A Minnow, An E. Coli and Ubiquinone: The story of Rectified Brownian Motion

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ABSTRACT

Rectified Brownian motion provides a mechanistic alternative to the *power stroke* model for the function of motor proteins and other enzyme complexes. ATP is the source of metabolic free energy for motor proteins such as kinesin and myosin V. In the power stroke models, the energy of ATP hydrolysis causes chemo-mechanical energy conversion although the precise manner by which this occurs has yet to be identified. In the rectified Brownian motion model, the energy released by ATP hydrolysis causes an irreversible conformational switch in the ATP binding protein that results in release of the motor protein head from the track along which it moves, and Brownian motion provides the power for the head to move to a new binding site. Asymmetric boundary conditions for this diffusive motion result in a directed motion on the average.

Keywords: rectified Brownian motion, power stroke, ubiquinone, kinesin, Langevin equation

1. THE CENTRAL PROBLEM

Motors proteins move along protein tracks and carry cargo from one cellular region to another. Kinesin moves along microtubule tracks and myosin V moves along actin tracks. How these proteins function is the central question. The mechanism may be much more general and also apply to rotary enzyme complexes, ATPases, polymerases, membrane transporters and other molecular systems. Two different mechanisms vie for recognition, the power stroke model and the rectified Brownian motion model. Andrew Huxley [1] introduced the rectified Brownian motion model many years ago but at a time when the detailed macromolecular structure of muscle fibers was not well determined. In the absence of an appreciation of the robustness of thermal energy at the macromolecular scale, the power stroke model is usually put forth and tends to dominate text book presentations [2]. The present author has addressed this dichotomy in the recent past and clearly favors the rectified Brownian motion mechanism [3,4]. The purpose of this paper is to make the case for rectified Brownian motion more widely known and to argue why it is a natural mechanism in the context of robust thermal energy. Moreover, this mechanism suggests how thermal energy can be constructively used in the design of nanotechnological devices.

2. THERMAL ENERGY

In order to appreciate the importance of thermal energy at the sub-cellular level, it helps to look at three examples that represent very different scales. These examples are a minnow, an E. Coli and ubiquinone.

The minnow is 16 cm long and has a cross-sectional diameter of 4 cm. It has a mass of 134 gm. Using its fins it can swim up to 100 cm/s. Throughout this paper we will assume that the ambient temperature is 25 ^{o}C. The viscosity of its environment is one centi-poise. The Reynolds number for the swimming motion is 80,000 which is very large. Normally this set of values would complete our description of the minnow in motion. However, for comparative purposes let us consider its thermal motion as well. The thermal velocity of the minnow's center of mass is 1.75×10^{-8} cm/s. This is ten orders of magnitude smaller than its swimming speed. It is a random motion that causes the center of mass to diffuse in addition to its secular, swimming motion. In one second the root-mean-square displacement caused by this diffusion is 3.7 nm. This is to be compared to the 100 cm it swims in one second. Thus, the thermal motion is entirely negligible for the minnow. The Reynolds number for this thermal motion is 1.4×10^{-6}, a very small value that will be characteristic of all examples to follow. The secular power expended by swimming is 5.96×10^{-4} W. This shows how efficiently the little fish can swim. Its thermal power is 5.7×10^{-23} W, 19 orders of magnitude smaller than the swimming power and showing in another way how insignificant the thermal energy considerations for the minnow really are.

An E. Coli is a bacterium that is two microns long with a cross-sectional diameter of one micron. It has a mass of 2×10^{-12} gm. Using its flagella, it too can swim. It is capable of "runs" of about one second duration, interspersed with "tumbles" of about 0.1 second duration. The runs have top speeds of 2×10^{-3} cm/s. The tumbles reorient the direction of motion in an effectively random way. The viscosity of its environment is 2.7 centi-poise. Because of the very small mass, the thermal velocity for the E. Coli is 0.14 cm/s, much larger than its secular, swimming speed. Its secular Reynolds number is 1.5×10^{-6} and its thermal Reynolds number is 10^{-3}, each of which is very small. What this means physically is

