

Solutions to 7.014 Quiz I

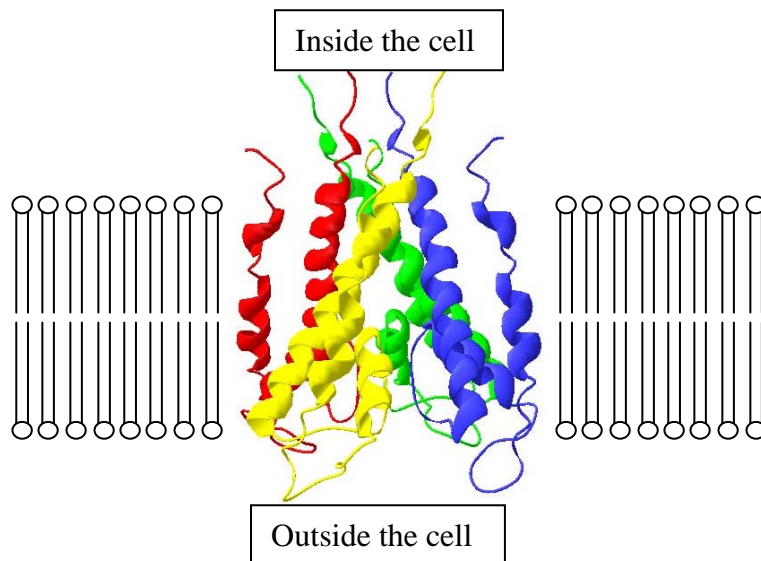
Class Average = 72

Median = 73

| <u>Grade</u> | <u>Range</u> | <u>%</u> |
|--------------|--------------|----------|
| A | 82 - 100 | 22 |
| B | 70 - 81 | 39 |
| C | 57 - 69 | 28 |
| D | 41 - 56 | 10 |
| F | 0 - 40 | 0 |

Question 1 (36 points)

Below is a ribbon representation of the K^+ channel, a membrane spanning protein made up of four copies of a single polypeptide. The K^+ channel allows K^+ ions to be shuttled through the membrane.

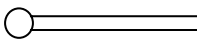


a) What protein secondary structure is part of the K^+ channel protein as shown above?

K^+ channel contains only α -helices.

b) Does the K^+ channel have quaternary structure? If yes, describe it.

Yes, the channel has quaternary structure. It consists of four interacting polypeptides. It appears that each polypeptide interacts with its two neighboring polypeptides, such that the overall shape of the channel is a barrel.

c) Using  as a schematic of a phospholipid, draw a cross-section of the membrane around the K^+ channel shown above.

d) What type(s) of amino acids do you expect to find on the K^+ channel polypeptides

i) next to the tails of the membrane lipids? (Circle all that apply)

Polar

Nonpolar

Positively charged

Negatively charged

Why?

The tails of the membrane lipids are hydrophobic, and it is energetically favorable for the protein to have hydrophobic amino acids in the hydrophobic environment.

ii) next to the heads of the membrane lipids? (Circle all that apply)

Polar

Nonpolar

Positively charged

Negatively charged

Why?

The heads of the membrane lipids have negatively charged phosphate groups, and it is energetically favorable for the protein to have positively charged amino acids that can form ionic bonds, or polar amino acids that can form hydrogen bonds in that environment.

Question 1, continued

- e) If you were trying to estimate the volume occupied by this protein, would the picture above provide all the information you need? Why or why not?

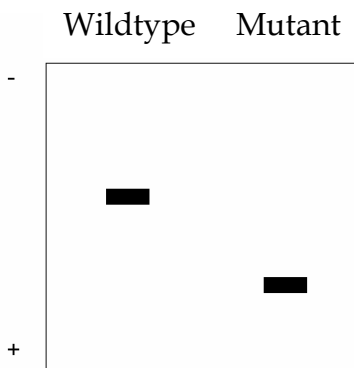
No, the picture above is of a ribbon representation, i.e. backbone trace. It does not show any of the sidechains, and thus does not give us a clear picture of how much space the protein occupies. In addition, for the atoms in the backbone, the vdW radii are not shown.

The positively charged K^+ ion is a very small soluble molecule.

