MOLECULAR MOTORS

The miracle of motion

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Abstract

Biological motion from the contraction of muscles to the transport of material within the cells, is for a large part powered by molecular motors in the form of motor proteins. These tiny machines convert chemical energy into mechanical force that causes motion. In this seminar I present different phenomenological and physical models that describe the operation of these motor proteins.
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1. INTRODUCTION

Movement is one of the defining properties of life. So what enables us to contract our muscles? The first one to propose an answer was the Greek anatomist and physician Erasistratos of Ceon[2]. He associated muscle motion with the so-called »pneuma«, some sort of fluid or gas which after being produced in the brain is pumped through the body along hollow nerves as pipelines. When this fluid reaches the muscle it makes it swell and shorten thus producing force. (The similarity of design is what gives the name to modern day pneumatic machines). Though it was soon found out that muscles contract at constant volume it was not until 1847 that Hermann von Helmholtz formulated the idea that a muscle is an isothermal chemical machine\(^1\). It uses the energy obtained from chemical reactions to produce force.

A similar question as with the motion of macroscopic organisms can be asked regarding the motion of their microscopic parts. How do (eukaryotic)cells manage to move necessary materials and organelles inside of them? The answer as we will see can be found in the form of so-called motor proteins, molecular machines that convert chemical energy derived from the hydrolysis of ATP into mechanical work.

2. MOLECULAR MOTOR PROTEINS

Motivation and goals

It is amazing to think that nature has by means of evolution produced such complex systems. Among other things molecules arose that were able to move inside a cell, ceaselessly performing duties like transporting molecular materials, copying and translating the genetic code into proteins, communicating with other cells, adapting the cell shape to the external environment and reorganizing its interior in such a way to allow for complex processes like cell division. The same molecules can be found in everything from bacteria to whales.

Modern microscopy has transformed our view of the cell interior from a relatively static environment to one that is full of activity not unlike the traffic in a large city. Understanding the transportational system of the cells can help prevent or heal problems related to its malfunction such as cardiovascular and neuronal diseases, and also developmental defects.

\(^1\) Hermann von Helmholtz; Über die Erhaltung der Kraft, Berlin 1847
Understanding the physical principles on which motor proteins or enzymes work is also fundamental for the design of man-made devices and "non-biological" molecular machines.

A brief history

The first motor protein that was isolated was myosin, which drives the contraction of the muscles. In 1864, Kuhne and coworkers isolated myosin as a complex with actin filaments and in 1940 it was dissociated into separate proteins, myosin and actin. Dynein which drives the beating of the sperm was identified in 1963 and Kinesin which moves organelles inside the cell was purified in 1985. These three constitute the main families of motor proteins. Since then the pace of research has accelerated and hundreds of specialized motor proteins were discovered. The sequences of their amino acids contain regions that are similar to the domains of the original proteins. They participate in a wide range of processes that occur in the cell.

The principles of operation

Basically motor proteins are molecules which use the energy gained from cyclical ATP hydrolysis to move along cytoskeletal filaments which constitute the backbone of the cell. A motor generally attaches itself to a certain spot on the filament, hydrolyses an ATP molecule and moves one step along the filament. After this it can either disconnect (linear motor proteins) or stay connected and repeat many steps (processive m.p.). It is also interesting to note that a motor protein can only move along the filament in one direction. Different proteins can move in different directions.

3. MUSCLE CONTRACTION AND MYOSIN

Muscle contraction

The discovery of myosin crossbridges by H.E. Huxley in 1957 provided a molecular basis for the contraction of muscle. Muscles contract by shortening each sarcomere. Thin filaments on each side of the sarcomere slide past each other until they meet in the middle. Myosin filaments have club-shaped heads that project toward the actin filaments.
Myosin heads attach to binding sites on the actin filaments. The myosin heads swivel toward the center of the sarcomere, detach and then reattach to the nearest active site of the actin filament. Each cycle of attachment, swiveling, and detachment shortens the sarcomere. Hundreds of such cycles occur each second during muscle contraction.

