Complex cooperativity of ATP hydrolysis in the F₁-ATPase molecular motor

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Abstract:

F₁-ATPase catalyses ATP hydrolysis and converts the cellular chemical energy into mechanical rotation. The hydrolysis reaction in F₁-ATPase does not follow the widely believed Michaelis-Menten mechanism. Instead, the hydrolysis mechanism behaves in an ATP-dependent manner. We develop a model for enzyme kinetics and hydrolysis cooperativity of F₁-ATPase which involves the binding-state changes to the coupling catalytic reactions. The quantitative analysis and modeling suggest the existence of complex cooperative hydrolysis between three different catalysis sites of F₁-ATPase. This complexity may be taken into account to resolve the arguments on the binding-change mechanism in F₁-ATPase.

Keywords: F₁-ATPase, molecular motors, ATP hydrolysis, binding cooperativity, chemomechanics.
1. Introduction

F1F0-ATPase is an enzyme complex that is vital to cellular energy conversion. It works as a dual-domain molecular motor powered by two types of driving forces [1-4]. The off-membrane F1 domain synthesizes adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi) driven by a proton-motive force from the in-membrane F0 domain. Alternatively, when operating in the reverse, it hydrolyzes ATP into ADP and Pi at F1 and releases energy. In both cases, coupling of the transduction of chemical energy from catalysis reactions with the conformational changes generates rotary mechanical torque at F1. Isolated F1-ATPase can also work independently as a rotating motor fueled by ATP hydrolysis [4]. Cloned F1-ATPases have been explored as biologically powered nano-machines because they work at an exceptionally high chemomechanical coefficient [5-9].

The functionally stable F1-ATPase is composed of subunits designated as α, β, γ, δ, and ε with a stoichiometry of 3:3:1:1:1 [10-12]. The α and β subunits alternate in an hexagonal arrangement around a central cavity containing the N- or C-terminal helices of γ, δ and ε subunits, as illustrated in Figure 1. Three alternative sites on the hexamer formed by subunits (αβ)3 are found to be catalytically active and responsible for ATP hydrolysis/synthesis [1,2,10,11]. These catalytic sites are located on the β subunits at the interfaces with the α subunits. The crystal structures of three (αβ) pairs are almost identical and have a strong symmetry, but the incorporation of the central γδε subunits creates a structural and functional asymmetry between the three catalytic sites [1,2,4,10,11].

Long before the molecular level structures and binding-states were determined, Boyer proposed a ‘bi-site’ binding change mechanism [13] for rapid hydrolysis of F1F0-ATPase based on its kinetics. As shown in Figure 1(a), three (αβ)3 catalysis sites are in different conformations at any given time, namely they bind with ATP, or ADP and/or Pi, or they are empty. The existence of alternative conformations depends on their respective positions relative to the concave, neutral, or convex sides of the central shaft γ subunit. The catalytic sites at three (αβ) pairs work in a sequential collaboration – while one site tightly binds ATP and undergoes hydrolysis, the next one loosely binds the hydrolyzed
ADP, and the third one opens to release the hydrolysis products and intake ATP. The collaborative conformational changes in \((\alpha\beta)_3\) induce a torque between the hexamer \((\alpha\beta)_3\) and the central stalk \(\gamma\) subunit, causing the F_1-ATPase motor to rotate [1-2]. Atomistic structural investigations [10,11], along with motor experiments of F_1-ATPase (especially the visualized rotation by Noji et al. [5,6]), confirmed Boyer’s mechanism. Subsequently other issues were raised concerning the multisite binding-change scheme and the cooperativity of hydrolysis reactions at multiple catalytic sites [12,14,15,30,31]. For example, the issue of whether or not a bi-site mechanism is universally valid remains unresolved [12,14]. Contrary to the belief of many researchers, the kinetics of a rotating F_1-ATPase motor does not follow Michaelis-Menten kinetics [6,7]. Considerable efforts have been reported to resolve these issues and to reveal the rotating chemomechanic nature of F_1-ATPase. These works include sophisticated single molecule measurements [6,7,9,16-18], chemomechanic modeling [19-23] and atomistic simulation using molecular dynamics methods [24-27]. However, the dynamic and interactive cooperativity at different ATP catalysis sites has not been convincingly demonstrated. The molecular coupling mechanism of ATP hydrolysis in F_1-ATPase is still beyond fully understanding.

