Simulation of proton movement in $F_oF_1$-ATP synthase by quantum-mechanical approach

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Abstract. Quantum-mechanical approach is applied to the description of proton transport through $F_oF_1$-ATP synthase which is the crucial process in ATP synthesis. Proton was described as a particle located in potential wells formed by charged centers along the half-channels. Energy spectra of bounded states were calculated using Bohr-Sommerfeld quantization, and the initial population of each quantum level was determined by Boltzmann distribution. Water molecules were stochastically distributed in an inlet half-channel taking into account atomic radii. Characteristic time of proton transition between the charged centers (amino acid or water molecule) was estimated and it revealed the critical areas needed to be full with water. All possible pathways were analyzed in Monte-Carlo simulation which allows calculating of a mean time of proton transfer through the inlet half-channel (23 ms).

1. Introduction

Adenosine triphosphate (ATP) plays a very important role in metabolism and energy exchange in all living organisms. It is a high-energy compound which appears to be a universal energy source for many physiological and biophysical processes. ATP is produced in cells from adenosine diphosphate (ADP) and inorganic phosphate ($P_i$) by means of protein complex $F_oF_1$-ATP synthase which is a unique energy transformer driven by electrochemical gradient of hydrogen/sodium ions [1]. Its catalytic reversibility is also a unrivalled complex feature which is not yet fully recognized. Structure of this enzyme is well known, but its precise working mechanism is still a point of actual scientific discussions.

Macromolecular complex $F_oF_1$-ATP synthase consists of two morphologically determined parts. One of them, $F_1$ (factor determined at first), is exposed to the aqueous phase, markedly extends from the membrane as a nearly spherical formation and it can be easily separated as a soluble fraction. The other one, $F_o$ (oligomycin-sensitive), is embedded into the membrane and represents a highly hydrophobic protein complex [2]. Although being a highly conservative protein, it has few peculiarities depending on a specie. For example, in bacterium E.Coli (the most investigated one) $F_1$ part consists of nine protein subunits ($\alpha_3\beta_3\gamma\epsilon\delta$). It carries three catalytic centers responsible for the reactions of synthesis and hydrolysis of ATP [3]. Transmembrane $F_o$ part ($\alpha_1\beta_2\gamma_{135}$) includes two proton-conducting half-channels across the membrane [4]. A proton passes through the inlet half-channel to the middle of membrane, then it gets bounded to the essential amino acid residue and...
moves in membrane during rotation of the rotor to the outlet half-channel which leads it to the other side of the membrane.

Key amino acid residues containing protonated groups capable of holding the protons and transmitting them to each other play the significant role in $F_oF_1$-ATP synthase half-channels functioning. Most essential amino acid residues were determined using site-specific mutagenesis. Two important ones belong to $a$-subunit – $a$Arg210 and to $c$-subunit – $c$Asp61 (in E.Coli) [5]. Presumably, $a$Arg210 is a positively charged residue, initiating rotation of $F_oF_1$-ATP rotor, but it is not directly involved to the conduction of proton through the membrane because of its location outside of the half-channels area [6]. $c$Asp61 is the final/initial point of proton movement through both half-channels and it is the residue bound to which the proton moves in membrane during the rotation process [7].

According to modern data, proton half-channels are formed by $a$- and $c$-subunits of $F_o$ factor. First 3D-structure of the protein, obtained by means of NMR, showed that $a$-subunit folded in membrane as five vertical transmembrane helices (TMH) and thus provided access of protons to $c$Asp61 residue [8]. However, recent research of rotating ATPase structures by electron cryomicroscopy has revealed refined structure of $a$-subunit. It occurred to consist of a pair of long, horizontal, membrane-intrinsic helices in $a$-subunit next to the $c$-ring rotor [9]. Meanwhile up-to-date there is no precise understanding of TMH 3D distribution in $a$-subunit, and the position of proton half-channels is still under consideration.

