Biology 5357: Chemistry & Physics of Biomolecules - Membranes & Membrane Proteins

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Lecture 7:
Membrane proteins: direct and secondary active transport

Mitchell, P. (1979). **Keilin's respiratory chain concept and its chemiosmotic consequences** Science 206(4423), 1148-1159. https://dx.doi.org/10.1126/science.388618

Skou, J. (1998). **The Identification of the Sodium Pump** Bioscience Reports 18(4), 155-169. https://dx.doi.org/10.1023/a:1020196612909

Transporters.

- Solute carrier transporters (SLCs)
- major facilitator superfamily
- ABC transporters
- ATPase pumps

For more in depth outline of families see http://www.tcdb.org/superfamily.php

Uniporters. This process is described as facilitated diffusion. The transport is driven by the concentration gradient of the substrate passing the membrane, and the end-point is equilibrated concentrations of the substrate on either side (provided it is not charged). In this way, it is very much like a channel, but involves binding of the substrate on the high concentration side, which couples to a conformational change that changes the access pathway to introduce the substrate on the other side, where the substrate can diffuse away. The transporter thus solves two issues. It can sequester the substrate that may be at low concentrations outside the cell, but even lower concentrations inside the cell. It can also do this specifically so only one type of substrate enters the cell. Second, it speeds up the movement of the substrate across the membrane, bypassing the kinetic barrier for free diffusion.

Note, for passive diffusion, the flux across the membrane is

$$J_A = -D\frac{dA}{dx} = P(A_{out} - A_{in})$$

where P = D/x. Thus, the flux is proportional to the concentration gradient across the membrane.

For facilitated transport, there is a binding reaction that is also involved. The reaction scheme for facilitated diffusion is the following:

$$R + A_{out} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RA \xrightarrow{k_3} R + A_{in}$$

Where the initial flux of the substrate A across the membrane is:

$$J_A = k_3[RA]$$

Here, the flux is proportional to the amount of bound receptor, i.e. substrate occupied receptor. According to mass balance:

$$R_0 = RA + R$$

At at initial time t = 0, $A_{out} = A_{total} = A_0$ and $A_{in} = 0$.

Also, the binding is defined as:

$$K_d = \frac{[A][R]}{[RA]}$$

Thus,

$$RA = R_0 \frac{A}{K_d + A}$$

and

$$J_A = k_3 R_0 \frac{A}{K_d + A}$$

Note, $J_A = J_{max}$ when $R_A = R_0$ (total amount of receptor), thus $J_{max} = k_3 R_0$. This is exactly analagous to the Michaelis-Menten kinetics from the study of enzyme activity, where v0 = JA, and Vmax = Jmax.

Note, this is a good model for the initial flux of the transport. However, the full reaction is more complex because in order to transport the second substrate, the transporter must reset

and return to the original conformation in the membrane. For unitary transporters, this means that the conformational changes can occur without any substrate.

Note, unitary transporters facilitate diffusion and are very similar to channels but differ in that they bind and sense the substrate and couple this to a conformational change that provides alternate access of the substrate to each side of the membrane. Therefore, by definition it must have an occluded state that it transitions through in order to constituted a transporter. If it provided a free diffusible path, it would technically be a channel.

Note, the overall steady-state transport depends on the kinetics of the full transport cycle, which may be different when occupied or in the absence of substrate. The different orientations of the transporter may have different binding affinities. Binding can typically be measured by Isothermal Calorimetry whilst the proteins are in detergent micelles or nanodiscs, provided the buffer condition is properly controlled for.

Some examples:

- sugar transporters: semiSWEET, GLUT

Secondary active transporters. The secondary active transporters are quite similar to the unitary transporters except that the move substrates uphill against their concentration gradient. In other words, they are used to establish concentration gradients, or perform uptake under conditions where the substrate is rare. In this way, it is indirectly linked to ATP hydrolysis, because it is the ATPases that set these driving ion gradients.

The way that this happens is that the transport cycle is linked to another substrate that moves down its concentration gradient. This is called the driving substrate. Thus, by utilizing the driving force of this substrate, the other one can sneak uphill.

There are two types of secondary active transport activity - symport, which moves the transport substrate in the same direction as the driving substrate, and antiport, which moves the substrates in opposite directions. Note, that conformational transitions are all spontaneous and reversible. The directionality of the net flux simply depends on the concentration gradients of the substrates.