Previously, we proposed a model of the F_1-ATPase motor based on enzyme kinetics and rotary Langevin dynamics [23]. In the model, the energy transduction and stepwise rotation of F_1-ATPase were regulated by a series of near-equilibrium reactions when nucleotides bind or unbind. For the case of F_1-ATPase driving an actin filament, the theoretical load-rotation profiles were analyzed against experiment and gave good agreement. The link from the molecular-scale hydrolysis reactions to the micro-scale mechanical rotation of F_1-ATPase was established without considering the possible effect from the non-Boyer schemes. In this work, we quantitatively analyze the complex cooperativity of ATP hydrolysis and describe non-Boyer chemomechanics of the F_1-ATPase molecular motor.

2. Kinetic model of hydrolysis cooperativity in F_1-ATPase

The binding conformations of the multiple catalysis sites of F_1-ATPase are identified as \(\alpha\beta_{TB}, \alpha\beta_{LB}\), and \(\alpha\beta_{O}\) [1,2,10-12] (Figure 1). The open (O) site has a very low affinity for
substrates of ATP, ADP or Pi and is catalytically inactive, whereas the other sites involve either a loosely bound (LB) substrate ADP or a tightly bound (TB) substrate ATP. The actual order of unbinding and release of ADP and Pi were proved to be Pi first, followed by ADP [4,17]. Therefore a complete enzymatic cycle of ATP hydrolysis in F1-ATPase occurs via the following pathway,

\[
\begin{align*}
\text{MM}^O & \xrightleftharpoons[k_{+\text{ATP}}]{k_{-\text{ATP}}} \text{MM}^{TB}.\text{ATP} \xrightleftharpoons[k_{\text{hyd}}]{k_{\text{syn}}} \text{MM}^{TB}.\text{ADP}.\text{Pi} \xrightleftharpoons[k_{-\text{Pi}}]{k_{+\text{ADP}}} \text{MM}^{TB}.\text{ADP} \xrightleftharpoons[k_{-\text{ADP}}]{k_{+\text{ADP}}} \text{MM}^O \\
\text{ATP} & \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \q
Here $[\text{ATP}]$, $[\text{ADP}]$ and $[\text{Pi}]$ are the concentrations of ATP, ADP and Pi respectively. $P_O$, $P_{\text{ATP}}$, $P_{\text{ADP,Pi}}$ and $P_{\text{ADP}}$ are the probability of the states when different pairs of $(\alpha\beta)_3$ are either empty or occupied by ATP, ADP, Pi or ADP molecules, respectively (as illustrated in Figure 1). Here we assume that the F$_1$-ATPase motor is under physiological conditions and ATP, ADP and Pi molecules are fully dissolved in solution. Eq. (2) is the steady-state cycle equivalent of Eq. (1), which is justified by the fact that the alternating binding states and the chemical reaction steps are repeatedly coupled. It should be noted that metal ions, in particular Mg$^{2+}$, have a crucial role in ATPase enzymatic activity for a physiological catalysis. The different concentration of Mg$^{2+}$ does affect the kinetics of F$_1$-ATPase by coordinating ATP catalysis [37]. However, since Mg$^{2+}$ changes the rate constants but does not alter the catalysis pathways, the present kinetic model naturally includes any possible effect of Mg$^{2+}$ via the rate constants.

