Monte Carlo simulation from proton slip to “coupled” proton flow in ATP synthase based on the bi-site mechanism

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ABSTRACT

ATP synthase couples proton flow to ATP synthesis, but is leaky to protons at very low nucleotide concentration. Based on the bi-site mechanism, we simulated the proton conduction from proton slip to “coupled” proton flow in ATP synthase using the Monte Carlo method. Good agreement is obtained between the simulated and available experimental results. Our model provides deeper insight into the nucleotide dependence of ATP catalysis, and the kinetic cooperativity in three catalysis subunits. The results of simulation support the bi-site mechanism in ATP synthesis.

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1. Introduction

ATP synthase is a universal enzyme in biological energy conversion that is present in the membranes of mitochondria, chloroplasts and bacteria. The enzyme can synthesize ATP from ADP and inorganic phosphate (Pi) using the energy of a transmembrane electrochemical gradient of protons, on the other hand, it also can hydrolyze ATP and release chemical energy stored in ATP. The enzyme is composed of two portions: the hydrophilic F1—portion catalyzes ATP synthesis/hydrolysis and the membrane-embedded Fo—portion performs proton transfer. The catalysis sites of ATP synthesis/hydrolysis are at three β subunits in F1 portions. F0 and F1 portions are mechanically coupled by a common central stalk, cα−ε−γ. The proton flux driven by the proton motive force (pmf) causes the rotation of central stalk [cβ−ε−γ]. In turn, the rotation of the asymmetric subunit γ induces conformational changes of α3β3 crown in F1 that lead to ATP release and binding of ADP and phosphate. Many recent investigations such as structure (Abrahams et al., 1994; Menz et al., 2001; Kagawa et al., 2004; Bernal and Stock, 2004; Stocker et al., 2007), single molecule (Yasuda et al., 1998; Yasuda et al., 2001; Nishizaka et al., 2004; Adachi et al., 2007), biochemistry (Weber and Senior, 2000; Mao et al., 2006; Toei et al., 2007; Steigmiller et al., 2008; Nakamoto et al., 2008), and theoretical modeling (Wang and Oster, 1998; Elston et al., 1998; Liu et al., 2003; Xing et al., 2005; Gao et al., 2005; Shu and Lai, 2008) have greatly deepened our understanding of the ATP synthase. However, some debate still exists about the detailed catalysis mechanism of the enzyme: for example, whether bi-site or tri-site occupancy induces rapid ATP synthesis (Weber and Senior, 2001; Boyer, 2002; Milgrom and Cross, 2005; Koga and Takada, 2006; Pu and Karplus, 2006; Bulygin and Milgrom, 2009), and whether ADP and phosphate (Pi) binds in a compulsory order or in a random order (Kothen et al., 1995; Banke and Rumberg, 1996; Al-Shawi et al., 1997; Scanlon et al., 2007).

The proton transport occurs through the ATP synthase in two different ways: (a) proton slip in the absence of nucleotides and (b) proton translocation coupled to ATP synthesis. Uncoupled proton slip has been investigated in the enzyme from the chloroplasts (Groth and Junge, 1993) and Rhodobacter capsulatus (Feniouk et al., 2005) in the absence of nucleotides. Proton slip is blocked by small concentrations of ADP (over 0.1 μM). In 1993, Groth and Junge studied proton conduction from proton slip to coupled proton flow in ATP synthase of chloroplasts at varying concentrations of ADP (0.01 μM–100 μM) by flash spectrophotometry (Groth and Junge, 1993). The transition process from an enzyme state with proton slip (no nucleotides) to a state with reduced proton conduction (about 0.1 μM ADP) and then to a state with ATP synthesis (>1 μM ADP) was investigated by detecting the decay rate of the flash-induced voltage which represents the proton current across the enzyme. Besides the investigation of dynamic behaviors in ATP synthase,
that, as ATP synthesis rate under different concentrations of ADP and Pi, this work is a good experiment to study the nucleotide dependence of catalysis in ATP synthase.