The mechanisms of symport and antiport are fundamentally different. In symport, there is a critical requirement of the occluded state when the substrates are bound. The concentration gradient of the driving substrate cannot be dissipated otherwise all of the energy is lost. This also means that the conformational changes cannot occur with a single substrate - it must be both or nothing. Binding can be cooperative, and only the binding of both in proper stoichiometry enables the conformational change. Also, in order to reset, conformational changes must occur in the apo or unbound state.

In an antiporter, the situation is slightly different. The requirement here is that the conformational change which provides alternating access is occurring with substrate is bound. Both substrates being bound at the same time is not a requirement, but the transition requires the binding of one or another. This can be referred to as a ping-pong type of mechanism and leads to the uphill movement of one of the substrates.

Alternatively, antiporters can have a mechanism that is more similar to symport where there is simultaneous binding of substrates. The movement of the substrate in opposite directions is thus a highly coupled process. Take for example the case of 2:1 Cl-/H+ transport in the CLC transporters. Also note that in this case, there are not large conformational changes that occur but rather rotameric changes of sidechains along the transport pathway. This is enough to provide the required occluded state.

Key questions about the mechanisms of alternating access. Alternating access requires a conformational teeter totter which is linked to specific substrate binding. Examination of the topology of these transporters reveals that this is somewhat encoded within the protein fold. It appears that in all transporters are structured so that they possess alternate orientations of the same folded unit. Thus, this conformational teeter totter stability is encoded in the fold. Still there are major question as to how the conformational stability is linked to the different steps of transport to yield faithful transport activity without dissipating gradients.

- same fold & function, different stoichiometry
- same fold - different function
- high affinity ligands often block & inhibit, do not transport

In order to understand this, we must understand the dynamics between these conformational states and the underlying energy landscape. Static structures cannot inform on these mechanisms.

Some examples:

- The major facilitator superfamily - LacY, lactose permease
- GltpH
- CLC
- Na+/Ca2+ exchanger

ABC transporters. ATP Binding Cassette transporters, which possess a nucleotide binding domain where ATP binding, and hydrolysis leads to conformational changes in the transmembrane domains. These are actively driven by ATP and are essentially pumps but are referred to as transporters and are workhouses of cells. Substrate selectivity is not as heavy a requirement since the alternating access is driven by ATP hydrolysis.

Some examples:

- ModBC-A (nutrients, sugar, amino acids)
- BtuCD (vitamin B12, heme, siderophores)
- P-glycoprotein (expels all substance from the blood brain barrier)
- CFTR (an ion channel though)

Pumps/ATPases. These are the true pumps that are driven by ATP hydrolysis and establish all of the essential ionic gradients. For example, the Na+/K+ ATPase is responsible for establishing the Na+ and K+ gradients that are present in cardiac myocytes.

There are many types of ATPases (E-type, F-type. e.g. Mitochondrial H+ ATPase, P-type. e.g. Na+/K+ ATPase, Ca2+ ATPase, V-type. e.g. Lysosomal H+ ATPase, A-type) which differ in their mechanisms, but in general, the conformational changes are linked to ATP/ADP binding, hydrolysis/phosphorylation. They can work forwards (ATPase) or backwards (ATP synthase).

These are the proteins that are central to the chemiosmotic hypothesis proposed by Mitchell. Here, the proton gradient produced by the electron transport chain leads to the fueling of the F0-F1 ATP synthase in mitochondria, yielding the production of ATP. The mechanism is incredible, linking transport to the rotation of the transmembrane domain to catalyze ATP production from ADP and Pi.

Multidrug export. One of the key problems in biomedical research today is the emergence of antibiotic resistant bacteria, largely due to human intervention and overprescribing antibiotics. The adaptations and evolutionary mechanisms underlying antibiotic resistance usually involves one of more types of transporter. These organisms employ one of each type of transporter, and MFS transporter, ABC transporter and ATPase to expel all unwanted molecules from the cell. Thus, these are the proteins that are typically modified during antibiotic resistance. The other strategy is to modify the outer membrane by changing the distribution of certain types of lipids like Lipid A.

Measuring transporter activity and dynamics. Transporters are far more complex that ion channels. The conformational changes are highly coupled. Transporters are slow and more challenging to study by electrophysiological methods. At the same time, the ways in which these transporters work are fundamentally interesting but also inherently important to physiology since transporters are responsible for the long-term homeostasis of every cell.

- Radioactive uptake
- electrical recordings of ionic changes in and out of liposomes
- planar bilayers

- single-molecule studies of FRET
- EPR & NMR