ATP binds to the cross bridges between myosin heads and actin filaments. The release of energy powers the swiveling of the myosin head. Muscles store little ATP and so must recycle the ADP into ATP rapidly. Creatine phosphate is a muscle storage product involved in the rapid regeneration of ADP into ATP.
A more detailed view of myosin

In the picture above we can see the structure of myosin crossbridge in the muscles of a chicken which was solved using X-ray spectroscopy. The typical size of these proteins is in the range of several tens of nm. Roughly speaking they have heads and tails. The head or motor domains are globular proteins with the functionality of “legs” interacting with the molecular track. Each head has a catalytic site which initiates the ATP hydrolysis. The coiled-coil tail is essential for biological function, by forming filaments (like myosin in muscles) or binding to cargo (like kinesin in neuronal transport). The two main parts are connected by a converter.

In the rotating crossbridge model (Fig 3), there are four phases of motor operation.

1. the myosin head binds to the actin filament, catalyzes the release of phosphate and a highly strained ADP state is created

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2 Raymont et al. 1993, Science 261:50-58
2. The strain drives the rotation of the converter domain, which in turn rotates the lever (or tail) domain, amplifying the motion and moving the load through the working distance.

3. Following ADP release, ATP binds to the motor domain and causes dissociation of myosin from the actin.

4. While dissociated the crossbridge recovers to its initial conformation which moves the motor to the next binding site on the filament.

It is especially interesting how small structural changes associated with the chemistry of ATP hydrolysis are mechanically amplified into much larger conformational changes of protein domains. This amplification is at least ten fold. From a few Angstroms which is the size of a phosphate ion, to several nanometers, which is the size of a working stroke.

4. KINESIN AND INTRACELLULAR TRANSPORT

The motor protein kinesin is mostly employed for intracellular transport. It pulls organelles (like mitochondria) to a part of the cell where they are needed. It is a processive motor protein, which means it can make up to a hundred strokes and stay attached to its biopolymer track (which is a microtubule, consisting of many protein units) before it comes loose again, as opposed to myosin that makes only one stroke and detaches. The interesting thing about kinesin is that it literally steps over a microtubule. It has two identical units that function as feet (they have been rather unfortunately named as heads). Each unit measures about 7 nm. They communicate with each other through the state of the linker at the neck so that when one unit is attached the other can detach and make a »step«.

5. ATP HYDROLISIS

As was said previously the underlying physical principle of motor proteins is the conversion of chemical energy into mechanical work. The energy needed comes from hydrolysis of ATP (adenosin triphosphate). In this reaction a phosphate group is dissociated from the ATP molecule producing excess energy. The Gibbs free energy released can be described by the equation.

![structure of ATP](Fig 5)
\[ \Delta G = \Delta G_0 - kT \ln \left( \frac{[ATP]}{[ADP][P_i]} \right), \quad \Delta G_0 = -54 \cdot 10^{-21} J \]  

(5.1)

and depends on the value of the so-called standard free energy \( \Delta G_0 \), as well as on the cellular concentrations of ATP, ADP and P. In cells, the concentration of ATP is \(~1\text{mM}\), that of ADP is \(~10\text{mM}\) and that of phosphate is app. \(1\text{mM}\). Therefore the value of Gibbs free energy is around \(100 \cdot 10^{-21} J\), which is about \(25 \ k_bT\). The energy of \(1k_bT\) corresponds to the work done by a force of \(1\) pN acting through \(4\) nm.

6. PHYSICS OF THE MICROSCOPIC REALM

For a normal sized object the mechanics used to describe its motion involve, mass, gravity and inertia. But things are quite different for an object the size of a few nanometers. Inertial forces become negligible compared to the frictional forces and the Brownian motion of molecules. Since the Reynolds number is effectively zero (0.05 for a protein) we call this the overdamped realm. The dominant force acting on an object inside a cell is that of drag, resulting from the viscosity of the intracellular fluid.

The drag force is equal

\[ F_d = \gamma v \]  

(6.1)

\( \gamma \) drag coefficient

\[ \gamma = 6 \pi r \eta \] for a sphere with radius \(r\)

The size of the drag force on a globular protein of \(r = 6\) nm moving with the average instantaneous thermal velocity \(8\) m/s at room temperature is \(480\) pN, which is a very large force compared to the gravitational force acting on the same protein which amounts only to \(10^{-9}\) pN.