One of the arguments about the catalysis mechanism of ATP synthase is whether the main kinetic enhancement occurs upon filling the second or the third catalytic site. Results from the laboratories of Weber, Senior, and co-workers indicated that all three catalytic sites must be filled with ATP or ADP in order to promote rapid ATP hydrolysis (Weber and Senior, 2001). However, Boyer maintains that rapid ATP synthesis (or hydrolysis) is attained when a second catalytic site is filled with ADP and Pi (Boyer, 2002). Recent experiments of Milgrom and Cross (2005) and Bulgin and Milgrom (2009) supported the bi-site activation mechanism. In this paper, based on the adapted bi-site mechanism proposed by Boyer, we numerically simulated the proton translocation from proton slip to coupled proton flow in ATP synthase. The simulation results agree well with the experimental results by Groth and Junge (1993). The analysis of our model supports the bi-site mechanism in ATP synthesis.

2. Model

2.1. Basic assumptions

In the ATP hydrolysis direction, it is generally accepted that the enzyme releases Pi before ADP after the hydrolysis step (Masaikake et al., 2000). In the ATP synthesis direction, the binding order of ADP and Pi to the catalytic sites is not confirmed. Some kinetic analyses of enzyme indicate a random binding order of ADP and Pi (Kothen et al., 1995; Panke and Rumberg, 1996). The other experiments (Al-Shawi et al., 1997) suggest that ATP synthesis is the exact reverse process of ATP hydrolysis. Here, we adopt this view, and assume that, in synthesis, Pi binds to the catalytic site after ADP. It should be noted that we can also obtain the similar simulated results upon the random binding assumptions (not shown in this paper) since more simulation parameters would be adjusted in this way, so we cannot determine the binding order of ADP and Pi in this work. The kinetic pathway in each subunit of ATP synthase in our model can be shown in Fig. 1, where different states are connected by thick arrows. In the main pathway, \( k_1 = k_{\text{ADP}} \) is the ADP-binding rate, \( [\text{ADP}] \) is the concentration of ADP; \( k_2 = k_{\text{Pi}} \) is the Pi-binding rate, \( [\text{Pi}] \) is the concentration of Pi; \( k_3 \) represents the ATP-synthesis (ATP-hydrolysis) rate; \( k_4 \) represents the ATP-release (ADP-release and Pi-release) rate; \( k_5 \) is the ATP-binding rate which is neglected in our simulation because of the very low ATP concentration in the experiment.

Our model is based on the bi-site mechanism as shown in Fig. 2. For net ATP synthesis site 1 binds ADP and Pi, site 2 catalyzes ATP synthesis and releases ATP, site 3 is empty and waiting for binding of ADP and Pi. During catalysis sites are converted sequentially into three different states accompanying rotation of the \( \gamma \). Site 1 with ADP and Pi bound could be closely represented by the ADP binding form with sulfate present as described by Menz et al. (2001). A form of site 2 with ADP and Pi present may be related to the \( \beta_{\text{DP}} \) form (Abrahams et al., 1994). Site 3 is an empty form \( \beta_{\text{E}} \) (Abrahams et al., 1994). Rapid ATP synthesis can occur when site 2 is already filled and site 1 binds ADP and Pi. When site 1 is not filled, the ATP synthesis in site 2 is very slow. The experiments by Stroop and Boyer (1985) indicated that the tightly bound ADP, Pi and ATP interconvert about 50 times before ATP is released when \( [\text{ADP}] \) is low, which supports our assumptions for nucleotide dependence of ATP synthesis. In addition, recent experiments by Tomashak et al. (2004) indicated that occupation of the third site further activates the rate of synthesis. Because the hinge bending of the \( \beta \) subunits are tightly coupled to rotation of \( \gamma \), it is easily understood that each catalysis site can influence the dynamics of other sites via the torsional stress transmitted through the \( \gamma \) shaft (Xing et al., 2005). In our model, we assumed that the occupation of site 1 increases the synthesis rate (\( k_4 \)) largely in site 2 and the occupation of site 3 further increases the ATP-release rate (\( k_4 \)) on a small scale in site 2. It is noted that our model is not entirely same as the Boyer’s mechanism. In Boyer’s mechanism (Boyer, 2002), site 1 binds ADP and Pi, site 2 catalyzes ATP synthesis, and the release of ATP is in site 3. However, if the ATP synthesis and the release of ATP are in different sites (sites 2 and 3), and site 1 contributes to the bi-site activation for site 2, there will be no site for realizing the further activation for the synthesis because of occupation of the third site as shown in Tomashak et al. (2004). Therefore, in our model, the ATP synthesis and the release of ATP are proposed to occur in one site (site 2). Furthermore, according to the Groth and Junge’s work (1993), we assumed that the proton slip occurs when all three sites are unoccupied.