- f) Explain why K^+ cannot come across the membrane without a channel protein.

The tails of the membrane lipids are hydrophobic. It is not energetically favorable for a charged molecule, such as K^+ to be in a hydrophobic environment. Thus, K^+ by itself can not come across the membrane.

You isolate a mutant of the K^+ channel that transports less K^+ than normal. You run both the wild-type and mutant proteins on a denaturing gel and get the following result:



- g) From the gel data we can conclude that (circle all that apply):

shorter than

- i) Each subunit of the mutant protein is the same length as in the wild-type protein.

longer than

Justify your answer(s)

The mutant protein shows only one band on the gel, meaning that it consists of only one type of subunit. Denaturing gel separates protein fragments based on their size, with shorter fragments traveling further. Since the mutant subunit travels farther on the gel, we conclude that the mutant subunits are shorter than the wild type ones.

primary

- ii) The mutant and wild-type proteins could differ in their secondary structure.

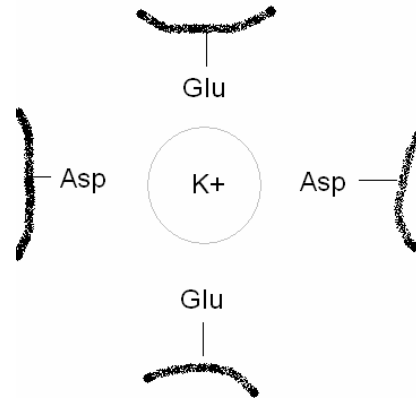
tertiary

Justify your answer(s)

Because the mutant subunits are shorter than wild-type subunits, it is clear that the primary structure is affected. If the lost fragment is of significant length, tertiary structure will most likely be affected too. And depending on where the fragment the lost in the mutant subunit is located in the wild-type protein, it is possible that the secondary structure would also be altered.

Question 1, continued

The K^+ channel has several binding pockets in which K^+ ions may associate. Below is an image of one of the binding pockets of the K^+ channel shown from above.



h) Circle the strongest type of interaction that exists between the K^+ ion and the Glu residues.

Covalent bond Van der Waals Hydrogen bond Ionic bond

i) You isolate a series of mutant K^+ channel proteins where the two Asp residues have been replaced by amino acid X (see table below). For each X, indicate whether K^+ binding in the resulting pocket will be stronger, weaker, or the same and give a brief explanation of your choice.

| X | Interaction (choose one) | Explain why |
|-----|-----------------------------|--------------------------------------------------------------------------------------------------------------------------|
| Asn | stronger <u>weaker</u> same | Same size, weaker interaction – H-bond instead of ionic bond |
| Leu | stronger <u>weaker</u> same | Approximately same size, weaker interaction – vdW instead of ionic bond |
| Phe | stronger <u>weaker</u> same | Weaker interaction – vdW instead of ionic bond. Phe is also significantly larger than Asp, so steric hindrance is likely |

j) Suppose you isolate another mutant that has four Glu residues instead of two Asp and two Glu residues in the pocket above. You find that the mutant has decreased K^+ transport. Explain this result.

Glu is a negatively charged amino acid, just like Asp. Therefore, mode of interaction, i.e. ionic bond would be preserved. The only difference between Glu and Asp is the extra CH_2 group in Glu. Since the function of the protein is impaired, we conclude that it is harder for the K^+ ion to fit into the new, smaller binding pocket.

Question 2 (36 points)

a) Fill in the blanks:

An autotroph is an organism that is capable of making its own food store, while a heterotroph must rely on getting food from the environment.

b) What types of organisms carry out

i) glycolysis (circle all that apply)

autotroph heterotroph prokaryotes eukaryotes

ii) photosynthesis (circle all that apply)

autotroph heterotroph prokaryotes eukaryotes

Below are simplified chemical flowcharts of glycolysis and the Krebs (citric acid) cycle.

