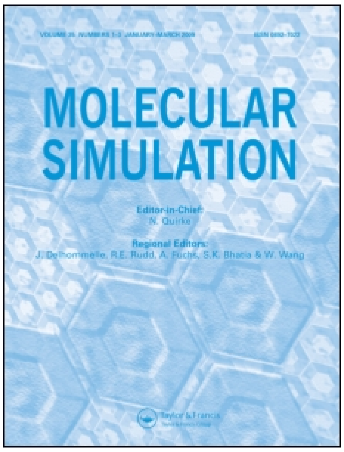

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Molecular simulation and theory for nanosystems: Insights for molecular motors

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Molecular simulation and theory for nanosystems: Insights for molecular motors

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Knowledge of self-assembling molecules such as deoxyribonucleic acid (DNA) and dendritic systems can provide beneficial insights for the development of tailor-made nano-scale devices. Another important category of nanosystems is molecular systems that provide a mechanism of molecular propulsion such a myosin, kinesin and adenosine triphosphate synthase (ATPase). Work on these so-called 'molecular motors' has been led largely by experimental investigations. The application of rigorous theoretical techniques such as molecular simulation is a considerable computational challenge. However, the reward for accepting the challenge can be a greatly improved theoretical insight, which benefits the development of nanotechnologies. In this work, we discuss recent progress in improving the theoretical understanding of an important molecular motor, ATPase.

Keywords: molecular simulation; molecular motors; nanosystems; adenosine triphosphate synthase

1. Introduction

A 'molecular motor' is a molecular system that is capable of exerting forces and motion at molecular and cellular scales. Molecular motors are expected to play an increasingly important role in nanotechnology by providing a means of controllable mechanics and functions [1,2]. The ultimate goal of building an artificial molecular motor is well beyond current scientific and technical capabilities. In contrast, there are many naturally occurring biomolecules that work as molecular motors [3–5], which could provide useful insights into the design and construction of purpose-built systems. Arguably the most important class of molecular motors is adenosine triphosphate (ATP)-fueled enzymes such as myosin, kinesin, and adenosine triphosphate synthase (ATPase).

One of the most important [3,4] molecular motors is F_1F_0-ATPase (Figure 1). The F_1F_0-ATPase motor is an enzyme protein complex, which is primarily responsible for the process of energy conversion in bacteria, chloroplasts and mitochondria. The two main sections of F_1F_0-ATPase contain multiple domains or subunits. The alternating domains, labelled α and β, are constructed as a hexamer around the linking central $\gamma\delta\varepsilon$ subunits. In common with many other molecular motors, F_1F_0-ATPase is powered by the hydrolysis and synthesis reactions of ATP. Reactions involving ATP trigger the $(\alpha\beta)_3$ subunits into a series of asymmetrical and sequential transitions through different conformational and binding states causing the central $\gamma\delta\varepsilon$ subunits to rotate [3,6]. It is this rotary motion that makes F_1F_0-ATPase the most appealing [7] system to investigate because rotation is an essential feature of all macroscopic human-made motors. In contrast other molecular motors such as myosin [8], kinesin and dynein [9] exhibit only translational motion, which cannot be readily adapted for a general-purpose motor.

Considerable efforts have been made to understand the behaviour of F_1F_0-ATPase. These include single-molecular measurements [10–13], and studies of both the functional microbiology [3,14] and the structural biology [6] of the system. In addition, theoretical studies [15] have been reported. However, the exact molecular level details of the operation of ATPase remain largely unresolved [13]. The mechanism of ATP binding states, nucleotide reaction and release from the protein subunits represents an important challenge to our understanding of the functions of molecular motors. Our understanding is also deficient for critical molecular processes such as the binding of ATP to active subunits, reaction, catalytic pathways, the dynamic conformations of subunits and mechanical transduction processes.

In common with work on other nanosystems, our understanding of molecular motors has been led by experimental investigations. The application of rigorous theoretical techniques such as molecular simulation [16] is a computational challenge. However, the reward for accepting the challenge can be a greatly improved theoretical insight, which benefits the development of nanotechnologies. In this work, we review some of our recent progress in understanding ATPase.

