Name Key

Chem 452 - Fall 2011 - Exam IV

Potentially useful facts:

Ideal Gas Law constant, $R = 8.314$ J/(mol•K) = 0.08206 (L•atm)/(mol•K)

Faraday's Constant, $F = 9.65 \times 10^4$ J/mol•V

1. We learned earlier in the semester that the First Law of Thermodynamics states that the total energy of the universe is a constant and therefore all processes involving energy must do so by transforming energy from one form to another. In our discussions of pumps, signal transduction pathways and molecular motors, we saw numerous examples of free energy transformations. In a sentence, describe the free energy transformation that take place for each of the following systems:

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   a. Lactose permease: The free energy that is released as protons (H^+) flow down both a chemical and electrical potential gradient across a plasma membrane is used to transport a lactose molecule up a concentration gradient and into the cell.

   b. The Na^+/K^+ pump: The chemical free energy released upon hydrolysis of ATP is used to transport a Ca^{2+} ion up a concentration gradient across the sarcoplasmic membrane.

   c. ABC transporter: The chemical free energy released upon hydrolysis of ATP is used to transport metabolites up a concentration gradient across cell membrane. The example that we discussed was the multi-drug resistance transporter that gave cancerous cells resistance agains chemotherapy drugs by actively transporting them out of the cell.

   d. Myosin: The chemical free energy released upon hydrolysis of ATP is used to mechanically move the myosin head group along an actin filament. The hydrolysis of ATP coupled to the conformational change that leads to this movement..

   e. The bacterial flagellum: The free energy that is released as protons (H^+) flow down both a chemical and electrical potential gradient across a membrane is used to drive the rotational motion of the bacterial flagellum.

   f. ATP synthase The free energy that is released as protons (H^+) flow down both a chemical and electrical potential gradient across a membrane is used to drive the rotational motion of the ATP synthase, which leads to the synthesis of ATP from ADP and Pi.

2. The action potential of nerve cells involves both Na^+ and K^+ selective channels. In a couple of sentences, describe how the K^+- channel is able to select K^+ ions over Na^+ ions, and how the Na^+-channel is able to be select Na^+ ions over K^+ ions. In order for these channels to transport either ion, the ions must first be dehydrated, which requires energy. To compensate for the energy required to dehydrate these ions, the channel must form favorable interactions with the ions during their transport across the membrane. In the case of the K^+ selective channel, because the Na^+ ion is smaller than the K^+ ion, it is unable to make these compensating interactions. In the case of the Na^+ selective channel, the channel's opening is smaller and thereby excludes the larger K^+ ion.

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3. Last June, Michael Oldham and Jue Chen published and article in the *Proceedings of the National Academy of Sciences* (*PNAS 108*, 15152–15156) that provided snapshots of the maltose transporter at steps along its reaction pathway. The abstract for their article reads:

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> ATP-binding cassette transporters are powered by ATP, but the mechanism by which these transporters hydrolyze ATP is unclear. In this study, four crystal structures of the full-length wild-type maltose transporter, stabilized by adenosine 5′-(β,γ-imido)triphosphate or ADP in conjunction with phosphate analogs BeF_3^{1-} , VO_4^{3-} , or AlF_4^{1-} were determined to 2.2- to 2.4-Å resolution. These structures led to the assignment of two enzymatic states during ATP hydrolysis and demonstrate specific functional roles of highly conserved residues in the nucleotide-binding domain, suggesting that ATP-binding cassette transporters catalyze ATP hydrolysis via a general base mechanism.

a. Is the maltose transporter described an example of *active* or *passive* transporter? active transport

b. How many molecules of ATP are hydrolyzed for each maltose transported? two

c. Your textbook described a functional difference between eukaryotic and prokaryotic ATP-binding cassette transporters. If I told you that in this case, the maltose is being transported into the cell, is the system described more likely to be *eukaryotic* or *prokaryotic*? prokaryotic

d. The abstract indicates that the authors looked at different crystal structures of the transporter with different ATP or ADP analogues bound to it. One of these was ADP plus VO_4^{3-}. What step along the reaction pathway does this combination of ligands mimic? the transition state

e. The abstract also indicates that ATP hydrolysis may be catalyzed by way of a general base mechanism. What is a "general base" and what might be an example of what they are alluding to?
We discussed general acids and bases back in our discussions on catalytic strategies. There, a general base catalyst was defined as a molecule other than water that accepts a proton in a reaction. The examples that we encountered were mostly ionizable amino acid side-chains, in particular those of aspartic acid, glutamic acid, histidine and cysteine.

