Single molecule studies of a biological motor F_1-ATPase: interplay of experiment analytic theory and computation

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Abstract
In this chapter we discuss the interaction between theory and single molecule experiments on a biological motor, F_1-ATPase. In particular we consider an interplay between the experiment, analytical theory, and computer simulations. The complementarity of the millisecond and microsecond experiments is noted. For example, the limited experiments on the latter indicate that the ATP binding in one β subunit precedes an ADP release in another β subunit, whereas the millisecond experiments do not time-resolve the two steps.

Text
It is a real pleasure to contribute this chapter to this book honoring a long time friend and colleague, Bill Goddard, in celebration of his 80th birthday. Bill's deep physical insight and understanding of complex and diverse problems and his enthusiasm are his trademark and a pleasure to behold.

We recall that F_1-ATPase is the part of the biological motor F_oF_1-ATP Synthase that is outside a bilayer membrane, the part inside the membrane being the F_0-ATPase. We recall that the F_oF_1-ATPase utilizes a proton gradient across a bilayer membrane to synthesize the energy rich compound adenosine triphosphate (ATP), used for many functions in biological systems. In the F_oF_1-ATPase a proton enters an offset channel from one side of the membrane bilayer and during its transfer via the offset in the ion channel to the other side. It causes the central γ subunit, a spindle, extending from the F_1 part, to rotate. This spindle, often termed as the rotor shaft, in turn presumably causes clefts between pairs of the α and subunits in the F_1 part to open and close, because of their asymmetric nature and the asymmetric nature of spindle and so permits entry of a reactant into a cleft, reaction, and departure of products into the medium on the F_1 side of membrane.

The single molecule experiments are on the F_1-ATPase and a large majority of them are in the millisecond time domain (stalling experiments, controlled rotation experiments and some free rotation experiments), and some are in the microsecond time domain. The individual steps in the former are describable by rotor-angle dependent rate constants. The steps in the microsecond time domain are, apart from one exception, the distribution of catalytic dwell times, not describable by rate constants. Instead for them one needs a formulation that gives the observable in

these microsecond experiments, the rotor angle as a function of time. A description of the reaction kinetics in the latter would involve instead the solution of a partial differential equation (Fokker Planck equation) or of the corresponding stochastic ordinary differential equation (Langevin equation). The timescale associated the F_oF_1-ATP Synthase itself is milliseconds, since its rotation rate is 100-150 cycles/s depending on the species. [Spetzler et al., 2006]

We discuss first the various experiments in the millisecond time domain. We recall that most of the relevant single molecule experiments are obtained using techniques whose natural time scale is milliseconds, and indeed, as already noted, that is also the natural timescale for the F_0F_1-ATPs Synthase. However, as a stand alone the natural timescale for the F_1-ATPase is microseconds. To adapt the latter to the former the overall process was slowed down in the single molecule experiments from microseconds to milliseconds, accomplished by modifying the nucleotides and the ATPase and in addition by reducing considerably the ATP concentration well below its physiologically relevant concentration.

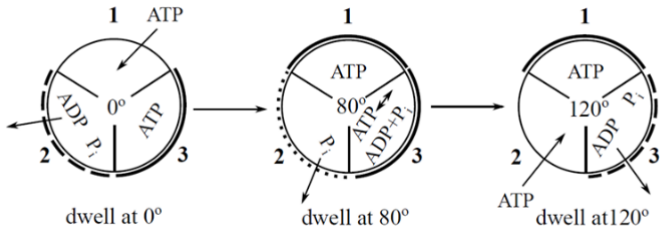

Fig. 1. Scheme of coupled processes in F_1-ATPase during free rotation.[Volkan-Kacso & Marcus, 2015] The dwell angle increases in the counter clockwise direction. The species occupying the pockets of ring β subunits 1, 2 and 3.

Under these millisecond conditions there is a series of reaction steps versus angle of rotation inferred from the data [E. g., Adachi et al., 2000, Adachi et al., 2007, Adachi et al., 2012] and depicted in Fig. 1. It is seen there that there is a "dwell angle" at 0°, (observable in single-molecule imaging experiments only when the ATP concentration is very low). It is the state of the F_1-ATPase just prior to an ATP binding in the β_1 subunit in Fig. 1. Simultaneously, on this millisecond timescale, an ADP exits from β_2 during the 80° step. This ADP, the product of a hydrolysis, will not exit from this β subunit on this millisecond timescale unless an ATP is entering another β subunit, β_1. After the next dwell angle at 80° (the catalytic dwell) in a subsequent 40° step, a hydrolysis of an ATP is beginning to occur in β_3. Meanwhile, a Pi is exiting from β_2, and the ATP in β_1 remains unchanged. After the next dwell angle at 120° an ATP enters β_2, from which the Pi just exited, and the ATP in β1 is unchanged. This cycle is continuously repeated.