The drag force is a result of **collisions with water molecules**. A huge number of such collisions takes place per second resulting in Brownian motion of particles and diffusion. The diffusion equation describes how the average concentration of a collection of molecules changes over time

\[ \frac{\partial c}{\partial t}(x,t) = D \frac{\partial^2 c}{\partial x^2}(x,t) \]  

(6.2)

The solution for free diffusion from a point source is a Gaussian function

\[ c(x,t) = \frac{1}{\sqrt{4\pi Dt}} e^{-\frac{x^2}{4Dt}} \]  

(6.3)

\[^3\text{Alberty and Goldberg, 1992, Biochemistry 31:10610-10615}\]
We can see that the root mean square displacement (which equals the standard deviation) is

\[ x_{\text{rms}} = \sqrt{4Dt} \]  \hspace{1cm} (6.4)

and it increases with the square root of time. This means that the larger the cell becomes, the more ineffective diffusion as a means of transport will be. This can easily be seen by calculating the time needed by a protein to diffuse a distance of 1 m (which is still less than the length of certain nerve cells). For a protein radius of 3 nm diffusing in water \((D = 100 \mu m^2/s)\) that time is approximately 8 years, which tells us that cells need active transport.

The diffusion coefficient \(D\) is a macroscopic parameter. The link between it and the microscopic parameter of drag coefficient \(\gamma\) is given by the Einstein relation

\[ D = \frac{k_B T}{\gamma} \]  \hspace{1cm} (6.5)

Another important formula in our case is Boltzmann’s probability distribution. Because the molecules are being constantly agitated they get elevated to states of higher energy. Boltzmann’s probability distribution

\[ p_i = \frac{1}{Z} \exp\left(-\frac{U_i}{k_B T}\right) \quad Z = \sum_i \exp\left(-\frac{U_i}{k_B T}\right) \]  \hspace{1cm} (6.6)

gives the probability \(p_i\) of finding a particle or a system in the state \(i\) with energy \(U_i\). So if we have just two states with energies \(U_1\) and \(U_2\), then the ratio of the probabilities of the system being in the two states is

\[ \frac{p_2}{p_1} = \exp\left(-\frac{\Delta U}{k_B T}\right) \]  \hspace{1cm} (6.7)

If a protein has two states, state A where it is attached to a filament and state D where it is detached, without other energy inputs there will be equilibrium fluctuations between these two states. The ratio of probabilities is then given as above by Boltzmann’s equation. It is important that the motor protein breaks the randomness of the fluctuation between states A and D, and it does so by coupling the binding and unbinding with the chemical process of ATP hydrolysis [3].
7. PHYSICAL MODELS OF MOTOR PROTEINS

With a physical model of a molecular motor we try to correlate the measured microscopic properties of a single motor to the microscopic properties of the same motors that were calculated from macroscopic measurements on a muscle or the motion of organelles in the cell. The macroscopic properties of the muscle and their averages are

1. Speed of contraction $V$ from which the speed of the actual motors relative to the actin filaments can be deduced ($v$)
2. Force produced (this can again be used to calculate average force per motor $<F>$)
3. The distance of contraction (displacement per ATP of a single protein $\Delta$)
4. Time of contraction (Time $T_c$ (per cycle))
5. Stiffness $K$ (per motor)
6. and work $W$ (also per motor)

So we are interested primarily on the averages per motor obtained from macroscopic measurements. The same single motor properties can also be measured. The link between the macro averages and micro measurements is the fraction of time that individual crossbridges spend attached to the actin filaments, since that is the time they can produce force. This is the so called duty ratio $r$ and it is equal to the ratio of the working distance $\delta$, and the average displacement per ATP $\Delta$ (since we know that one ATP is consumed per cycle).

$$r = \frac{\delta}{\Delta} \quad (7.1)$$

Powerstroke model

The powerstroke model assumes that the force that generates the rotation of the head comes form an elastic element inside the protein (this element has not yet been identified). The conformational change in the crossbridge produces strain in the spring (immediately) which stretches beyond its resting length. We will take a closer look at one version of the powerstroke model and we begin by making a few assumptions

1. The actin filaments are moving past a crossbridge at a constant speed $v$
2. The crossbridge can only attach to the filaments at certain places which are a distance $d$ apart
3. At these places there is a high attachment rate $k_{on}$

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4 Adapted from A.F. Huxley, 1957 Prog. Biophys. Biophys. Chem. 7:255-318
4. The detachment rate rises quickly (a step) at a distance $\delta_+$ from the attachment position.

\[ k_{on} \quad \quad k_{off} \quad \quad \cdots \quad \quad 0 \quad \quad \delta_+ \quad \quad d \]