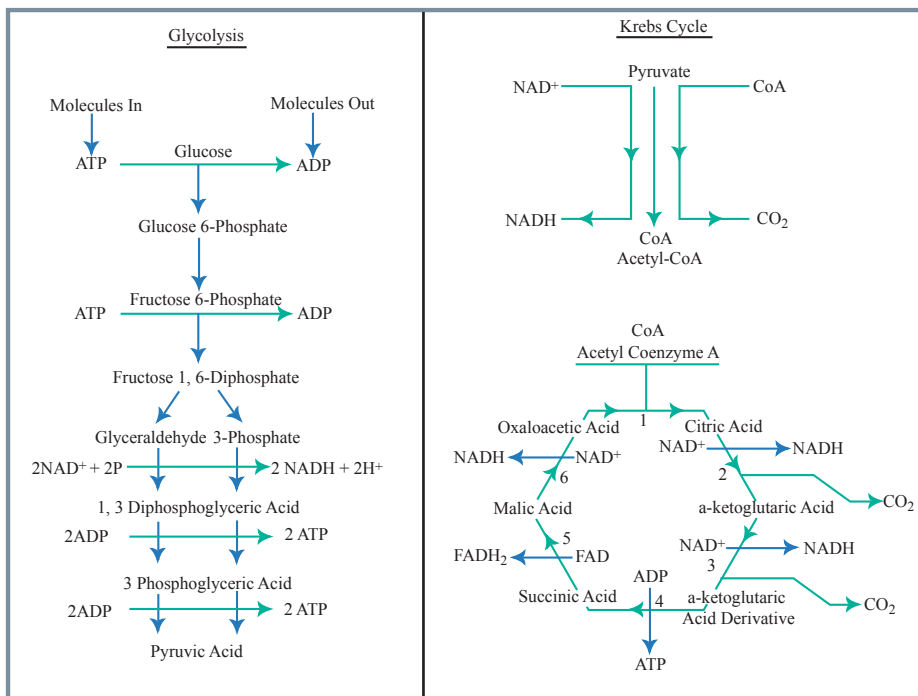


Figure by MIT OCW.

In the course of glycolysis, NAD⁺ is reduced to NADH.

c) In glycolysis, what is the original source of the electrons that are used to reduce NAD⁺?
The original source of electrons in glycolysis is glucose.

Question 2, continued

The glycolysis pathway produces energy from glucose.

- d) In what molecule is this energy stored? *ATP*
- e) Where in that molecule is the energy that is used to perform work stored? Be specific.
In ATP, the energy used to do work is stored in the phosphate bonds.

Breakdown of the molecule (in d) is often coupled with other reactions in the cell, making the new, coupled, reaction proceed at an appreciable rate.

- f) Describe one mechanism that is commonly used in such coupled reactions.
One common mechanism involves transferring the third phosphate of the ATP onto one of the reactants, thus raising its energy state such that the new, coupled reaction is now favorable. Another mechanism involves performing ATP hydrolysis on the same enzyme, but in a different active site than the reaction being catalyzed. In this mechanism, ATP hydrolysis often causes the enzyme to change conformation, allowing it to catalyze the main reaction.

S. oxyphiliae is an organism that can undergo fermentation or respiration.

- g) You take equal aliquots of the same *oxyphiliae* culture and supply both with equal amount of food. You place aliquot A into an airtight bottle and aliquot B into an open shallow container.
- i) Are the cells in cultures A and B deriving all of their energy in the same way?
Explain.
No. Culture A derives all of its energy from glycolysis, while culture B derives additional energy (ATP) from Krebs cycle and oxidative phosphorylation.
- ii) Will one culture run out of food faster? If yes, state which culture, and explain why.
If no, explain why not.
Yes. Because culture A derives all of its energy from glycolysis, it only produces 2 molecules of ATP/molecule of glucose. Thus, it will have to utilize more glucose to perform same amount of cellular functions as culture B that derives 36 molecules of ATP/molecule of glucose through respiration.
- iii) At the time when both cultures run out of food, will there be approximately the same number of cells in each? Why or why not?
No. Because culture B utilizes its food stores more efficiently, it will be able to maintain cellular functions for longer on the same amount of food. Therefore, it will have more time to divide, and it will have more cells than culture A.
- h) In the Krebs cycle itself, only one ATP molecule is generated per molecule of pyruvate. Yet we know that respiration overall yields more additional ATP molecules. Beginning with the products of the Krebs cycle, briefly outline how that additional ATP is generated.
Krebs cycle is run in mitochondria, where e^- rich NADH and $FADH_2$ unload electrons onto the electron transport chain (ETC). As electrons are passed down the ETC, protons are pumped into the space between the inner and outer membrane, forming gradient. Because of the gradient, it is favorable for H^+ to get back across the membrane. That proton motive force powers ATP synthase to add a P_i to an ADP, creating ATP.

