F₀F₁-ATPase STATOR REGULATION STUDIED WITH A RESONANCE MODEL

Yao-Gen Shu and Zhong-Can Ou-Yang

Institute of Theoretical Physics, Chinese Academy of Sciences, Beijing 100190, China

Keywords: Molecular motor, F₀F₁-ATPase, Stator regulation, Binding change mechanism, Resonance model.

Abstract: The F₀F₁-ATPase activity was regulated through external links on the exposed stator. The regulation tendency of synthesis was the same with that of hydrolysis. A resonance model has been proposed to illustrate these regulation phenomena. The novel model not only has deepened our understanding of the "binding change mechanism", but also was very useful to develop the rotary motor into a biosensor.

1 INTRODUCTION

F₀F₁-ATPase is the ubiquitous rotary motor that uses the transmembrane electrochemical potential to synthesize ATP in bacteria, chloroplasts and mitochondria. The holoenzyme is a complex of two rotary motors, F₀ and F₁, which are mechanically coupled by a common central stalk ("rotor"). c₃-e-γ. The membrane embedded F₁ unit converts the proton motive force (p.m.f) into mechanical rotation of the "rotor", thereby causing cyclic conformational change of α₃β₃ crown ("stator") in F₁ and driving ATP synthesis. A striking characteristic of this motor is its reversibility. It may rotate in the reverse direction for ATP hydrolysis and utilize the excess energy to pump protons across the membrane. In F₁ motor, the eccentric rotation of γ subunit and the cyclic conformational change of α₃β₃ crown, are mechanically coupled at near 100% efficiency (Boyer, 1997; Leslie and Walker, 2000; Weber and Senior, 2003; Kinosita et al., 2004; Junge, 2004; Wächter et al., 2011).

In the proof-of-principle demonstrations, the holoenzyme activity was regulated by changing of the transmembrane gradient of proton (Panke and Rumberg, 1996; Turina et al., 2003; Diez et al., 2004; Steigmiller et al., 2008). Moreover, the F₁ activity was studied in detail through an actin filament or a bead on the "rotor" (Yasuda et al., 1998; Itoh et al., 2004). On the other hand, several pioneering investigations (Boyer, 1993; Abrahams et al., 1994; Noji et al., 1997; Nishizaka et al., 2004) have revealed that the cyclic conformational change of α₃γβ₃ crown "stator" is tightly coupled with the rotation of γ "rotor". A fascinating question arises: can we regulate the holoenzyme activity through a convenient method? Yue Group’s recent experiments (Cheng et al., 2010) about virus detection have demonstrated that the holoenzyme activity can be regulated by the external links on the exposed α (or β) subunits. The external links not only inhibit the enzyme but also activate it, and the regulation tendency of synthesis is the same with that of hydrolysis. However, what is the implied mechanism?

It is impossible for the two movements to transfer energy at near 100% efficiency (Yasuda et al., 1998) unless they work in a resonance fashion through an elastic element. Actually, Boyer’s "binding change mechanism" (Boyer, 1993), Walker’s structure (Abrahams et al., 1994) and Oster’s hinge bending model (Wang and Oster, 1998) have partly implied that F₁-ATPase works in a fashion of driven oscillation (Kargovsky et al., 2009; Wächter et al., 2011). The driving force is supplied by the eccentric rotation of γ subunit for synthase or by the binding of ATP for hydrodase, whereas the passive oscillation is the cyclic conformational change of α₃β₃ crown, whose frequency is the driving frequency. If we regard α or β subunit as a harmonic oscillator, α and β subunits will vibrate at its natural frequency. Once the driving frequency matches the natural frequency, resonance occurs. At resonance frequency, the amplitude of steady state response is the biggest in proportion to the amount of driving force, which is the most convenient for binding of substrates and releasing of products. The driving force is essentially just pumping energy back into the system to compensate for the loss.
of energy due to ATP synthesis or protons pumping as well as damping, which is the most efficient way for motor to work. The rotary speed of motor, which corresponds to the measurable holoenzyme synthesis activity, is equal to the resonance frequency of “stator”.