**Fig 6.** Attachment and detachment rates

The probability of a crossbridge being attached at a certain point $x$ is given by

\[ v \frac{dp_{on}}{dx}(x) = k_{on}(x)p_{off}(x) - k_{off}(x)p_{on}(x) \]  \hspace{1cm} (7.2)

The solution is

\[ p_{on}(x) = \frac{1}{\Delta} \exp \left( -\frac{x}{\delta_-} \right), \quad x > \delta_+ \]  \hspace{1cm} (7.3)

\[ p_{on}(x) = \frac{1}{\Delta}, \quad 0 < x < \delta_+ \]

**Fig 7.** The solution to the model

The total probability of being attached is given by integration over all positions.
If the speed of motion is slow the myosin heads will have a higher probability of attaching to the filament and conversely. The probability of attaching depends on the speed

$$P_{on} = \int_{-\infty}^{\infty} p_{on}(x)dx = \frac{1}{\Delta} (\delta_+ + \delta_-) = \frac{\delta_+}{\Delta} = r$$  \hspace{1cm} (7.4)

and since the next opportunity to attach again will occur after a distance $d$ the average path traveled by a motor between attachments is

$$\Delta(v) = \frac{d}{P_a}$$  \hspace{1cm} (7.6)

We can also calculate the average force produced by the motors on the filament

$$<F> = \frac{\delta}{\kappa} <x> = \frac{\delta}{\Delta} \kappa (p_+ <x_+> - p_- <x_->) =$$

$$= \frac{1}{2} \frac{\kappa \delta^2}{d} \left( 1 - \exp \left( - \frac{k_0 x_0}{v} \right) \right) \left[ 1 - \frac{2v^2t^2}{\delta^2} \right]$$  \hspace{1cm} (7.7)

This gives us an explicit formula for the force-velocity curve in terms of the measurable parameters. This formula has been found to be in good agreement with the measurements on whole muscles (Fig 8).

![Fig 8. Force-velocity curve for a myosin crossbridge in skeletal muscle. The circles are data from rabbit muscle (Pate et al. 1994, Biophys. J. 66:1554-1562) and the solid curve is the prediction of the powerstroke model.](image-url)
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Thermal ratchet models

Another complementary way to look at this process is by thermal ratchet models. The motor is considered as a Brownian particle diffusing freely in an asymmetric but periodical potential that is being switched on and off. When the potential is on it forces the motor to move to the nearest energy minimum. After that the potential is turned off and the protein moves with thermal diffusion. Because of the asymmetry of the potential it is more likely that the protein will fall in the closer minimum when the potential is switched on again. Thus directed motion is produced. The energy in this model is being used to power the switching of the potentials.

The thermal ratchet model has one major problem however. The maximum force it can generate against viscous loads is only around $2kT/d$ which for kinesin amounts to around 1 pN, much less than the measured value which is around 5 pN. Inspite of this the model was used successfully on certain simpler motor proteins.

8. EXPERIMENTAL METHODS

Experiments on muscle tissue

As said earlier we can get information on the macroscopic averages of motor protein properties from experiments on muscle tissue. The muscle is attached to an apparatus and activated by electrical stimulation. The force and speed of contraction are turned into an electrical signal by a transducer and recorded by a computer.

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5 Hunt et al., 1994 Biophys.J., 67:766-781
In vitro motility Assays

These are solutions in which purified motor proteins are attached either to a fixed substrate or to small balls. In the first case it is observed that when we put filaments on such a surface filled with motor proteins they move around and their speed can be measured. It was found that motor proteins can indeed produce movement at rates consistent with the speeds of muscle contraction.

Single molecule technics

It is also possible to attach a load (usually a bead with the diameter of about 1 micrometer) to a single motor protein and observe its motion along a microtubule. The position of the bead is measured by imaging it onto a photodiode detector. The maximum force that the motor can produce is obtained with the help of an optical trap.

![Diagram of motor protein and microtubule](image)

**Fig 9.** A force opposing that of a motor is applied by positioning an optical tweezer just behind the bead. The amplitude of the steps measured corresponds to the spacing of tubulin dimers along the protofilament (After Visscher et al., 1999.)

9. CONCLUSION

As we have seen we are indebted in many ways to motor proteins. Because of them we can move around, run and even think. Their structure is complex and their operation is a delicate interplay of many processes. We can hope that with a close collaboration between theory and experiment an increasingly realistic and quantitative description of these motors can be achieved and with it better understanding of living organisms.

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10. BIBLIOGRAPHY