In reference of Groth and Junge (1993), the proton conduction in ATP synthase is described by the decay rate of the flash-induced voltage as a function of ADP and Pi. According to the reference of Groth and Junge (1993), the decay of the transmembrane voltage is discussed in terms of the capacitor equation

\[
i = -\frac{dU}{dt} \quad (1)
\]

where \( i \) is the current density in A/m², \( c \) is the specific capacitance in F/m², \( U \) is the voltage and \( t \) is the time. The current density is related to the voltage by Ohm’s law:

\[
i = gU \quad (2)
\]

where \( g \) is the specific conductance in S/m². In the experiment, the specific conductance \( g = g_s + g_l \), where \( g_s \) is the proton conductance by ATP synthase related to slip, and \( g_l \) is the proton conductance by ATP synthase related to ATP synthesis. It is noted that the leak conductance \( g_l \) in \( g \) is corrected in the description of decay rate.
of the transmembrane voltage (Groth and Junge, 1993), so here we neglect it in formula. The voltage decay then is calculated by

\[
\frac{dU}{dt} = \frac{U}{\tau} (g_\text{s} + g_\text{A})
\]  

(3)

Here the voltage \( U \) and the specific capacitance \( c \) is constant in the experimental circumstance. It is easily understood that \( g_\text{s} \) is directly proportional to the conducted protons related to slip in unit time, and \( g_\text{A} \) is directly proportional to the conducted protons related to ATP synthesis in unit time.

2.2. Monte Carlo simulations

Based on the kinetic pathways (Fig. 1), we let the model system evolve according to the Monte Carlo algorithm. The Monte Carlo method is a powerful one to simulate the kinetic cycle of a molecular motor (Dimroth et al., 1999; Stahlberg et al., 2001; Singh et al., 2005; Qian et al., 2009), because state transitions of motor are always stochastic. In order to simulate the kinetic cooperativity in three subunits, different kinetic parameters for Subunits 1, 2, and 3 were adopted, as shown in Table 1. The ADP-binding rate \((k_\text{b}^{\text{ADP}})/[\text{ADP}]\), Pi-binding rate \((k_\text{b}^{\text{Pi}})/[\text{Pi}]\), and the ATP-release rate \((k_\text{A}^{\text{Pi}})/[\text{Pi}]\) in Subunit 2 are higher than the other two subunits. When Subunit 1 is unfilled, the ATP-synthesis rate \((k_\text{A}^{\text{Pi}})\) in Subunit 2 is low. When Subunit 1 is filled by ADP and Pi, the ATP-synthesis rate \((k_\text{A}^{\text{Pi}})\) in Subunit 2 increases by \(N_1(=1000)\) times. When Subunit 3 is filled by nucleotides, the ATP-release rate \((k_\text{A}^{\text{Pi}})\) in Subunit 2 increases by \(N_2(=2)\) times. In simulation, when all three subunits are unoccupied, the time of proton slip (which is proportional to the slip protons) in unit time is counted as \(G_\text{s}\): the synthesized ATP number in unit time is counted as \(G_\text{A}\). The proton conductance \(g_\text{s} = aG_\text{s}\), and \(g_\text{A} = bG_\text{A}\) where \(a\) and \(b\) are the constants determined by comparison between the initial experimental results and the simulation results. According to Eq. (3), the relative decay rate was calculated by \(aG_\text{s} + bG_\text{A}\). Each datum of our results is calculated from our simulation with the duration of 50 s.