In single-molecule controlled rotation experiments [Adachi et al. 2012] fluorescence microscopy was used to monitor individual ATP binding and release events. Using magnetic tweezers, a slow, constant rate of rotation of the rotor shaft was induced the individual binding and release events of a fluorescently labeled ATP species

were monitored. For rotor angles θ between −45° to +45° the controlled rotation data overlaps with another single molecule experiment using a stalling technique. Important differences between the controlled rotation and stalling experiments are that (1) in the former a fluorescent species, Cy3-ATP and Cy3-ADP, is used instead of the regular ATP and ADP and (2) in the former the nucleotide occupancy was at most 1 while in the latter it varied between 2 and 3. Assuming that these differences affected the absolute rate constants, but left the angle-dependence unchanged the rate constants in the controlled rotation experiments can be predicted, without using adjustable parameters.[Volkan-Kacso & Marcus, 2016] The comparison is given in Fig. 2, in the θ-range of overlap, in which the points (symbols) are the experimental data and the solid curves are theoretical calculations.

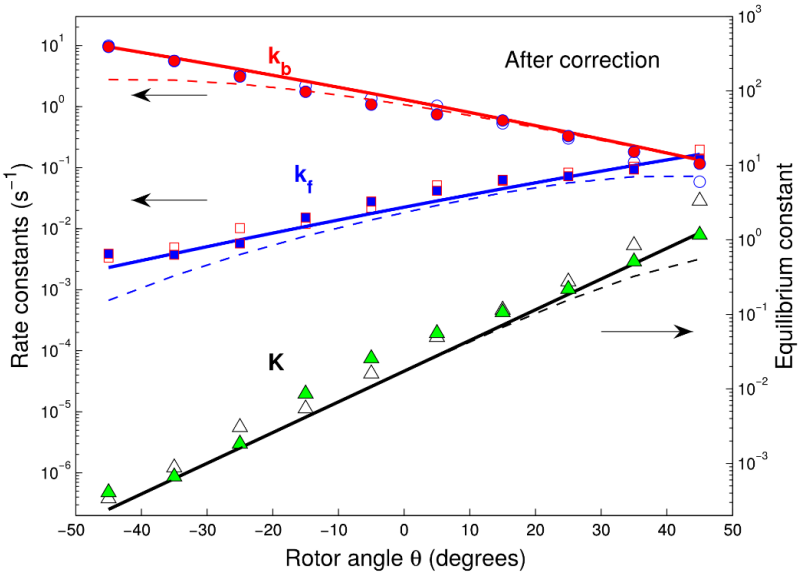

Fig. 2. Corrected binding and release rate and equilibrium rate constants versus θ angle for Cy3-ATP in the presence (solid squares, circles and triangles) and absence of Pi (open symbols) in solution adapted from Volkán-Kacsó and Marcus [2016]. The experimental data of Adachi *et al.* [1012] corrected for missed events (and an error due to replacing the time spent in the empty state by total time of a trajectory) are compared with their theoretical counterparts (solid lines). Dashed lines show the data without corrections.

For comparison we consider next the microsecond experiments. Here, the only dwell that is observed experimentally is the catalytic dwell. [Sielaff et al., 2016] As a consequence the end of that dwell is used to define the 0° for the rotor angle for that experiment. This convention for a 0° in these microsecond experiments differs by 40° from the convention for the 0° used for the millisecond experiments (the time at which ATP at its very low concentration enters a β subunit). The distribution of dwell times before the release of Pi, the catalytic dwell times in the microsecond type experiments using nanorods [Spetzler et al. 2006], was observed to obey an exponential decay and the corresponding rate constant of 130 s^{-1} was found to agree with rate constant observed in ensemble E. Coli F_1 –ATPase experiments.

The hydrolysis dwells in the microsecond type experiments were separated by brief transition periods during which all the other substeps, Pi release, ATP binding and ADP release followed in quick succession. The plot of angular velocity versus angle from 0° to 20° in these microsecond experiments was the same for the two ATP concentrations studied and is attributed to the PI release from a β subunit. From the very limited data for the effect of ATP concentration on this plot and other considerations one may infer that during the 20° to 80° there is an ATP binding, followed on this microsecond timescale by ADP release occurring likely between 80° to 120°, so completing the 120° cycle. Thereby, on this microsecond time scale the ATP binding and the ADP release are time-resolved but are not time-resolved on the millisecond timescale. If confirmed by additional studies at lower ATP concentrations this result suggests that when an ATP enters a β-subunit it creates a "crowdedness" that causes the ADP to leave another β-subunit. Thereby, a tendency to conserve total occupied space in the β-subunits [Volkan-Kacso & Marcus, 2019] represents an equilibrated condition, rather than being fulfilled instantaneously.

