of an electron would always make the channel directed to this site to be more open for the positive charge of the protons. However, in complex structures, such as the cytochrome *c* oxidase complex, additional charge of even single electron leads to conformational changes and redistribution of many charges in the protein environment.

A computational analysis of the energetics of the cytochrome *c* oxidase was presented in [16–20] with molecular models reproducing energetic barriers for the proton transfer steps [16, 17]. The obtained energetic map

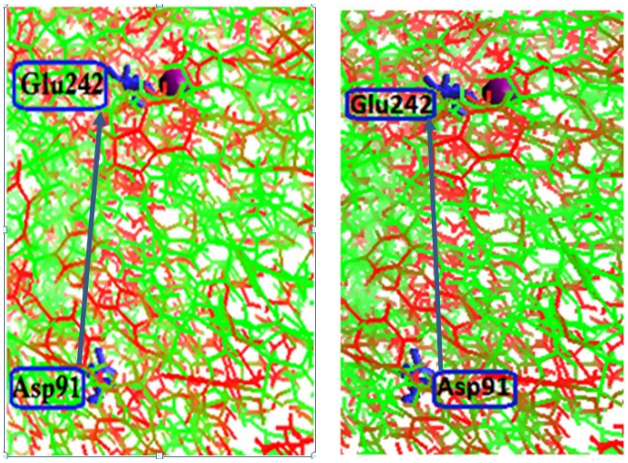

Figure 2. Electrostatic potential in the vicinity of the D-channel for the fully oxidized (left panel) and fully reduced (right panel) forms of the cytochrome *c* oxidase complex of bovine heart. The green color corresponds to the positive potential, whereas the red color represents the negative potential.

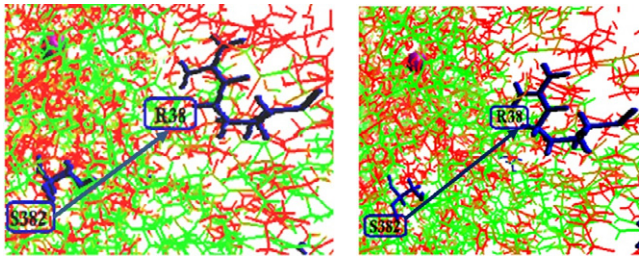

Figure 3. Electrostatic potential in the vicinity of the H-channel for the fully oxidized (left panel) and fully reduced (right panel) forms of the cytochrome *c* oxidase complex of bovine heart. Only the part of the channel near the binuclear center is shown.

of the proton and electron pathways in the enzyme can be converted into a set of rate constants, which qualitatively explains the kinetics and unidirectionality of the pumping process. Specific models of the electron–proton energy exchange accompanying particle transfer between the sites were proposed in [21, 22]. In the later, we revealed all the kinetic phases observed in the experiment [15]. In the present work, we examine only proton transport along corresponding channels, without the analysis of the channel population and depopulation events with an ultimate goal to combine these two approaches in future research.

3. Electrostatic potential of the cytochrome *c* oxidase

To determine actual electrostatic potentials in the vicinity of D- and H-channels, we use the APBS package [7] package coupled to VMD [6]. The complex under interest can be downloaded from the protein database (PDB) according to its unique code and thereafter the Poisson equation is solved for the specific structure providing the electrostatic potential distribution. We compare two forms of the cytochrome *c* oxidase complex, the fully oxidized one, with electrons removed from all four metallic sites (PDB code 3ASO), and the fully reduced one (PDB code 3AG1). Corresponding potentials are shown in figure 2 for the D-channel and figure 3 for the portion of the H-channel located near the metallic sites.

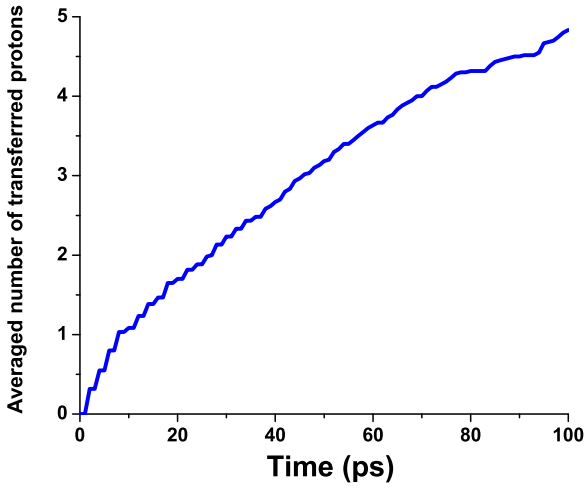

Figure 4. Time dependence of the averaged number of protons transferred through the D-channel of the fully oxidized form of the cytochrome *c* oxidase of the bovine heart (PDB code 3ASO).

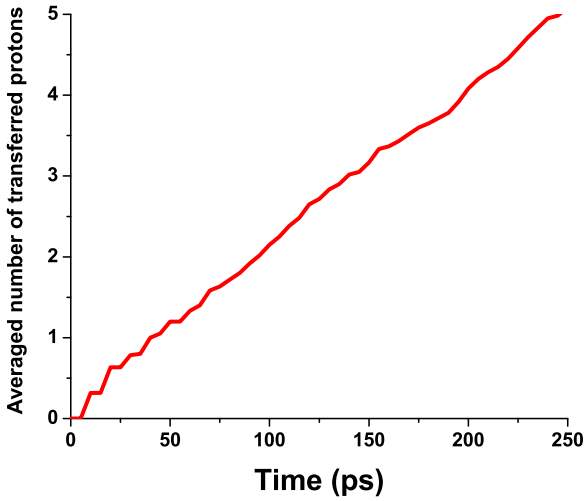

Figure 5. Time dependence of the averaged number of protons transferred through the D-channel of the fully reduced form of the cytochrome *c* oxidase of the bovine heart (PDB code 3AG1).