There are three main approaches to description of proton transport through different media: Grotthuss mechanism, stochastic modeling, and quantum-mechanical approach. In Grotthuss mechanism, proton could hop from one molecule of water to another, in cases when formation of Zandel cation ($H_2O_2^+$) or Eigen cation ($H_4O_4^+$) is possible [10]. Stochastic modeling is based on calculation of random trajectory of a particle which is modeled by finite difference equations giving an approximate solution of the equation of motion [11]. The most comprehensive approach is a quantum mechanical consideration which takes into account the energy of electrostatic interaction of all charged particles and also purely quantum effects, inherent only to microparticles (tunneling effect and localization of bound states) [12].

In this work combined approach is used for description of proton transport through half-channels of $F_oF_1$-ATP synthase. Probability of proton transfer from one charged center to another one is determined in quantum-mechanical model of one-dimensional motion. The general problem of proton transport through the sequence of charged centers in the half-channel is solved using stochastic approach. This combined method could be further applied for description of energy transfer in protein channels and could lead more profound understanding of energy transforming mechanisms.

2. Methods

The main force acting on a proton is due to its electrostatic interaction with fixed charges of protein and with polarized water molecules. In the paper these charged particles, that can affect proton movement and create short-lived bound states, are called charged centers.

2.1. Structural analysis of proton half-channel

Based on 1c17 PDB structure (X-ray or NMR protein structure databank) we determined modeling areas as the region of TMH 2-5 of $a$-subunit in the inlet half-channel including all essential amino acid residues which were proposed to take part in proton transport (figure 1). In order to take into account the interaction of a proton with polar water molecules present in the half-channel, coordinates of each water molecule should be determined (position and orientation). In the present study voids in the molecular structure of the half-channel were stochastically filled with water molecules using Van der Waals radii of atoms.
2.2. Quantum-mechanical description of proton transport

Potential of interaction between proton and charged centers should be determined to formulate
quantum-mechanical description of proton transport mechanism. Disregarding effects of polarization
of atoms and orientation of electron orbitals, sum of Coulomb potential and Lennard-Jones potential
were used as interaction potential:

\[ U(r) = \frac{kq_1 q_2}{\varepsilon r} + \varepsilon \left( \frac{r_{\text{min}}}{r} \right)^{12} - 2 \left( \frac{r_{\text{min}}}{r} \right)^{6}, \tag{1} \]

where \( q_1 \) – proton charge, \( q_2 \) – uncompensated charge of the center, \( \varepsilon \) – permittivity, \( \varepsilon \) – binding
energy between the proton and the charged center, \( r_{\text{min}} \) – bond length.

Probability estimation of the proton hop between neighbor centers was obtained by using
approximation of quasi-one-dimensional proton movement along the line, connecting the centers, in
the potential given by the sum of potentials of both centers.

In the case of bounded state of proton there is a discrete spectrum of possible energies which can be
calculated by solving of Sturm-Liouville problem on eigenvalues of particle Hamiltonian. Analytical
solution occurs only in a few cases, for example in a rectangular quantum well, so one can use more
simple quasi-classical approach – a Bohr-Sommerfeld quantization. Condition for existence of allowed
quantum state is equality of the following:

\[ \frac{1}{\pi \hbar} \int_a^b \sqrt{2m(E-U(x))} \, dx = n + \frac{1}{2}, \tag{2} \]

where \( a \) and \( b \) – classical turning points, \( n \) – integer number, defining the level number with energy \( E \)
in quasi-classical case.

The probability of proton occurrence at the given quantum level is determined by Boltzman
distribution:

\[ N = N_0 \exp \left( \frac{E}{kT} \right). \tag{3} \]

Despite indefinite proton localization in the potential well in terms of classical approach, in the
case of quantum-mechanical description the penetration of particles into the next well is allowed.
through a sufficiently thin barrier. Tunneling probability depends on proton energy and could be defined as:

\[
p = \exp\left(-\frac{2}{\hbar} \int_{x_1}^{x_2} \sqrt{2m(E-U(x))} \, dx \right),
\]

where \(x_1\) and \(x_2\) are subject to condition \(U(x_1) = U(x_2) = E\).