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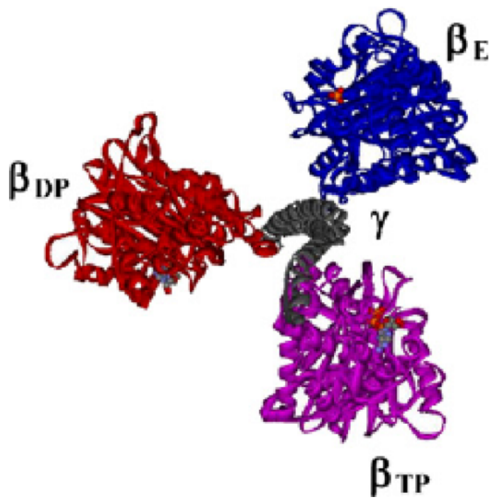

Figure 1. Schematic top view of the β protein domains of the F_1F_0-ATPase molecular motor.

2. Theory and simulation

Details both theory and simulation are available elsewhere [17–20]. Therefore, only the salient features are outlined here.

2.1 Chemo-mechanical properties

The chemo-mechanics of the F_1-ATPase motor were modelled using an appropriate reaction scheme [17] for the hydrolysis. The resulting rotary motion (Figure 2) was calculated via Langevin dynamics. The master equation for the Langevin dynamics of the rotating system was:

$$\left.\begin{aligned} \boldsymbol{\varpi} &= \frac{\mathrm{d}\theta}{\mathrm{d}t}\hat{\theta} \\ \frac{\mathrm{d}\mathbf{L}}{\mathrm{d}t} &= \tau_{\mathrm{mm}} + \tau_{\mathrm{ext}} - \zeta\boldsymbol{\varpi} + \tau_{\mathrm{B}}(t) \end{aligned}\right\}, \qquad (1)$$

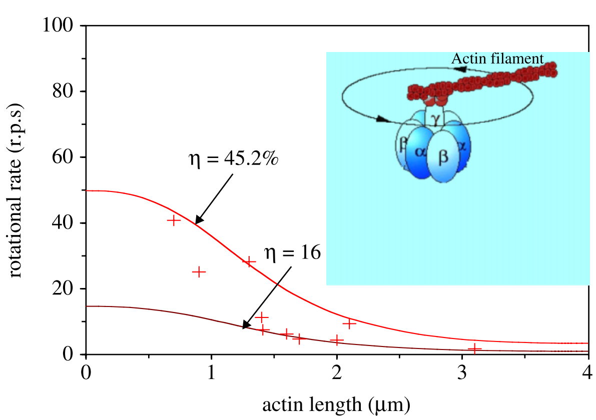

Figure 2. F_1-ATPase drives an actin filament. The theoretical lines (red solid) indicate that the chemomechanical coefficients of the F_1-ATPase motor fall between 0.165 and 0.452 with an effective 'ratchet' coefficient of 1.07 pN nm s. The cross symbols are from single motor experiments [17].

where, $\boldsymbol{\omega}$ is the angular velocity and θ the mechanical position (with rotation only occurring in the x–y plane), $\mathbf{L} = \mathbf{I}\cdot\boldsymbol{\omega}$ is the angular momentum of the system, and $\mathbf{I}$ is the moment of inertia tensor, $\boldsymbol{\tau}_{\mathrm{mm}}$ is the torque generated directly from the hydrolysis reactions at corresponding functional subunits of the F_1 motor, $\tau_{\mathrm{mm}} = -(\partial U(\theta)/\partial\theta)$ and $U(\boldsymbol{\theta})$ is the corresponding chemical potential energy at position $\boldsymbol{\theta}$ of the molecular motor. It is expected that the molecular motor will switch between a series of $U(\boldsymbol{\theta})$ when it rotates and undergoes continuous conformational changes, $\boldsymbol{\tau}_{\mathrm{ext}}$ is the torque on a load applied by an external force (if any), $\zeta\boldsymbol{\omega}$ is the torque via frictional drag in a viscous medium, with ζ the drag coefficient of the load against the media, $\boldsymbol{\tau}_{\mathrm{B}}$ is the Brownian motion term due to thermal fluctuation-dissipation of the acting subunits of F_1-ATPase and its load.

2.2 Cooperativity of ATP hydrolysis

The hydrolysis reactions at the three binding sites of F_1-ATPase were modelled as a series of fast equilibrium reactions [18], with the reaction rate (R) determined by applying a steady state condition. The enzymatic cooperativity can be measured (Figure 3) using a modified Hill equation [21]

$$\log(R/(R_{\max} - R)) = h\log[\mathrm{ATP}] - \log K, \qquad (2)$$

where $R_{\max}$ 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. This means that only one ATP molecule is hydrolysed at any time and the