A comparison of the progression of events during F1-ATPase rotation monitored in the millisecond and microsecond timescales is summarized in Table 1. We note that the catalytic dwell is the only dwell clearly resolved in both, although in the millisecond experiments low ATP concentrations permitted the resolution of the binding dwell also (cf. Fig. 1). The catalytic dwell is used as the reference rotor angle (0°) in the microsecond experiments, while in the millisecond experiments the reference rotor angle (0°) is the binding dwell. The latter follows the former by a 40° delay. While the natural timescale under physiological condition is of milliseconds, the microsecond experiments have the potential to resolve the sequence of coupled steps, such as ATP binding and ADP release (from a different pocket), which appear to occur in concert on the slower timescales. We note that the rotor angle used in Table 1 provides a partial information about the state of the F1-ATPase, and so angular ranges were provided to show where the events would occur with greatest likelihood. For example, the millisecond experiments indicate that Pi release occurs within a 40° range (80°—120°) while the microsecond experiments indicate a more limited range (0°—20°).

Table 1. Comparison of elementary events observed at the millisecond and microsecond time scales.

Event	Catalytic dwell	ATP hydrolysis	Pi release	Binding dwell	ATP binding	ADP release	Catalytic dwell
Timescale: milliseconds	80° (-40°)	80°—120° (0°)		0°	0°—80°		80° (-40°)
Timescale: microseconds	0°		0°—20°	Not observed	20°—80°	80°—120°	0°

In the transitions between the catalytic dwells in the microsecond experiments the time behavior of the average rotation velocity was complex, and a simple rate description is not feasible for the treatment of the velocity vs. rotor angle data yielded by these high-resolution experiments. Under different experimental conditions, such as low ATP concentration, ATP binding dwell times were resolved

to also follow an exponential distribution, and the ATP binding was describable by a rate constant under such millisecond conditions.

We comment next on some simulations. The atomic-scale resolution of the F_1-ATPase structure with the three active subunits in different nucleotide occupancy and conformational states [Braig et al, 2001] yielded an opportunity to study the individual processes by atomistic simulations. The hydrolysis reaction was studied using embedded quantum-classical methods [Dittrich et al., 2004] that focused on the cleavage reaction inside the catalytic pocket of the tightly bound conformation of the β subunit. These studies treated the degrees of freedom of the amino acids lining the pocket and the internal waters using a classical force-field approach (CHARMM27) reserving the quantum degrees of freedom to the ATP molecule. A possible clue to the coupling between the cleavage (hydrolysis) reaction and the conformational change of the protein was found in the importance of an arginine "finger" to move into close proximity of the bond being ruptured, thereby reducing the reaction free energy barrier.

Numerical methods based on a Fokker-Planck approach were also developed in an attempt to treat the full cycle of the chemo-mechanical coupling [Wang and Oster] and to explain the mechanical coupling between the rotation of the F_o and the F1 [Panke et al, 2001].

Single molecule experiments have subsequently provided detailed information about the substeps along the active chemo-mechanical pathway. In an effort to bring together structure and function revealed in experiment, several groups have developed atomistic molecular dynamics methods to study how the conformational change in the ring subunits leads to a rotation of the shaft. Steered molecular dynamics studies have demonstrated that the closure of the subunit hinges induces a rotation of the shaft and that the 80/40 degree substeps in the rotation can be reproduced.[Pu and Karplus, 2008] Computer simulations using advanced electrostatic interactions between atom groups have successfully demonstrated a 2-dimensional free energy profile showing that a minimum energy path results in the 80/40 substeps.[Mulherjee and Warshel, 2011] These simulations were unbiased, they did not require the application of external forces to induce a conformational changes in the ring subunits. More recent studies have focused on individual substeps and the nature of the coupling between molecular displacements in the pockets and conformational changes in the whole ring-rotor structure. In particular, it was shown, using all-atom MD simulations, that during Pi release, the diffusion of the Pi molecule induces a significant conformational change in the β subunit [Okazaki and Hummer, 2013].

For the treatment of the microsecond experiments by a Fokker-Planck or Langevin Equation knowledge of some constant such as the diffusion constant of the Pi in the Pi release in a cleft is needed. This Pi, the $H_2PO_4^{2-}$, diffuses along an internal channel along a path that leads from the binding pocket towards a peripheral binding site from which then it can be released into the solution. Molecular dynamics

simulations provided an estimation of the diffusion constant [Hummer 2005] which for the Pi diffusion was found to be about 1.5×10^{-7} cm^2/s, during the diffuses in the binding channel [Okazaki and Hummer 2013]. This diffusion constant is nearly two decades slower than the diffusion of Pi in water (0.9×10^{-5} cm^2/s).

In summary, this field of biological motors and the efforts to treat them theoretically are rich in their possibilities and offers continually interesting features and challenges. It is very much in a state of growth.

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