Quantum-mechanical analysis of amino acid residues function in the proton transport during $F_0F_1$-ATP synthase catalytic cycle

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Quantum-mechanical analysis of amino acid residues function in the proton transport during F\textsubscript{o}F\textsubscript{1}-ATP synthase catalytic cycle

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Abstract. Implications of quantum-mechanical approach to the description of proton transport in biological systems are a tempting subject for an overlapping of fundamental physics and biology. The model of proton transport through the integrated membrane enzyme F\textsubscript{o}F\textsubscript{1}-ATP synthase responsible for ATP synthesis was developed. The estimation of the mathematical expectation of the proton transfer time through the half-channel was performed. Observed set of proton pathways through the inlet half-channel showed the nanosecond timescale highly dependable of some amino acid residues. There were proposed two types of crucial amino acids: critically localized (His245) and being a part of energy conserving system (Asp119).

1. Introduction
In 1941 Fritz Lipmann discovered that the main storage of energy in the cell is adenosine triphosphate (ATP). These molecules belong to the so-called macroergic compounds containing bonds, hydrolyzed with a release of a significant amount of energy. In living organisms, ATP is synthesized by substrate-level, oxidative and photosynthetic phosphorylation of adenosine diphosphate (ADP) [1].

There are several types of ATPases, which can hydrolyze ATP – F-type, V-type, A-type. Nevertheless, the only enzyme capable of synthesizing ATP in the process of oxidative phosphorylation is F\textsubscript{o}F\textsubscript{1}-ATP synthase [2]. F\textsubscript{o}F\textsubscript{1}-ATP synthase is a complicated molecular motor that is a unique energy transformer in a living cell. This highly conserved protein complex uses the energy of the electrochemical gradient of hydrogen ions created by the electron transport chain [3].

Despite the long-term biochemical and structural studies and theoretical modeling [4-7], many issues remain unanswered. Among them are the mechanisms of energy, time and proton transfer coupling. In addition, the question of the precise enzyme structure also remains open. It is assumed that the membrane part of the enzyme contains two half-channels for proton transport, however, direct measurement of any characteristics of this process is challenging [8]. The key unresolved issue is how the process of proton transfer through the membrane is coupled with the synthesis of ATP.

One of the most precise ways to describe deep molecular processes is the application of quantum-mechanical principles. It can be widely used for current calculation in nanoscale field-effect transistors, drug design, enzyme-ligand docking, protein folding and many other processes. Wave function of proton in gramicidin channel, which is a pore in a lipid membrane, was previously described elsewhere [9]. Meanwhile processes in more complicated biological objects consisting of a complex channels or half-channels can be also characterized using quantum-mechanical approach.

It is generally accepted that proton transport is carried out along the chain of amino acid residues of the enzyme. Therefore, the problem of identifying the essential amino acids for proton transport became
very important in the last decade [5]. Studies of the protein structure were performed by X-ray diffraction analysis, cryo-EM, site-specific mutagenesis and cross-linking [10, 11]. Thus, a number of amino acid residues that are supposedly involved in proton transport has been identified. Being a part of a channel wall and taking part in the cumulative charge forming these residues can also serve as intermediate energy storages or sources.

New data delivered using quantum-mechanical approach can be very useful not only for description of proton movement in F-ATP synthase half-channels but also for the understanding of other transmembrane ion movement processes.

2. Methods
Currently, there is a list of different pdb-structures of F\(\nu\)-ATP synthase, but many of them are incomplete or have low resolution (7-8 Å). In this paper we use one of the first pdb-files 1C17 for E.Coli, which contains the \(a_{12}\) subunits [12].

Thorough study of the published data revealed the following amino acid residues important for proton transport in the inlet half-channel: Gln234, Asn116, Asp119, His132, Ser144, Asn148, Ser152, Asn214, Glu219, Ser233, Asn238, His245, Thr250, Gln252 [13, 14, 15].

There are different approaches to the description of proton transport in various environments: Grotthuss mechanism, the stochastic modeling, the molecular dynamics and so on. In this paper, in order to simulate proton transport through the input half-channel, a combined approach is used. It allows obtaining information that is more detailed. Probability of proton transfer from one charged center to another is determined in quantum-mechanical model of one-dimensional motion. This allows us to consider the purely quantum effects inherent in microparticles. The general problem of proton movement through the sequence of charged centers in the half-channel is solved using stochastic approach [16].

In order to describe the mechanism of proton transport it is necessary to define the potential of interaction between a proton and charged centers (amino acid residues and water molecules), which can bind protons on its way through the half-channel. Energy spectra of bound states in potential wells were determined by Bohr-Sommerfeld quantization. Occupation numbers of the quantum levels were given by Boltzmann distribution. Hopping motion including only amino acid residues leads to rather low probability values of a transition from one charged center to another due to their considerable distance. Therefore, the distribution of water molecules was obtained in the input half-channel. Possible pathways of proton movement through half-channel were revealed using Monte-Carlo simulation. An exact description of the model and the spatial arrangement of water molecules were previously described elsewhere [16].

To determine the direction of a proton motion when choosing from adjacent centers, it is necessary to calculate the characteristic times of elementary steps. Using Monte-Carlo simulation, the transfer from the current center to another is selected considering the probability of the transition, which is inversely proportional to the calculated transfer time.