The steady-state condition of Eq. (1) gives the overall reaction rate of ATP hydrolysis at given physiological conditions (such as, of given $[\text{ATP}]$, $[\text{ADP}]$ and $[\text{Pi}]$) by,

$$R = k_{\text{hyd}}P_{\text{ATP}} - k_{\text{syn}}P_{\text{ADP,Pi}}$$

For F$_1$-ATPase following Boyer’s mechanism, this leads to [23],

$$R = \frac{(-k_{-\text{ATP}}k_{\text{syn}}k_{\text{ADP}}[\text{ADP}][\text{Pi}]+k_{-\text{ADP}}k_{-\text{Pi}}k_{\text{hyd}}k_{\text{ATP}}[\text{ATP}])}{(k_{-\text{ADP}}k_{-\text{Pi}}k_{\text{hyd}}+(k_{\text{hyd}}k_{-\text{Pi}}+k_{-\text{ATP}}k_{-\text{Pi}}+k_{-\text{ATP}}k_{\text{syn}}+(k_{-\text{ATP}}+k_{\text{syn}}+k_{\text{ADP}})k_{\text{Pi}})k_{\text{ADP}}[\text{ADP}]+k_{\text{hyd}}(k_{-\text{ADP}}+k_{-\text{Pi}}+k_{\text{Pi}})k_{\text{ATP}}[\text{ATP}]+(k_{-\text{ADP}}k_{-\text{Pi}}+k_{-\text{ADP}}k_{\text{syn}}+k_{\text{ADP}}k_{\text{Pi}}[\text{Pi}])k_{\text{ADP}}[\text{ATP}])}

However, the symmetric $(\alpha\beta)_3$ structures and nearly identical catalysis kinetics can not guarantee that the catalysis at three sites takes place in a highly ordered fashion. To check the possibility of complex cooperativity between three catalytic sites of F$_1$-ATPase, we may carefully investigate the cooperativity from its enzyme kinetics or motor chemomechanics. From a kinetics point of view, the enzymatic cooperativity may be quantitatively measured by a modified Hill Equation [28],

$$\log\left(\frac{R}{(R_{\text{max}} - R)}\right) = h \log[\text{ATP}] - \log K$$
where $R_{\text{max}}$ is the saturation rate (or motor speed if applicable) in F$_1$-ATPase, $K$ is a constant and $h$ is the Hill number. When $h = 1$, there is no cooperative interaction between different catalytic sites; only one ATP molecule is hydrolyzed at any time and the kinetics follows the Michaelis-Menten mechanism, i.e. $R = R_{\text{max}}[\text{ATP}]/(K_m + [\text{ATP}])$ with $K_m$ the Michaelis constant. When $h > 1$, there is positive cooperative hydrolysis between different sites and more than one ATP molecule is simultaneously undergoing catalysis reactions. Values of $h < 1$ indicate negative cooperative behavior where there might be some inhibition between the multi catalysis sites.

The calculations involving Eq. (2) to Eq. (4) were programmed using the Mathematica library [36] and executed on a standard pentium-4 PC or Unix workstation. The overall computing time is of the order of CPU minutes. This represents a considerable advantage over time-costly atomistic simulation [24-27].

3. Results and discussions

In Boyer’s ‘bi-site’ activation (Figure 1(a)), three catalysis sites are in different reaction stages (binding states), and on average only one catalytic site carries out a specific hydrolysis reaction step at any one time. Therefore in Hill’s plot, bi-site catalysis should give $h = 1$. There is currently little evidence that the chemical reaction steps in ATP synthase take place on average at three different states at a time. On the contrary, solid evidence from chloroplast [29], *E. Coli* [30,31], and bovine mitochondrial F$_1$-ATPase [12] suggests that two or more sites may be active simultaneously, as schematized in Figure 1(b). For example, in chloroplast F$_1$-ATPase [29], binding states of an ADP analog to cooperating sites depends on nucleotide concentration. Due to very strong cooperativity, two of the three sites were found simultaneously to be in the tightly bound state. In addition, both the two $K_m$ constants fit to the reaction rate [6,7] and our simulation (Figure 4 of ref. [23]) of the mechanochemistry of *Bacillus PS3* F$_1$-ATPase implied that more than one ATP molecule are needed at a time for the steady operation of the F$_1$-ATPase motor. These considerations strongly suggest that a non-Boyer mechanism does occur in F$_1$-ATPase.
To determine the difference between Boyer-like and non-Boyer-like cooperative hydrolysis in F$_1$-ATPase, we firstly re-analyze the motor dynamics of *Bacillus PS3* F$_1$-ATPase [6,7] for possible enzymatic cooperativity schemes. The kinetic simulations from both Boyer-like and non-Boyer-like mechanisms are performed to interpret the complex cooperativity. We also calculate the occupation probability of different binding states. This calculation may help to determine the bi-site or tri-site catalysis mechanism for F$_1$-ATPase operating in different [ATP] regions.