that for the E. Coli inertia is of no importance and its motion is dominated by viscosity. This is graphically exhibited by the fact that if it is moving at top speed and the flagella are suddenly turned off then the E. Coli will come to a complete stop after moving a distance of only 1.3 x 10^{-10} cm. This remarkable result was noted by Howard Berg [5]. Clearly, the E. Coli world is dominated by thermal energy and viscosity. While the thermal velocity is nearly 100 times greater than the swimming velocity, it does not persist in a single direction for any appreciable length of time like in the case of a secular run. Instead it causes a diffusion of the center of mass of the E. Coli that amounts to a root-mean-square displacement of half a micron in one second. In that same one second the secular motion moves the E. Coli 20 microns. This example lends some credence to the idea of a power stroke in that it shows how the E. Coli is able to overcome a very robust thermal environment and achieve a secular motion in spite of all the thermal agitation. Moreover it can do this at the relatively low power of 1.23 x 10^{-17} W compared to the thermal power of 1.92 x 10^{-13} W.

Ubiquinone is a ubiquitous molecular species that is found in aerobic bacterial membranes and in the organelles, mitochondria and chloroplasts. It plays a central role as an intermediate in electron transport chains. These systems of membrane embedded protein complexes are made up of a predominately iron-sulfur protein complex and a predominately cytochrome complex that are coupled together by diffusive shuttling of ubiquinone between the two complexes. In bacteria, iron-sulfur proteins reduce oxidized ubiquinone near the surface of the membrane that is adjacent to the interior of the cell. Cytochromes oxidize the reduced ubiquinone near the surface of the membrane that is adjacent to the external environment. Since ubiquinone oxidation and reduction involves a pair of electrons and a pair of protons, the location of the reduction near the inside and the location of the oxidation near the outside results in protons being translocated from inside the cell to outside the cell while electrons are passed along the electron transport chain. This process is a paradigm for rectified Brownian motion. Ubiquinone has a molecular weight of 862 when oxidized (864 when reduced). This makes its mass 1.44 x 10^{-21} gm. This is nine orders of magnitude smaller than the mass of E. Coli and 23 orders of magnitude smaller than the mass of the minnow. It moves in the lipid interior of the membrane where the viscosity is 25 centi-poise. It has a spherical conformation with a radius of 0.75 nm. Its motion inside the membrane lipid interior can be described by the Langevin equation, a stochastic differential equation [6]. It has no fins or flagella and therefore is unable to "swim" through the membrane. Instead the only source of motion available to ubiquinone is thermal motion. The relaxation time for the Langevin equation for ubiquinone is 4 x 10^{-15} s. This is such a short time that the Langevin description may be replaced by an equivalent diffusion process for all times long compared with this short relaxation time. The ubiquinone diffusion constant is 1.2 x 10^{-7} cm^2/s. For a membrane with a thickness of 8 nm, the expected time for ubiquinone to cross the membrane thickness is 2.8 x 10^{-6} s. This is nine orders of magnitudes longer than the relaxation time. Thus, the ubiquinone motion in the membrane is in the extreme limit of diffusion for the Langevin equation. Therefore, in this case, rectified Brownian motion is described by diffusion with asymmetric boundary conditions.

The asymmetry of the boundary conditions for diffusion is the key to rectified Brownian motion. This situation should not be confused with "Brownian ratchets" in which an asymmetric saw-tooth potential that is usually oscillated plays a central role [7]. For ubiquinone the boundary conditions are produced by the non-equilibrium concentrations of electron donors on the one side and of electron acceptors on the other. Specifically, the reduced form of the electron donor is kept in excess over the oxidized form by metabolism, as is the oxidized form of the electron acceptor compared to its reduced form. As long as metabolism is operating, these disequilibria are maintained and asymmetric boundary conditions for ubiquinone diffusion function. The result is that on the average there is a non-zero flux of reduced ubiquinone (UQH_2) from the inside surface of the membrane to the outside surface, and a non-zero flux of oxidized ubiquinone (UQ) from the outside surface back to the inside surface. These two fluxes create a ubiquinone cycle. The physical motion of the ubiquinone molecule is provided by the thermal agitation. The thermal power associated with this motion is 3 micro-Watts. While this may at first seem small, compare it with the secular power of the minnow.