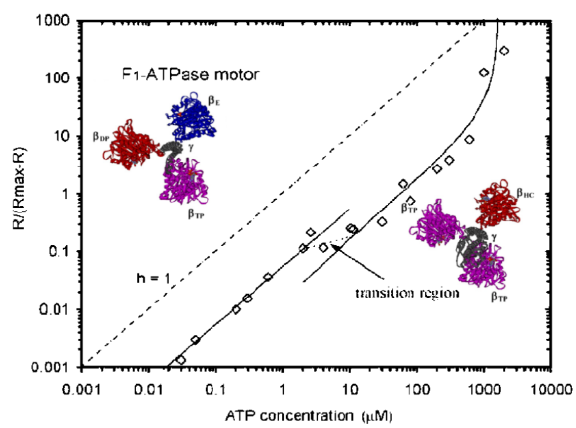

Figure 3. Hill plot of the rate of ATP hydrolysis. Cooperativity is a key dynamic feature for the steady operation of molecular motors. F_1-ATPase with different cooperativity (diamonds are from motor experiments and solid lines are from our molecular simulation results) shows different rotation schemes of 'bi-site' activities (Left) and 'tri-site' activities (Right) [18].

kinetics follows the Michaelis–Menten mechanism [21], i.e. $R = R_{max}[ATP]/(K_m + [ATP])$, where K_m is 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.

2.3 Structural properties

As detailed elsewhere [20], the structure adopted by ATP (Figure 4) was determined using canonical ensemble molecular dynamics (MD) simulations with the CHARMM force field [22]. The TIP3P model [23] was used for the interaction of The systems were equilibrated using MD simulations that included constant temperature control via Langevin dynamics. The velocity Verlet algorithm [16] was used for integrating the equations of motion.

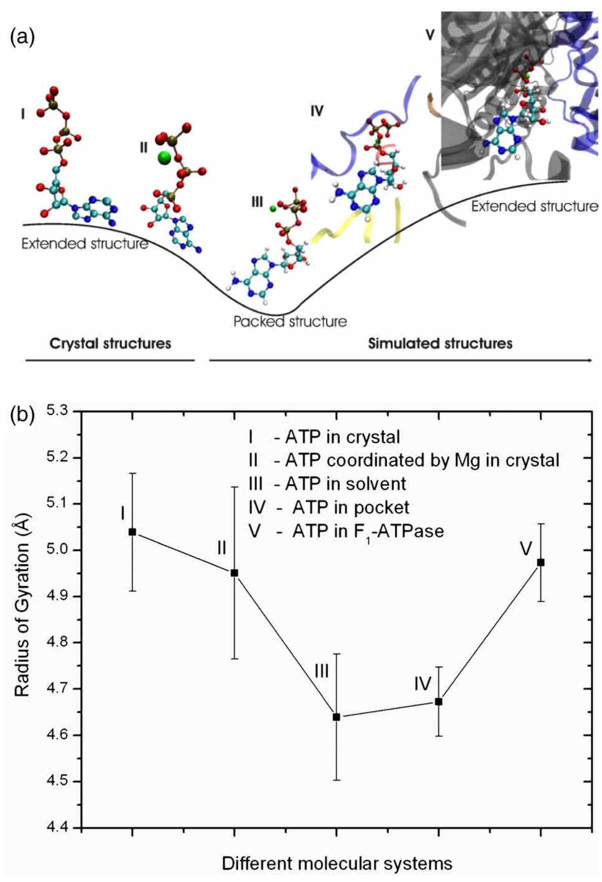

Figure 4. (a) Illustration of the five different average structures of ATP in ATPase in different environments: (I) crystal without Mg^{2+}; (II) crystal structure when Mg^{2+} coordinates the phosphate region; (III) Mg:ATP in solution; (IV) Mg:ATP in a small pocket; and (V) Mg:ATP situated in F_1-ATPase. (b) Radii of gyration for ATP/Mg:ATP in the various environments at a temperature of 300 K [20].

The flexibility and particularly the degree of extensibility of ATP/Mg:ATP can be described by determining the radius of gyration radius (r_g), as defined in the following expression:

$$r_g^2 = \frac{\sum_{i=1}^{n} m_i(\vec{r}_i - \vec{r}_c)^2}{\sum_{i=1}^{n} m_i}, \qquad (3)$$

were m_i is the mass of the ith atom, $\vec{r}_i$ is the position of the ith atom and $\vec{r}_c$ is the position of the centre of mass of the ATP/Mg:ATP molecule.