### 3.1 Complex cooperativity of ATP hydrolysis for multiple catalysis sites of F$_1$-ATPase

Figure 2 shows the Hill plots, $\log(R/(R_{\text{max}}-R))$ versus $\log[\text{ATP}]$, of ATP hydrolysis rate for the F$_1$-ATPase motor. The experimental results (diamonds [6,7]) are re-produced in the Hill manner. Both the experiments and our simulations of strong cooperativity (solid line) indicate that ATP hydrolysis in F$_1$-ATPase does not simply follow the Michaelis-Menten mechanism, which should be parallel to the dot-dashed line (i.e., $h = 1$). There is different cooperativity of ATP hydrolysis from multiple catalysis sites occurring across the whole [ATP] region.

From the Hill plot (Figure 2) of the experimental data, the cooperativity of hydrolysis reactions in F$_1$-ATPase might be classified into three concentration regions. Region 1, for the lower region ([ATP] = nM ~ 10 µM), F$_1$ obeys Michaelis-Menten kinetics and there are no cooperative hydrolysis reactions. Region 2 is in the intermediate pre-saturated region ([ATP] = 10 µM ~ 100 µM), and there is a negative cooperativity. Region 3 is in the saturated region ([ATP] > 100 µM), and there is a strong cooperativity between the hydrolysis reactions from different sites. In Region 1, a Boyer scheme simulation (the crossed line, with a full set of rate constants from Panke et al. [21]) is sufficient if we take the binding rate of ATP as determined by experiment [6,7]. However, from the pre-saturated Region 2 to the saturated Region 3, a complex cooperativity of ATP hydrolysis is apparent.

To perform the simulation, we applied the hypothesis that the number of multiple catalysis sites is unknown except that their pathway of hydrolysis reactions is known as Eq. (1). These catalytic sites are also assumed to have the same rate of association and dissociation of nucleotides of ATP, ADP.Pi or ADP. When three ATPs are hydrolyzed
simultaneously in the same pathway at three catalysis sites, the solid line in Figure 2 gives a much better fit to the experimental data (in Region 3). This indicates that strong cooperativity of ATP hydrolysis is the case for the F\textsubscript{1}-ATPase motor, at least for the saturated [ATP] region. Region 2 seems to have a transition from bi-site to tri-site catalysis. A chemomechanical and molecular structural mechanism for this transition remains unknown.

For comparison, Figure 3 illustrates the simulations of the overall hydrolysis reaction rates of F\textsubscript{1}-ATPase versus [ATP] for different schemes of hydrolysis. For Region 3 ([ATP] > 100 µM), the simulation of ATP hydrolysis with strong ‘tri-site’ cooperativity (solid lines in Figures 2 and 3) agrees much better with experimental data for the \textit{Bacillus PS3} F\textsubscript{1}-ATPase motor (diamonds [6,7]), compared to the bi-site catalysis simulations (dash and crossed lines in Figures 2 and 3). It is interesting to note that complex cooperativity of ATP hydrolysis does not only exist in F\textsubscript{1}-ATPase, but also occurs in other ATP-fueled multi-domain molecular motors, such as myosin (Liu, M.S., Todd, B.D. and Sadus, R.J., unpublished data). This appears to be a common chemomechanical character for the ATP-fueled molecular motors.