This description can be made quantitative by explicitly using the diffusion equation. Let $f(x,t)$ denote the probability density at time t for reduced UQH_2 and let $g(x,t)$ denote the probability density at time t for oxidized UQ. The inside surface of the membrane is located at $x = 0$ and the outside surface is located at $x = d$. In steady state, it is expected that the probability density for UQH_2 at the inside surface, denoted by Q_{in}^r, and the probability density for UQH_2 at the outside surface, denoted by Q_{out}^r, satisfy $Q_{in}^r > Q_{out}^r$, because UQH_2 is produced at the inside surface and is converted at the outside surface. Similarly, in steady state it is expected that the probability density for UQ at the inside surface, denoted by Q_{in}^o, and the probability density for UQ at the outside surface, denoted by Q_{out}^o, satisfy $Q_{out}^o > Q_{in}^o$, because UQ is produced at the outside surface and is converted at the inside surface. The reduced species satisfies the diffusion equation

$$\frac{\partial}{\partial t} f(x,t) = D \frac{\partial^2}{\partial x^2} f(x,t)$$

with the boundary conditions at steady state given by

$$f_{SS}(0) = Q_{in}^r \quad and \quad f_{SS}(d) = Q_{out}^r$$

where the subscript SS denotes the steady state values. Similarly, the oxidized species satisfies the diffusion equation

$$\frac{\partial}{\partial t} g(x,t) = D \frac{\partial^2}{\partial x^2} g(x,t)$$

with the boundary conditions at steady state given by

$$g_{SS}(0) = Q_{in}^{o} \quad and \quad g_{SS}(d) = Q_{out}^{o}$$

These equations are easily solved and have the steady state solutions

$$f_{SS}(x) = f_{SS}(0) - \frac{x}{d}\left(f_{SS}(0) - f_{SS}(d)\right)$$
$$g_{SS}(x) = g_{SS}(0) - \frac{x}{d}\left(g_{SS}(0) - g_{SS}(d)\right)$$

The probability currents, or fluxes, are defined by

$$-D\frac{\partial}{\partial x} f_{SS}(x) = \frac{D}{d}\left(f_{SS}(0) - f_{SS}(d)\right) > 0$$
$$-D\frac{\partial}{\partial x} g_{SS}(x) = \frac{D}{d}\left(g_{SS}(0) - g_{SS}(d)\right) < 0$$

wherein the left-hand sides define the fluxes in the manner that is standard for diffusion, and the right-hand sides are the results for the particular steady state solutions given above. The inequalities result from the boundary conditions. The meaning of these fluxes is simple, the reduced species goes from 0 to d and the oxidized species goes from d to 0, thereby creating the ubiquinone cycle.

3. MOTOR PROTEINS

The preceding considerations set the stage for a discussion of the function of motor proteins. In this presentation, the focus will be on kinesin, a motor protein that moves along microtubule tracks. Kinesin is a rather large molecule with a molecular weight around 500-600 kD [8]. It is made up of two heavy chains and two light chains. The heavy chain contains the head, i.e. the motor unit, that is comprised of about 340-350 amino acid residues. This region has a rough size of 7.5 nm x 4.5 nm x 4.5 nm. The head makes direct contact with the microtubule. The bulk of the heavy chain, called the neck, is a dimerized alpha-helical coiled coil. The total length of the complete kinesin is around 100 nm. The light chains are associated with the end of the molecule that binds the load. All of the catalytic activity is in the heavy chains. The ATP binding and hydrolysis takes place on the catalytic core of the heads. The neck is attached to the catalytic cores by segments of 15 amino acids called neck linkers.