In quasi-classical approach flux of particle from the given well to the adjacent well is equal to product of classical frequency of particle oscillation in the well and its tunneling probability. In a model system the possible proton way can be located at the chain of charged centers so the total time of the proton transport through the channel could be determined as the sum of characteristic times of proton transfer between each pair of adjacent centers.

3. Results

3.1. The spatial arrangement of water molecules in the inlet half-channel of \(F_6F_1\-ATP synthase\)

Statistical set of one million of water molecules distributions was obtained due to previously described approach. Since coordinates of each molecule were chosen randomly, there were distributions with a small number of molecules and a rather dense packing as well. Two areas with a large number of water molecules were discovered. They were located at the beginning and the end of the half-channel. In the central region of the half-channel an obtained small number of water molecules was associated with a quite dense structure of this protein area. To consider the majority number of possible proton ways through half-channels, the distribution with the maximum number of water molecules which amounted to 66, was further used in the model.

3.2. Quantum-mechanical calculation of the proton transfer between the two binding sites

In the described system oxygen atoms of water molecules (H\(_2\)O), oxygen atoms (R–O–) of carboxyl and hydroxyl groups, nitrogen atoms (R–N–) of the amine groups of amino acid residues can be considered as the proton binding centers. The proton can move between these centers in a random sequence. Full time of proton movement through half-channel will depend on the number of involved charged centers in the chain and the distance between them, and also on types of these centers (water or amino acid residue). Therefore, to solve the problem of proton transport it is necessary to calculate the proton transfer time between all possible pairs of the adjacent centers.

In case of sufficiently close centers location at a distance up to 5 Å, two separate wells do not appear, and a proton has common states. The characteristic time of transfer between the centers is determined by the size of well and the momentum of the proton and is about \(\tau = 15\pm30\) μs. When the distance between the centers is more than 5 Å, separate wells are formed, and the proton has a localized state at each center. The characteristic time of transfer between the centers is about \(\tau = 0.49\pm3.58\) ms (figure 2).

Analyzing the proton behavior in the system depending on the distance between centers the one can easily notice a sharp increase in the characteristic time of protons transport at the distance more than 5 Å when it becomes higher than the physiological timescale of 10 ms that logically leads to the restriction of the critical center distance.

All types of present in the model system distances and types of centers were analyzed and the critical distances for all combinations of center types were obtained to provide the possibility of the continuous transport of the protons.

The characteristic times of transitions between the charged centres are calculated for each considered geometrical configuration and are used to estimate the total proton transfer time via the inlet half-channel and to determine the optimal transport pathways.
3.3. Proton transport pathway analysis for inlet half-channel of $F_0F_1$-ATP synthase

All obtained pathways with different number of hops and the characteristic times of movement were carefully analyzed. Pathways with a small number of hops were unlikely and the occurrence probability increased along with a number of hops that was caused by the possibility of cyclic transitions in the applied algorithm. However, it turned out that the proton had reached the end of the half-channel in a finite time (figure 3 (a)). The average time of the proton movement through inlet half-channel was 22.83 ms. In figure 3 (b, c) there are histograms of pathway frequencies with a different number of all types of centers and a different number of water molecules in the transfer chain respectively.

**Figure 2.** Potential energy of proton between two binding centers. Dash line shows potential energy of proton in field of each center given by Coulomb and Lennard-Jones potential. (a) Distance between centers (H$_2$O$\rightarrow$H$_2$O) is $3.75\ \text{Å}$, $\tau = 3.26 \times 10^{-3}\ \text{s}$. (b) Distance between centers (H$_2$O$\rightarrow$H$_2$O) is $5\ \text{Å}$, $\tau = 4.88 \times 10^{-4}\ \text{s}$. (c) Distance between centers (H$_2$O$\rightarrow$R$-\text{O}^-$) is $3\ \text{Å}$, $\tau = 1.58 \times 10^{-5}\ \text{s}$. (d) Distance between centers (H$_2$O$\rightarrow$R$-\text{O}^-$) is $4.75\ \text{Å}$, $\tau = 3.58 \times 10^{-3}\ \text{s}$.