3.2 Bi-site vs tri-site hydrolysis for F\textsubscript{1}-ATPase

Strong cooperativity of ATP hydrolysis in F\textsubscript{1}-ATPase will require ‘tri-site’ catalysis, such as depicted by a possible scheme in Figure 1(b). In a tri-site mechanism, ATP hydrolysis probably plays a dominant role over ATP binding in producing rotary torque, and the bound nucleotide persists on the enzyme through more than one turnover for a subunit rotation step. An additional fifth conformation might have to be considered beyond the conformations in Eq. (1). This would most likely be a loosely-bounded site with enhanced affinity for Pi plus ADP and concomitantly lowered affinity for tightly bound ATP or ADP.Pi. The net result would favor Pi + ADP binding rather than ADP binding (to an empty site). This new scheme might help to explain the half-open ADP-bound structure [12] and the sub-steps of rotation of the F\textsubscript{1}-ATPase motor [7]. Nevertheless, such a mechanism may lead to incompatible chemomechanics with some motor experiments [32], in which the same ‘bi-site’ mechanism is believed to prevail throughout the whole [ATP] region [6,7]. Boyer argued [32] that the third site occupation...
during high [ATP] hydrolysis can be explained by rebinding or retention of ADP. He emphasized that bi-site activation manifests the characteristics of only one catalytic pathway in F1-ATPase [32]. However, even if only one type of catalytic site exists at any one time, it does not exclude the possibility of two or more sites simultaneously carrying out the same reaction pathway. The question is how are the hydrolysis reactions of F1-ATPase coupled to changes from uni-site, bi-site or tri-site binding-states? The answer is currently hindered by the absence of direct determination of the binding states and their changes during steady hydrolysis [14].

It is biochemically straightforward for F1-ATPase to undergo both unisite catalysis and multisite catalysis. When [ATP] is in sub-stoichiometric quantity, it binds to the first site with very high affinity. As this ATP is hydrolyzed to ADP + Pi, the products are released slowly (k_off <10^{-3} s^{-1}). The reversible hydrolysis/synthesis reaction occurs with an equilibrium constant close to 1, and F1 undertakes a unisite catalysis. Multisite catalysis occurs when there is high enough [ATP] and ATP begins to bind to the next site. Therefore, we need to re-examine the above question for F1-ATPase, namely, whether the binding is ‘bi-site’ or ‘tri-site’, and how do the binding-state changes couple with rapid hydrolysis. For the distribution of occupancy of catalysis sites in E. Coli F1-ATPase, Senior and coworkers [14,18,30,31] found that at lower [ATP] (< 1 \mu M), when primarily the high affinity site was occupied, the equilibrium reaction is a unisite catalysis. At high [ATP] (>>1 \mu M), all three catalysis sites are occupied and filled with ATP or ADP and tri-site hydrolysis takes place. The probability of an empty site is rare. Thus Senior concluded that a ‘bi-site’ mechanism does not exist [31].

Our theory permits one to calculate the occupation probability of different binding states during steady ATP hydrolysis. The simulations, as shown in Figure 4, indicate that the binding state distribution changes dramatically as a function of [ATP] before the hydrolysis is saturated. In other words, the hydrolysis mechanism is [ATP] dependent. Figure 4 shows the probability of binding state versus [ATP] for any single catalysis cycle at a given site of chloroplasts F1-ATPase (when three sites follow the same kinetics pathway of Eq. (1)). Even if no cooperativity between three catalysis sites occurs, Boyer’s bi-site scheme (where the probability of \( P_{\text{open}} = P_{\text{ADP}} = P_{\text{ATP+P_{ADP,Pi}}} = 1/3 \)) is only valid for a limited region. It is approximately at [ATP] of 100 \mu M ~ 200 \mu M for
the chloroplasts F$_1$-ATPase motor, which is the pre-saturated region. For higher [ATP], F$_1$-ATPase undergoes saturated rapid hydrolysis and the probability of a site being empty is small (10% or less at saturated region). In this case, $P_{ADP} \cong 2/3$ and $P_{ATP^+P_{ADP}.Pi} \cong 1/3$ and all three catalysis sites are in bounded states. This is consistent with the experimental observation [30,31], and in this concentration region F$_1$-ATPase undertakes tri-site hydrolysis.