2 EXPERIMENTAL RESULTS

A series of harmonic oscillators have been designed to link to the exposed \( \alpha \) subunits. The schematic illustration of this activity regulation assay is shown in Fig.1. \( F_oF_1 \)-ATPase is embedded in a chromatophore, in which the transmembrane p.m.f is stored. The first external harmonic oscillator is the \( \alpha \) antibody, the second one is the streptavidin, the third one is H9 avian influenza virus (hereafter called H9 virus) antibody and the fourth one is the H9 virus.

The holoenzyme activity with different links has been measured as follows: Firstly, each \( \alpha \) subunit was bound by an \( \alpha \) antibody, the holoenzyme was inhibited, and the relative synthesis and hydrolysis activity decreased to 0.71 and 0.84 respectively. Secondly, the streptavidin was linked to \( \alpha \) antibody through a small biotin, the enzyme was inhibited continuously, and the relative activities of synthesis and hydrolysis declined to 0.60 and 0.73 respectively. Thirdly, H9 antibody was linked to streptavidin through biotin, the holoenzyme was inhibited again, and the relative activities of synthesis and hydrolysis dropped to 0.51 and 0.61 respectively. However, when H9 virus bound to its antibody finally, the modified enzyme was activated, and its relative activities jumped to 0.90 and 0.93 respectively. It is very amazing that the activity of the modified holoenzyme, in which a series of external proteins and virus are linking on “stator”, can resume to a native level. These results are correspondingly indicated in Fig.2 No. 2, 3, 4 and 5. The activity of No. 5 is about two times that of No. 4, which means that the No. 4 complex is a sensitive biosensor of virus detecting.

To identify whether H9 virus affects \( F_oF_1 \)-ATPase activity directly, we measured the activity of native buffer incubated with H9 virus. The result shown in Fig.2 No.6 has proved that the H9 virus doesn’t have any direct effect on the holoenzyme activity no matter the enzyme functions as a synthase or hydrolase.

\( F_1 \) hydrolysis activity can be expectably regulated by varying the load on the “rotor” (Yasuda et al., 1998). With increasing of the length of filament attached on the “rotor”, the damping force will increase, thereby inhibiting the \( F_1 \) hydrolysis activity, which has been approvingly interpreted by traditional models (Sun et al., 2004; Xing et al., 2005; Jonathan et al., 2006). Our experiment has revealed that the holoenzyme activity also can be regulated by the external links on the “stator”. The regulation tendency of synthesis is the same with that of hydrolysis. However, it is very difficulty for us to understand this regulation mechanism using traditional model. Here, we introduce the resonance mechanism to statistically illustrate these phenomena.
3 RESONANCE MODEL

The “stator” of F1 motor is a crown with alternating α and β subunits. Each catalytic site located in β subunit is at different stage of the enzyme kinetic cycle: one binds ATP, another binds ADP, and the third keeps empty. All three sites coordinate for the next 120° step. The different stage of catalytic site is tightly coupled to the different conformation of β and α subunit(Nishizaka et al., 2004; Diez et al., 2004; Itoh et al., 2004). We define the cyclic conformational change of αβ1 crown as an oscillation. The oscillations of three α subunits are identical with the exception of 120° phase. Hence, the activity regulation assay may be simply cartooned in Fig.3 with one α subunit.

Each link, including α subunit, functions as a spring with its inertial mass, and is simply regarded as a harmonic oscillator. Five harmonic oscillators have constructed five systems. Each harmonic oscillator is a harmonic oscillator. Five harmonic oscillators have

\[ M = \begin{pmatrix} m_0 & \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots & \vdots \\ m_i & \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots & \vdots \end{pmatrix}, \quad X = \begin{pmatrix} x_0 \\ \vdots \\ x_i \end{pmatrix}, \]

and

\[ K = \begin{pmatrix} k_0+k_1 & -k_1 & 0 & \ldots & \ldots \\ -k_1 & k_1+k_2 & -k_2 & 0 & \ldots \\ 0 & \ldots & \ldots & \ldots & \ldots \\ \vdots & \ldots & \ldots & \ldots & \ldots \\ 0 & \ldots & \ldots & \ldots & k_i \end{pmatrix}, \]

\[ \Lambda = \begin{pmatrix} \lambda_0 \\ \vdots \\ \lambda_i \end{pmatrix}, \quad F = \begin{pmatrix} f_0(t) \\ \vdots \\ f_i(t) \end{pmatrix}, \quad \Gamma = \begin{pmatrix} \Gamma_0(t) \\ \vdots \\ \Gamma_i(t) \end{pmatrix}. \]