f. If the free energy for the hydrolysis of ATP is -51 kJ/mol and the plasma membrane potential is -60 mV, with the outside more positive than the cytoplasmic side, what ratio of maltose concentrations, inside to outside, can be maintained across the membrane at 37°C? (Be sure to refer back to your answer to part b. in doing this calculation.)
Since there are two moles of ATP involved in the transport of each mole of maltose, there is 2(-51 kJ/mol) = -102 kJ/mol of free energy available to establish the gradient. Therefore:

$$\Delta G = RT\ln\left(\frac{[maltose]_{in}}{[maltose]_{out}}\right) + z\mathcal{F}\Delta V \qquad (z = 0 \text{ for maltose}); \qquad \left(\frac{[maltose]_{in}}{[maltose]_{out}}\right) = e^{\left(\frac{\Delta G}{RT}\right)} = e^{\left(\frac{102\ \text{kJ/mol}}{(8.314 \times 10^{-3}\ \text{kJ/mol·K})(310\ \text{K})}\right)} = 1.5 \times 10^{17}$$

4. A major theme of the signal transduction pathways is *signal amplification*. Describe what this means and how it is effected. A common theme of all the signal transduction pathways is the marked amplification of the signal. The binding of a single signal molecule, called the primary messenger, to its cognate cell surface receptor can lead to a large number of secondary messengers being produced. This arises because the primary messenger activates enzyme activity, which can produce many secondary messengers in response to a single receptor binding event. Many pathways also involve the activation of protein kinases, which can also activate a number of proteins in response to a single receptor binding event. Many signal pathways have multiple amplification, so the total amplification can become quite large.

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5. In class we focused on four examples of signal transduction pathways. Based the descriptions below, identify the components for each pathway that fit the descriptions. "None" is a valid option:

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signal	epinephrine	EGF	insulin	angiotensin II
Type of receptor (7TM or tyrosine kinase)	7TM	Tyrosine Kinase	Tyrosine Kinase	7TM
Secondary Messenger(s)	cAMP	None	Phosphoinositide 3,4,5-trisphoshate (PIP_3)	Inositol 1,4,5-trisphosphate (IP_3) Diacylglycerol (DAG) Ca^{2+}
Pathway involves a G-protein (Yes/No)	Yes	Yes	No	Yes
Pathway involves an SH2 domain (Yes/No)	No	Yes	Yes	No
Name of a protein kinase involved in the pathway	Protein Kinase A (PKA)	Raf, MEK, ERK	PIP_3-dependent protein kinase (PDK), Akt (PKB)	Protein Kinase C (PKC)

6. Describe the model that explains how the free energy of a membrane proton gradient is coupled to the rotation of the bacterial flagellum. The same model is used to describe the rotation that occurs in the enzyme ATP synthase, which leads to the synthesis of ATP from ADP and P_i.

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There is a transmembrane protein, in this case, MotA-MotB proteins, which contain two half-channels, one that is open to the outside of the membrane, while the other is open to the inside. For a proton to make it across the membrane, it must first travel down the one half-channel, then jump onto the MS ring, which is made up of FliG proteins. The MS ring then rotates to release the proton into the other half-channel so that it can continue its travels across the membrane. In this way, movement of protons down a concentration gradient is coupled to the rotation of the MS ring. The MS ring is connected to the flagellum, so when it rotates so does the flagellum.

7. In our discussions, we have run across multiple examples of *P-loop NTPases*. For each of the following P-loop NTPases, indicate which NTP is used, and in a sentence, describe what happens when NDP is exchanged for NTP:

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System	Nucleotide that is bound (GTP or ATP?)	In a sentence, describe what happens when NDP is exchanged for NTP
Myosin	ATP	The myosin head group dissociates from the actin filament.
β-adrenergic receptor G_α protein	GTP	The G_α subunit dissociates from the $G_{\beta\gamma}$ subunits and from the β-adrenergic receptor.
Tubulin	GTP	The polymerization of the microtubules becomes more favorable.
Actin	ATP	The polymerization of the actin filaments becomes more favorable.
Kinesin	ATP	The kinesin head group associates with the associated microtuble.

8. We have also discussed a number of examples for the involvement of signal transduction pathways and molecular motors with diseases. In a sentence, explain the molecular role that each of the following has in relationship to a disease that is associated with either a signal transduction pathway or molecular motor:

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a. Taxol: Taxol is an anticancer agent that inhibits the proliferation of cancer cells by binding to and stabilizing microtubules, and thereby interrupting cell division.

b. The Ras protein: The Ras protein is a monomeric G-protein in the EGF signal transduction pathway, which when bound with GTP, activates the Raf protein kinase. Leaving it in an activated state can lead to uncontrolled cellular growth.

c. Tumor repressor genes: For many of the signal transduction, phosphorylations lead to an "on" state, which can lead to uncontrolled cell growth. Tumor repressor genes code for the phosphatases that turn off theses signal transduction pathways.

d. Chlolera toxin: Cholera toxin catalyses the ADP-ribosylation of the G_a protein and stabilizes it "on" state. This leaves the protein kinase A in the "on" state and ultimately to the inhibition of Na^+ ion take up by Na^+/H^+ exchanger. This leads to a loss of NaCl and water and consequently to diarrhea and dehydration.

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