3. Results and discussion

In Fig. 3, we show our simulation results for the decay rate of the transmembrane voltage as a function of ADP and Pi. The simulated results agree with the experimental results. The simulated results of the decay rate versus the [ADP] with [Pi] = 0.5 mM are shown in Fig. 3A. As Fig. 3A shows, the decay rate decreases with [ADP] when [ADP] is very small. In this case, proton slip is blocked by the binding of ADP to empty subunit. When [ADP] is larger than 1 \(\mu\)M, the decay rate increases with [ADP] because of the proton conduction coupled to ATP synthesis. In Fig. 3C, we show the results for the dependence of decay rate on [ADP] without Pi. It is seen that ADP blocks proton slip even in the absence of phosphate, but the value of [ADP] that entirely blocks the proton slip is larger by almost 50 times than that in Fig. 3A, which can be explained as follows. From the kinetic pathway of Fig. 1, we can see that the binding of Pi converts the state ADP to state ADP-Pi then reduces the transition probability from state ADP back to state Empty. Therefore, compared with the case in the presence of phosphate, the relative affinity of ADP to subunit 1 without Pi is decreased. Fig. 3B shows the results of decay rate versus [Pi] with [ADP] = 1 \(\mu\)M. It is evident that that phosphate is required to diminish proton slip as it is required for coupled proton flow.

Recent experiment by Tomashek et al. (2004) indicated that ATP synthesis is not a simply bi-site activation mechanism. Their experimental results showed that occupation of the third site further activates the rate of synthesis. It was found that the rate of synthesis does not obey the Michaelis–Menten kinetics with respect to ADP. In our model, the activation for synthesis due to the occu-

Fig. 3. Dependences of decay rates of the transmembrane voltage on [ADP] and [Pi]. (A) Simulated results (filled squares) of decay rates versus [ADP] with [Pi] = 0.5 mM. In calculations of decay rates, \(a = 0.4, b = 0.22\). (B) Simulated results (filled squares) of decay rates versus [Pi] with [ADP] = 1 \(\mu\)M. In calculations of decay rates, \(a = 1.3, b = 0.22\). (C) Simulated results (filled squares) of decay rates versus [ADP] without Pi. In calculations of decay rates, \(a = 0.4\). The unfilled symbols are the corresponding experimental data taken from Groth and Junge (1993).

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pation of the third site is adjusted by parameter \( N_2 \) in simulations. In Fig. 4, we show the simulated results of partial experimental data by Tomaszek et al. (2004). It is seen that, the rate of synthesis obey the Michaelis–Menten kinetics with respect to ADP when \( N_2 = 1 \), which means that occupation of the third site does not activate the rate of synthesis; the simulated results agree well with the experiment when \( N_2 = 2.8 \), which means that occupation of the third site increases the rate of synthesis (i.e. ATP-release rate \( k_4 \)) by 2.8 times. The parameters for simulation of the experiment are listed in Table 2. Different rates of ADP binding are used in three subunits in order to simulate the activation for site 2 due to the occupations of sites 1 and 3.