**Figure 3.** (a) Histogram of pathway frequency with a different transfer time. (b) Histogram of pathway frequency with a different number of centers. (c) Histogram of pathway frequency with different number of water molecules.
The most likely are the pathways with 27-29 proton binding centers. The number of water molecules located in half-channel during the protein structure assembly need not to be maximal. 19 water molecules occurred to be sufficient for proton transfer through half-channel. However, the probability of the pathways with such number of water molecules centers was not high although this type of centers was the most common. Comprehensive research revealed eight water molecules occupied in all the pathways which can be considered “essential” (figure 4) [13].

Proton transport time through inlet half-channel of F$_{0}$F$_{1}$-ATP synthase has not been yet experimentally estimated. Nevertheless, the proton transport time through the membrane could be indirectly evaluated from the enzyme turnover rate. Direct comparison of calculated in the model mean time of proton transport through inlet half-channel (22.83 ms) with estimations of published data (0.16-19.47 ms) [14-17] is challenging. Observing mismatch of defined time values can be easily accounted by an insufficient level of experimental technical possibilities and consequent low interest to the considered issue. The existing experimental values are in a wide range of time due to various approaches of their executing and a large scope of obtained characteristics. Yet mean time of proton transfer calculated in the present work is still in physiological timescale and complains with turnover rate for the whole enzyme.

4. Discussion

ATP is one of the renewable compounds in mammalian organism. The stock of ATP is not kept due to high rate of its utilization in all types of energy consuming physiological processes. Therefore, the new knowledge about the precise mechanism and regulation principles of F$_{0}$F$_{1}$-ATP synthase, a protein complex that catalyzes the synthesis of ATP, may have important scientific implications.

The main point of interest is the mechanism of electrochemical transmembrane gradient and rotational energy transformation into the chemical energy of ATP. The energy coupling can be localized in the areas of proton transfer in the transmembrane domains of the enzyme. Direct simulation and experimental study of this process are not fully realizable at the current stage of scientific technology.

Theoretical modeling of the proton transport using described combination of quantum-mechanical and stochastic approaches allows evaluating not only the time of proton movement through protein structure, but also the proton energy level and possible ways of energy transformation.

As a result of the modeling, various proton pathways were obtained. The total transfer times highly depend on the type and number of centers involved in a specific pathway. Including of hops with critical and near-critical (in the model) distances between the centers leads to a sharp increase in the total time. The low prevalence of amino acid centers in the proton pathways can be caused not only by its lower distribution but also by their localization and a deeper energy well. The exact definition of the upper boundary of the proton hopping distance is an important task which allows the determination of the maximum energy passed by the proton during its movement. Analysis of obtained set of
pathways revealed critical areas inside the intramembrane part of the enzyme which are to be occupied with water molecules during the protein assembly. Any failure in this physiological process may lead to irreversible breakthrough of the proton pathway and thereafter to corruption of normal ATP synthesis.

The obtained in the model mean proton transfer time occurred to be 22.83 ms and is on the upper limit of the time range estimated using various experimental data. Any ion transfer time is obviously highly dependent on its transmembrane potential and electrochemical gradient (the quickest time of 0.16 ms [14] was measured at 100 mV across the membrane). A further consideration in the model of the proton-motive force and membrane potential will not only improve the accuracy of the calculated proton transfer time under real biological conditions, but will also be useful to analyze the mechanism of transformation and accumulation of energy in the process of the proton transfer which is an important step in understanding the entire energy conversion mechanism during the F, F\textsubscript{1}-ATP synthase catalytic cycle.

This study is the first attempt to apply the quantum-mechanical approach to describe the proton transport in half-channels of F, F\textsubscript{1}-ATP synthase. The obtained new data on the mechanism and parameters of the protons movement in the protein half-channels along with method application on other subject can also have implications in explaining the functioning of many biological structures.

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