The motion of kinesin is processive, i.e. many sequential steps occur before kinesin is completely released from the microtubule track. In one cycle ATP is bound, hydrolyzed and released while the trailing head detaches from the microtubule, moves forward to the next binding site and reattaches. The issue here is whether ATP powers a *power stroke* or whether ATP facilitates *rectified Brownian motion*. In the former view, the energy released by hydrolysis of the γ-phosphate of ATP causes a conformation change in the kinesin that results in the movement of one head by a distance of 16 nm in order for this head to move from one binding site to the next. In the latter view, ATP hydrolysis causes an irreversible switch of the bound head to the unbound state, and the movement of the head from one binding site to the next is caused by Brownian motion. No mechanism for the power stroke has been supported by experiment to date.

From the rectified Brownian motion point of view, it is important to note that the motion of the detached head is in the diffusion regime for reasons that parallel the situation for ubiquinone. Kinesin heads are larger than ubiquinone but only by about 40-fold, and they are much smaller than an E. Coli. The diffusion time for a kinesin head to move 16 nm, in the absence of a load, is 1.7 x 10^{-6} s. This is much faster than the chemical reaction steps for ATP hydrolysis and release, that are longer than milliseconds in duration. Thus, the head diffusion is far and away the fast step in the process [4]. In fact, phosphate release is the slowest step according to biochemical assays [9].

The motion of a kinesin head can be modeled as a mean first passage time process with a reflecting boundary condition at one end and a absorbing boundary condition at the other end. These boundary conditions reflect detachment and rebinding respectively. The Langevin equation for this process is reduced to a Langevin equation for the equivalent diffusion process and the backward Fokker-Planck equation for this process is used to solve for the first passage time distribution [4]. The mean first passage time as a function of load can be determined analytically but the first passage time distribution itself must be obtained numerically [4]. For diffusion constant D, a distance d and a load c, the solution for the mean first passage time, T(d), is

$$T(d) = \frac{1}{c}\left\{\frac{D}{c}\left(\exp\left[\frac{c}{D}d\right] - 1\right) - d\right\}$$

This formula has an exponential character that implies that while the value for no load is 1.7 x 10^{-6} s, the value for a load of 5 pN is 4.3 s, more than a million times longer. Indeed, 5-6 pN is the size of the stall load according to measurements made using laser tweezers [10]. In calculating detailed load-velocity profiles the head diffusion step requires use of the entire first passage time distribution rather than a mere insertion of the mean first passage time into the rate formula. This is a consequence of the broad, exponential tail in the mean first passage time distribution function [4]. The results

obtained agree quantitatively with experimental results and the use of the entire distribution moves the stall force from 5 pN to 6 pN for ATP concentrations of 2.0 mM. Changes in the ATP concentration change the load-velocity profile by altering the maximum velocity and the stall force, so long as these changes are below the saturation concentration of roughly 2.0 mM. For example, an ATP concentration of 8 μM gives a maximum velocity of only 80 nm/s and a stall force of 5 pN whereas for a 2.0 mM concetration the maximum velocity is 700 nm/s and the stall force is 6 pN. That these results are quantitatively consistent with the measured values is strong support for the model.

Evolutionary evidence also exists for the rectified Brownian motion model. In the rectified Brownian motion model ATP binding and hydrolysis cause the kinesin head to switch from microtubule binding to release. In the power stroke model this conformation change also results in the complete translocation of the head from the old binding site to the new one. In the rectified Brownian motion model diffusion of the head creates the translocation. Is there a precedent for nucleotide stimulated switching activity? The answer is yes. The G-proteins, that use GTP in place of ATP, are such switches. These proteins are central to the second messenger mechanism of hormone action [2]. Study of the amino acid sequences of the nucleotide binding sites for G-proteins, kinesin heads and myosins has revealed strong similarities. It has been proposed [8] that there was an ancestor protein that gave rise to G-protein switches on the one hand and to kinesin heads and myosin heads on the other. If so, this strongly suggests that kinesin and myosin heads function as switches too. The importance of nucleotide hydrolysis in these systems is that it ensures irreversibility of the switch rather than being a source of energy for a power stroke. The energy require to move a kinesin head is supplied by Brownian motion, and the directed, processive motion of kinesin results from asymmetric boundary conditions for the head diffusion.

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