4. Conclusion

For ATP hydrolysis of the F$_1$-ATPase motor, our study and the comparison with experimental data indicate that there is: 1) a complex cooperativity between three catalytic sites for rapid ATP hydrolysis; 2) different rate constants of association, $k_{+ATP}$, might exist (at least for very low [ATP]); and 3) the binding-change mechanism is [ATP] dependent, changing from uni-site for lower [ATP], to bi-site for pre-saturated [ATP] and to tri-site at high [ATP]. The complexity of cooperative ATP catalysis is at present difficult to be revealed experimentally (mainly limited by the lack of suitable technology), given that ATP reactions at different catalysis sites are affected strongly by dynamic changes of binding affinity and constant fluctuation in a confined space. The complex cooperativity in F$_1$-ATPase is expected to be resolved with more comprehensive atomistic structural investigations and single molecular measurements, such as to probe disordered kinetics and enzymatic dynamics at the single molecular level [35].

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Figure captions:

Figure 1. Conceptual schemes of the time-averaged binding states occupation during steady hydrolysis of F₁-ATPase. (a) the ‘bi-site’ activation proposed by Boyer, and (b) one possible scheme of non-Boyer ‘tri-site’ activations. \((a\beta_T) (a\beta_L)\) and \((a\beta_O)\) refer to the conformational tight-binding, loose-binding, and open states of \((a\beta)\) pairs, respectively.

Figure 2. The Hill plots of ATP hydrolysis rate, \(\log(R/(R_{max}-R))\), versus \(\log[ATP]\) show non-Michaelis-Menten kinetics in F₁-ATPase. The diamonds refer to the experimental results [6,7]. The dashed and crossed lines are simulations according to Boyer’s bi-site scheme. The solid line indicates simulation when strong cooperativity of ATP hydrolysis occurs (three ATPs are hydrolyzed simultaneously). The rate constants are the same as in Figure 3.

Figure 3. Overall hydrolysis reaction rate, \(R\), of F₁-ATPase versus ATP concentration. The dashed line is the simulated hydrolysis rate according to Boyer’s bi-site scheme [23] with \(k_{ATP} = 2.08 \times 10^6\) M\(^{-1}\)s\(^{-1}\), \(k_{ADP} = 8.90 \times 10^6\) M\(^{-1}\)s\(^{-1}\), \(k_{Pi} = 8.10 \times 10^5\) M\(^{-1}\)s\(^{-1}\), \(k_{-ATP} = 2.70 \times 10^2\) s\(^{-1}\), \(k_{-ADP} = 4.90 \times 10^2\) s\(^{-1}\), \(k_{-Pi} = 2.03 \times 10^3\) s\(^{-1}\), and \(k_{hyd} = 4.5 \times 10^5\) s\(^{-1}\), \(k_{syn} = 1.15 \times 10^3\) s\(^{-1}\) (from Panke et al. [21]). The crossed line is again the bi-site simulation with a determined association rate [7] of ATP, \(k_{ATP} = 3.0 \times 10^7\) M\(^{-1}\)s\(^{-1}\). The solid line is the simulation for the case of strong cooperativity between three catalysis sites, and the diamonds refer to experimental data [6,7]. ([ADP] and [Pi] are set at cellular physiological condition of 10 µM and 1.0 mM, respectively).

Figure 4. Occupation probability of binding-state changes versus [ATP]. ATP-dependent binding states imply complex cooperativity of ATP hydrolysis for three different catalysis sites. Boyer’s bi-site catalysis scheme only holds for a limited [ATP] region, around 150 µM ~ 250 µM in this case. The rate constants are the same as in Figure 3 ([ADP] and [Pi] are consistently set as 10 µM and 1.0 mM, respectively).
Liu et al., Figure 1
Liu et al., Figure 2

R/(R_{\text{max}} - R) vs. ATP concentration (\mu M)

h = 1
Liu et al., Figure 3
Liu et al., Figure 4

![Graph showing occupation probability of binding-state as a function of ATP concentration.](attachment:image.png)