The universality of this approach consists in the ease including and excluding any number of essential amino acid residues based on the analysis of published data and depending on the specific research tasks. In this paper, we consider the case of 15 amino acids in the input half-channel.

3. Results
The model described in this paper allows one to obtain sets of pathways for the transport of a single proton through an inlet half-channel. The motion of a proton is not limited in direction and is determined only by the probability of a transition. The existence of adjacent charged centers in some regions of the half-channel lead to the set of equiprobable transfers that may cause possible cyclic transitions, thus increasing the total number of steps. The calculation of the proton path was carried out 100,000 times in each set. The number of steps varied from 600 to 200,000 in one pathway, however, more than 60% of the pathways included no more than 5000 steps. The estimation of the mathematical expectation of the proton transfer time through the half-channel was \((1.66±0.06)\times 10^{10}\) s. Errors were estimated from the obtained sample using the formula of an unbiased dispersion estimate.
Figure 1. Histogram of the observation frequencies of individual centers in the resulting set of paths. Black bars represent water molecules and red bars represent amino acid residues. (a) For all centers; (b) For amino acids.

Figure 1 shows a histogram of the observation frequencies of individual centers in the resulting set of paths. Black bars represent water molecules, the first four of which are located at the beginning of the half-channel. Therefore, participation of one of them is inevitable in every pathway. Red bars represent amino acid residues, numbered in the order described above. It is easily seen that water molecules are encountered more frequently in the proton pathways. However, the effect of some amino acid residues on proton transport is also significant. All amino acid residues can be divided into three groups according to their observation frequencies. Often found (frequencies > 0.004) are 5(Gln234), 8(His132), 14(Ser233); rarely found (frequencies > 0.002) are 7(Asp119), 11(Ser152), 16(His245), 18(Gln252). All the other amino acids practically do not participate in proton transport. To analyze the contribution of the specific amino acid the presented data should be analyzed in details. An amino acid residue is assumed essential in the transfer process if its exclusion from the pathway will lead to the increase in overall proton transport time. However, it should be mentioned that in this model the proton transport could not be seized by exclusion of any charged center due to stochastic mode of motion and high density of centers in the half-channel.

In two cases of exclusion of an amino acid residue from the second group, the significant changes in the time of movement through the input half-channel were observed. When Asp119 was excluded the time of proton transport increased by (23±7)%, however, no visible redistribution of observation frequencies of the other centers occurred. Therefore, there is no transport stoppage, however, the increase in time may indicate the non-optimal character of the observed paths, which may be due to the need for energy accumulation at this step. Asp119 is known to be one of the important amino acid residues in the proton pathways. Substitutions of this residue leads to the reduce but do not the abolishing of proton conductivity [15].

Another key amino acid residue is His245. Due to its highly conservative localization, it is often used to reconstruct the structure of the a-subunit obtained by the cryo-EM method [10]. The exclusion of this residue in the model calculations also led to an increase in proton transport time by (28±8)%. Observed redistribution of the participation frequency for both water molecules and amino acid residues consisted of an increase in the frequency of the centers located in the first half of the channel (1-30) along with the decreased frequency of the centers at the end of the channel (35-80) (Figure 2). This caused to the elongation of the pathway set. His245 located in the center of the membrane with a rather dense protein structure seems to be an important link between the inlet and the end of the half-channel.
4. Discussion

The proton transport via F_{o}-ATP synthase half-channels is an important step of energy formation in the living cell. Meanwhile the energy storage and transformation mechanism is still under consideration. Quantum-mechanical approach appeared to be fully applicable to the description of ion transport in this system. Observed set of proton pathways through the inlet half-channel showed the nanosecond timescale highly dependable of the protein structure. Most of the considered essential amino acid residues revealed to be an important part of the channel wall. Nevertheless, the observation frequencies of Asn116, Ser144, Asn148, Asn214, Glu219, Asn238, Thr250 tended to the zero that can be caused by its localization and water environment.

The often and rarely found amino acid residues are considered to be significant in the proton transfer. They can be crucially localized (His245) or being a part of energy conserving system (Asp119). Each separate pathway may consist of a huge number of steps, but short pathways (up to 5000 steps) are more often. A prevalence of water molecules as participating charged centers leads to the appearance of possible cyclic transitions regions located in the inlet part of the half-channel and near Asp61 (Figure 3).

Figure 2. Histogram of the observation frequencies of individual centers in the resulting set of paths without His245. Black bars represent water molecules and red bars represent amino acid residues.

Figure 3. Illustration of a proton movement through the inlet half-channel. The ribbons are 2-5 TMC of α-subunit and the nearest c-subunit. Red circles are the water molecules, essential amino acids are pointed. Lines show the trajectory of a proton.
The data discussed in the paper provide a quantitative estimation of proton movement through the inlet half-channel of F$_{o}$F$_{1}$-ATP synthase. Its stochastic behavior leads to a huge variability of certain pathways meanwhile conserving a high sensitivity to the types and the number of charged centers on the proton way. Further investigations of the system and its parameters under the different conditions and modifications can be an important step in the comprehensive theory of energy storage and transformation.

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