3. Results and discussion

It is generally accepted that strong dynamically regulated conformational transitions and cooperativity are primarily responsible for the operation of the ATP-fuelled molecular motors. However, a molecular understanding of the mechanisms and underlying dynamics remains unclear [7,24]. Efforts to determine the operating mechanism and dynamics of F_1F_0-ATPase, include single molecular measurements [10,12,13], functional microbiology [3,14] and structural biology [6] studies. Theoretical studies have also been reported [15,25]. However, the exact molecular level mechanisms and underlying dynamics of F_1-ATPase remain largely unresolved [14,24]. As partially detailed below, our work [17–20] on molecular motors indicates that the critical deficiency is in our knowledge of molecular processes such as the binding of ATP to the active subunits, nucleotide reactions and catalytic pathways, the structural transitions of subunits and mechanochemical transduction processes. In particular, the main issues affecting molecular motors are dynamic fluctuations, correlations and allostery across different spatial and time scales.

In general, understanding the functioning of biomolecules requires knowledge of the relationships between structure, dynamics and functions, e.g. protein folding and enzymatic catalysis [26]. Recent studies suggest that dynamics plays critical roles in regulating certain protein functions via allostery and cooperativity [27,28]. Therefore, the issues involved in understanding molecular motors are part of the broader challenges encountered in both protein dynamics and protein functions.

We [17–20] have made considerable progress in understanding the mechanism of molecular motors by studying F_1-ATPase as a prototype molecular motor. F_1-ATPase hydrolyses ATP into adenosine diphosphate (ADP) and inorganic phosphate (Pi) and converts chemical energy into mechanical rotation. We have developed [17,18] a computational model based on enzyme kinetics and Langevin dynamics that accounts for the chemomechanics of rotation and the load-dynamics of F_1-ATPase. It was demonstrated that the torsional energy and stepwise rotation is regulated by a series of near-equilibrium reactions involving the binding and unbinding of nucleotides. The model also uses an effective 'ratchet' drag coefficient and a chemomechanic coefficient. We have analysed the theoretical load-rotation profile when the motor drives an actin filament. As illustrated in Figure 2, comparison with experimental data indicates that this model is a reasonable approximation. It is capable of both qualitative and quantitative agreement with experiment.

The cooperativity of other ATP-fuelled molecular motors such as kinesin and myosin have also been analysed. Significantly, it was discovered [18] that the catalysis of these molecular motors does not follow a simple Michaelis–Menten mechanism (Figure 3). Instead motor activities such as the hydrolysis or processive rates of kinesin, myosin and F_1-ATPase have a complex ATP-dependent cooperativity. Our work indicates that conformational coupling of binding states of different subunits induces cooperativity.

In response to these insights, a conformation correlation theory of cooperativity has been developed [29] to predict this phenomenon in the ATP-fuelled motor proteins. In F_1-ATPase, the rotation of the $\gamma\delta\varepsilon$ subunits against the hexameric $\alpha_3\beta_3$ subunits is highly coordinative, driven by ATP hydrolysis and structural changes at three β subunits. However, the dynamical and coordinating structural transitions in the β subunits are not fully understood at the molecular level. We examined structural transitions and domain motions in the active subunits of F_1-ATPase via dynamical domain analysis of the $\alpha_3\beta_3\gamma\delta\varepsilon$ complex. The domain movement and hinge axes and bending residues were identified and determined for various conformational changes of the β-subunits. It was observed [29] that the P-loop and the ATP-binding pocket play essential mechanical functions, which is in addition to their catalytic roles. The cooperative conformational changes pertaining to the rotary mechanism of F_1-ATPase appears to be more complex than Boyer's 'bi-site' activity. These findings provide unique molecular insights into dynamic and cooperative domain motions in F_1-ATPase.

MD simulation [16] has a useful role in determining the structure adopted by ATP in the F_1-ATPase molecular motor. The structure of ATP changes in response to its immediate environment (Figure 4(a)). These structural changes provide valuable insights into the mechanisms driving the rotation of ATPase. In a MD simulation the size of the molecule in various environments can be quantified [20] by determining the radius of gyration. Values of the of the radius of gyration are summarised in Figure 4(b). It is clearly apparent from these data that ATP in F_1-ATPase (case V) adopts a considerably more relaxed configuration compared than either in an aqueous environment (case III) or inside an ATPase pocket (case IV).

4. Conclusions

The application of rigorous theoretical models and simulation provides valuable insights into the behaviour of nanosystems. Using such an approach, have identified complex cooperativity of ATP hydrolysis in F_1-ATPase. Significantly, for the first time, activity cooperativity induced by conformational correlations was also found in other ATP-fuelled molecular motors, namely myosin and kinesin. We have also identified the dynamic and coordinating domain motions in the F_1-ATPase molecular motor. These results illustrate the usefulness of theory in improving our fundamental knowledge of nanosystems.

Acknowledgements

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