It is evident from these figures that the potential profiles for the fully oxidized and fully reduced forms are quite different, although it is impossible to conclude anything about the openness of these channels for proton transport.

4. Proton transport through channels

For more quantitative analysis, we examine the proton transfer along the D- and H-channels in the presence of an actual electrostatic potential obtained from the APBS software. Corresponding equation of motion has the form:

$$m_p \frac{\mathrm{d}^2 x}{\mathrm{d}t^2} = -\gamma \frac{\mathrm{d}x}{\mathrm{d}t} + eE_x(\vec{r}) + \xi(t), \tag{1}$$

where m_p and e are the proton mass and charge, γ is the friction coefficient, and $\xi(t)$ is the fluctuation source with zero mean value and the correlation function given by

$$\langle \xi(t)\xi(t_1)\rangle = 2\gamma T\delta(t - t_1). \tag{2}$$

In the overdamped regime, equation (1) can be rewritten in the form of the Langevin equation, as

$$\gamma \frac{\mathrm{d}x}{\mathrm{d}t} = eE_x(\vec{r}) + \xi(t). \tag{3}$$

We solve this stochastic equation numerically taking the coordinates of the beginnings and ends for the channels from corresponding PDB files with the results being averaged over many realizations. We assume that only one proton can populate the channel at specific moment of time and the proton, which reaches the end of the channel, leaves it immediately and simultaneously the next proton starts to move along the channel from its beginning. Accordingly, the obtained results do not provide actual proton transfer rates but just characterize the openness of the channel.

The time dependencies of the averaged numbers of protons transferred through the D-channels of the fully oxidized and fully reduced forms of the cytochrome *c* oxidase of the bovine heart are shown in figures 4 and 5, respectively.

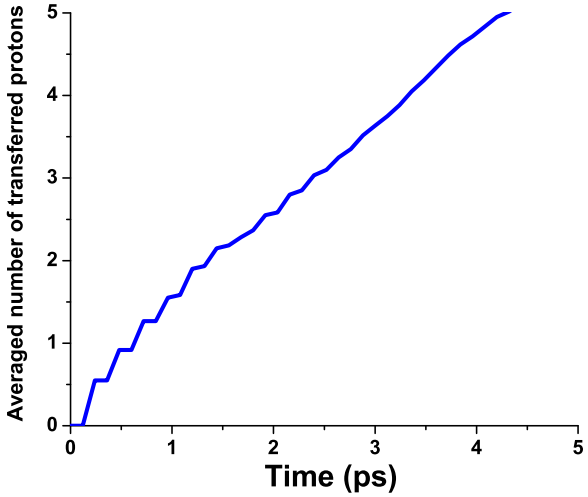

Figure 6. Time dependence of the averaged number of protons transferred through the H-channel of the fully oxidized form of the cytochrome *c* oxidase of the bovine heart (PDB code 3ASO).

In contrast to naive expectations, electrons added to metallic sites do not lead to the opening of the channel. Instead, the proton motion in the reduced form is about 2.5 times slower than that of the oxidized form. The reason for that is the conformational changes accompanied by the charge redistribution in the protein environment.

In similar figures for the H-channel (figures 6 and 7), one can see that the conformational changes accompanying the reducing of the metallic sites are favorable for the proton transfer, which is 1.5 times faster than that of the oxidized form.

5. Conclusions

In conclusion, we examined the proton transfer through the D- and H-channels of the cytochrome *c* oxidase of the bovine heart in its fully oxidized and fully reduced forms. We solved numerically the Langevin equation in the presence of the

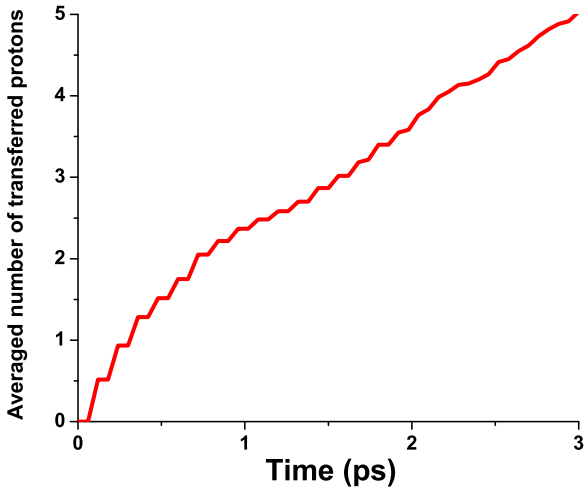

Figure 7. Time dependence of the averaged number of protons transferred through the H-channel of the fully reduced form of the cytochrome *c* oxidase of the bovine heart (PDB code 3AG1).

electrostatic potential which was determined using VMD and APBS software packages. It was shown that the reducing of metallic sites does not open the D-channel but opens the H-channel, in agreement with studies of [8, 9]. We believe that the combination of simple models with determination of their parameters using sophisticated numerical methods, such as molecular dynamics, is a promising approach to studies of complicated systems.

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