I is an identity matrix. MX is the inertial force and is usually ignored in linear motors motility study due to overdamping. i = 0, 1, 2, 3, 4 represents α subunit antibody, streptavidin, H9 antibody and H9 virus respectively, the inertial mass, mi, is in proportion to its Dalton molecular weight, that is, mi = 55, 170, 60, 170 and ∞ (KD) for i = 0, 1, 2, 3, and 4 respectively. xi is the displacement of the ith oscillator.

\[ F \] is the restoring force. AX is the damping force. \[ \dot{X} \] is the driving force (\( f_i(t) = 0 \) for \( i > 1 \)). \[ \dot{X} \] is the random force characterized by a Gaussian distribution with zero mean (\( \langle \Gamma \rangle = 0 \)). The general solution of Eq.(1) is a sum of a transient solution that depends on initial conditions, and a steady state one that depends only on driving frequency, driving force, restoring force and damping force. Of course, we only discuss the latter.

F1 motor consists of two rotary motors acting in opposition: F1 motor generates a mechanical torque using the hydrolysis energy of ATP, while F0 motor generates a rotary torque in the opposite direction using a transmembrane p.m.f. When the motor functions as a synthase, the driving force of α subunit, \( f_{\alpha}\gamma(t) \), is generated by the eccentric rotation of γ subunit that is impelled by the transmembrane proton flux of chromatophore, whereas the incurred counterforce, \( f_{\alpha}\gamma(t) \), comes of the releasing of ATP in β subunit. The net driving force then \( f_0(t) = f_{\alpha}\gamma(t) - f_{\alpha}\gamma(t) \). When the motor functions as a hydrolase, the driving force of α subunit, \( f_{\alpha}(t) \), is generated by the binding of ATP in β subunit, whereas the incurred counterforce, \( f_{\alpha}(t) \), comes of the transmembrane protons pumping, the net driving force then \( f_0(t) = f_{\alpha}(t) - f_{\alpha}(t) \). At steady state, F0F1-ATPase converts directly chemical energy at nearly 100% efficiency(Öster and Wang, 2000; Kinostat et al., 2000) between p.m.f and energy currency, ATP, which means that motor doesn’t output any mechanical work and dissipates hardly heat in medium, hence, the net driving force \( f_0(t) \approx 0 \).

Resonance is the tendency of a system to oscillate with the maximum amplitude at a certain fre-
The average value. Here, the native holoenzyme, $P_\text{h}$, is equal to the average holoenzyme activity. For simplicity, we consider that motor equiprobably matches every resonance frequency, the ensemble statistic time average resonance frequency of $i$th system can be calculated statistically by

$$
\langle \omega_i \rangle = \int \cdots \int P_i(\langle \omega_i \rangle)(\omega_i) d\omega_i dk_1 \cdots dk_i
$$

(Systematic kinetics study of FoF1-ATPase(Shu and Lai, 2008) has derived the formula of saturated rotary speed. The rates of ATP synthesis and hydrolysis have been given by

$$
v_s^h = \frac{3k_{i,T}k_{i,P}}{k_{i,D} + k_{i,P}}$$

respectively, where $k_{i,T}$ is the releasing rate of corresponding product, and $T, D$ and $P$ represent ATP, ADP and phosphate respectively. Because phosphate is too small to be blocked in the catalytic cleft, we consider that the releasing rate of phosphate, $k_{i,P}$, doesn’t rely on the conformation of $\beta$ subunit and is constant, $k_P$.

The releasing of ATP(ADP) is tightly coupled to the conformational change of $\beta$ subunit because the size of ATP(ADP) can be compared with the size of catalytic cleft, that is, $k_{i,T}(k_{i,D}) = \langle \omega_0 \rangle / 2\pi$. The relative synthesis and hydrolysis activities are defined as

$$
\rho_s^h = \frac{v_s^h}{v_0^h} = \frac{\langle \omega_0 \rangle}{\langle \omega_i \rangle} \quad \rho_s^h = \frac{v_s^h}{v_0^h} = \rho_s^1 + \frac{b}{\rho_s^1 + b}
$$

respectively, where $b \equiv k_P / (\langle \omega_0 \rangle / 2\pi)$. Eq.(4) predicts that $\rho_s^h > \rho_s^1$ if $\rho_s^1 < 1$ as indicated in Fig.2, otherwise, $\rho_s^h < \rho_s^1$ which has also been demonstrated by our recent experiment with links on $\beta$ subunit. The fitted $b = 1.58$, a reasonable value.