According to above discussions, our model does not support the tri-site mechanism of ATP synthesis, which means that all three sites must be filled in rapid ATP synthesis. If the tri-site mechanism operates, the activation for synthesis due to the occupation of the third site cannot be observed. Furthermore, our simulation can also rule out the possibility of uni-site mechanism for ATP synthesis, which means that rapid ATP synthesis can occur in one site in spite of the occupation of the other two sites. If we set the ATP-synthesis rate \( (k_3) \) in site 2 as high as \( 5^*N_5 s^{-1} \) whether or not the other two sites are filled, the simulated results of decay rates versus [ADP] are shown in Fig. 5. The simulated results do not agree with the experiment. As shown in Fig. 5, without the activation of the second site for ATP synthesis, the decay rate does not decrease in small

![Fig. 4](image1.png)

**Fig. 4.** Simulated results (filled symbols) of the rate of synthesis versus [ADP] with \( N_2 = 2.8, 1.8, 1.0 \) (squares, triangles, and circles, respectively). It is noted that the value of 0.5 μM/min/mg of membrane protein translates into a turnover number at room temperature of 160 s\(^{-1}\) according to the experiment. The unfilled squares are the corresponding experimental data taken from Tomaszek et al. (2004).

![Fig. 5](image2.png)

**Fig. 5.** Simulated results (filled squares) of decay rates of the transmembrane voltage versus [ADP] with [Pi]=0.5 mM based on uni-site mechanism. In calculations of decay rates, \( a=0.4, b=0.17 \). The unfilled triangles and circles are the corresponding simulated results of \( k_{G_c} \) and \( k_{G_s} \).

\[ [\text{ADP}] \], however, it monotonously increases over concentrations of \[ \text{ADP} \].

In the work of Groth and Junge (1993), the transition from closed (proton slip) to coupled (ATP synthesis) state of the ATP synthase was investigated as a function of the concentration of nucleotides and phosphate on the one hand and as a function of the proton motive force on the other. Both components of the proton motive force, the electrical potential difference \( \Delta \psi \) and the pH difference \( \Delta pH \), seemed to be equivalent in the experiment, while Kaim et al. (2002) found that no c ring rotation during ATP synthesis was observed in the presence of ion gradient only (without membrane potential). In our simulations, we tracked the proton flow and calculated the contribution of protons to membrane conductance without distinguishing the difference of \( \Delta \psi \) and \( \Delta pH \).

4. Conclusion

Based on the adapted bi-site mechanism proposed by Boyer, we numerically simulated the proton translocation from proton slip to coupled proton flow in ATP synthase. Moreover, the activation of ATP synthesis due to the occupation of the third site is also considered in our model. The simulation results agree well with the experimental results by Groth and Junge (1993). It is interesting to study the kinetic cooperativity in three catalysis subunits in ATP synthase through the proton flow experiment. Our simulations can rule out the possibility of uni-site mechanism in ATP synthesis, and support the bi-site mechanism in ATP synthesis. The theoreti-

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<th>Table 1</th>
<th>Kinetic parameters used in the simulations of the decay rate as a function of ADP and Pi (Fig. 3).</th>
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<td>( k_a^{\text{(ADP)}} (\text{M}^{-1} \text{s}^{-1}) )</td>
</tr>
<tr>
<td>Subunit 1</td>
<td>0.4 × 10(^4)</td>
</tr>
<tr>
<td>Subunit 2</td>
<td>1 × 10(^5)</td>
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<tr>
<td>Subunit 3</td>
<td>0.5 × 10(^5)</td>
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<th>Table 2</th>
<th>Kinetic parameters used in the simulations of the rate of synthesis versus [ADP] (Fig. 4).</th>
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<tbody>
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<td></td>
<td>( k_a^{\text{(ADP)}} (\text{M}^{-1} \text{s}^{-1}) )</td>
</tr>
<tr>
<td>Subunit 1</td>
<td>0.48 × 10(^8)</td>
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<tr>
<td>Subunit 2</td>
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<tr>
<td>Subunit 3</td>
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catical method presented here can be easily applied to studying other experimental results of ATP synthase, such as the results of single molecule and biochemical experiments in ATP hydrolysis.

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