### 4 DISCUSSIONS

Different from previous activity regulation assays(Yasuda et al., 1998; Panke and Rumberg, 1996; Turina et al., 2003; Diez et al., 2004; Steigimiller et al., 2008; Itoh et al., 2004), our regulation based on the stator links. The external links not only inhibit the enzyme but also resume it, and the regulation tendency of synthesis is the same with that of hydrolysis. It is very difficulty for power stroke model(Wang and Oster, 1998) to interpret this regulation mechanism. The
resonance mechanism, however, has satisfactorily illustrated these phenomena. Each external link serves as a harmonic oscillator. The more oscillators are linked in series, the more resonance frequencies may be selected. If the system does in a resonance fashion, the motor works in a most efficient way. Here, we consider that motor will equiprobably match every resonance frequency. The average resonance frequency is statistically equal to the ensemble experimental activity. Actually, the lowest resonance frequency will be closer and closer to zero with adding of oscillator or the increasing of inertial mass. However, it is impossible for \( F_1 \) motor to stop in virtue of the driving of \( F_o \) or binding of ATP. This lowest frequency that is close to zero has to be cancelled in statistics and the average resonance frequency then will jump. This is one of reasons that the value of No.5 is higher than that of No.4 in Fig.2. The critical lowest resonance frequency is waiting to be determined by further experiments. Our novel resonance model together with systematic kinetics study (Shu and Lai, 2008) has predicted that the relative activity of hydrolysis is higher (lower) than that of synthesis if the latter is less (higher) than 1, which has been experimentally demonstrated.

The frequency spectrum indicated in Fig.4 is waiting to be tested by a mass of single molecule experiments. If there is enough single molecule experimental data, we can firstly confirm whether the distribution of activity of single motor is the same with that of our model. Secondly, we can affirm whether the motor equiprobably matches every resonance frequency or not. Finally, we can estimate the standard deviation, \( \sigma_i \), for each Gaussian distribution. Here, for simplest, we consider that motor equiprobably matches every resonance frequency. The fitted elastic coefficient can be used to estimate the elasticity of linking protein. It seems reasonable that \( k_i \) is much higher than others in view of the stiff capsid of H9 virus. The fitted rate of \( F_1 \) releasing is also logical because it is higher than \( (\langle 0 \rangle \theta 0) / 2\pi \).

It is clear that the mechanical movement of \( F_1 \) comprises the eccentric rotation of \( \gamma \) subunit and the conformational change of the \( \alpha \beta \gamma \) crown, and they are tightly coupled with each other. However, previous theoretical study only focus on the rotation of \( \gamma \) subunit. In fact, the conformational change of the \( \alpha \beta \gamma \) crown is very important for the entire motor, and plays an active role in hydrolysis. On the other hand, the role that \( \alpha \) subunit plays in the conformational change of the \( \alpha \beta \gamma \) crown has not been studied although the structure of \( \beta \) subunit resembles that of \( \beta \) subunit with the exception of catalytic site. This work is try to investigate what \( \alpha \) subunit functions in the rotary motor and has expanded the “binding change mechanism”.

In contrast with Langevin equation that ignores the inertial force due to overdamping, our resonance model does neglect the damping force because the entire motor doesn’t output any mechanical work and dissipates hardly heat in medium. Additionally, the scale of conformational change of the \( \alpha \beta \gamma \) crown is very small, thereby the average velocity of oscillation is so low that \( \chi_i \langle \dot{x}_i \rangle \ll m_i \langle \dot{x}_i \rangle \), which is possible although the values have not been estimated. It should be noted that the elastic coefficient is independent of the additional links. Although the elastic coefficient can in fact depend on the additional links due to electrostatic interaction, in the simplest version of the model, we assume that all elastic coefficients are links-independent.

**ACKNOWLEDGEMENTS**

This work is partially supported by the NSFC grant No.91027045 and No.11075015, and the National Basic Research Program of China (973 Program) under the grant No.2007CB935903.

**REFERENCES**