Question 2, continued

- i) What is the main overall product of the dark reactions of photosynthesis?

The overall reaction of photosynthesis is $6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6(\text{glucose}) + 6\text{O}_2$, and the main product is glucose.

All enzymatic reactions, including those in glycolysis and the Krebs cycle, are reversible.

You decide to study where in the cell glycolytic enzymes are found. You are surprised to find that these enzymes are not only found in the cytoplasm, but also in chloroplasts.

- j) Explain why these enzymes are found in chloroplasts.

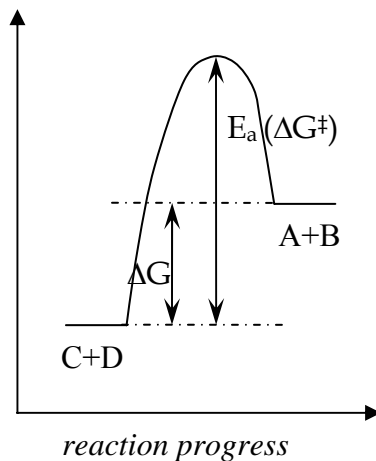
In the process of generating glucose from CO_2 , the cell runs some of the reactions of the glycolytic pathway in reverse. Because all enzymatic reactions are reversible, enzymes used to run the pathway backward in chloroplasts are the same that are used to run glycolysis in cytoplasm.

- k) Explain, in evolutionary terms, why two species that diverged billions of years ago still use many of the same enzymes and reactions.

Once a pathway is developed, any mutant (M) of the pathway that accidentally arises is likely to be less efficient. Even if some descendant of M, M1, if it was to acquire another mutation, might end up being significantly better than the original non-mutant organism, M itself is not likely to survive or generate enough progeny to allow M1 to ever appear. Thus, evolutionary pressure works against changes to key pathways, ensuring significant conservation throughout evolution.

Question 3 (14 points)

Below is the energy diagram for the reaction $\text{C} + \text{D} \rightarrow \text{A} + \text{B}$.



- a) On the energy diagram above, label the following:

• $E_a(\Delta G^\ddagger)$

• ΔG

• A+B

• C+D

- b) Based on the diagram above,

$\Delta G < 0$

$\Delta G = 0$

$\Delta G > 0$

Question 3, continued

c) How would the enzyme change

i) ΔG ?

Explain

ΔG is unaffected by the presence of an enzyme. This is because it is a thermodynamic property of the reaction, and is determined by the amount of energy available to perform the work of converting reactants to products and vice versa. Since the energy is stored in the bonds, it is independent of the path the reaction takes, or the rate at which it occurs.

ii) E_a (ΔG^\ddagger)?

Explain

Activation energy will be lower in the presence of the enzyme. Enzymes lower the activation energy of the reaction, allowing it to proceed.

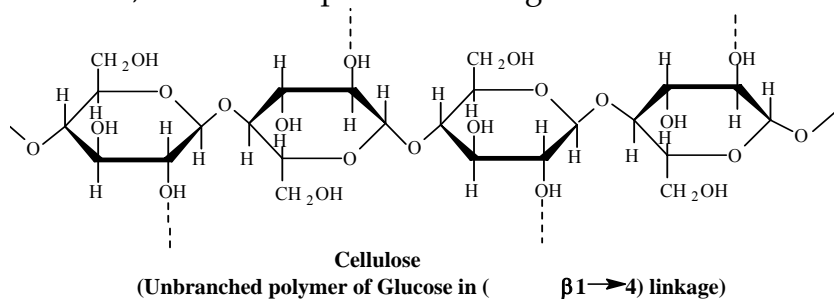
iii) speed of reaction?

Explain

The rate of reaction is affected. Reactants reach the transition state due to random fluctuations in energy caused by molecular motion. If E_a is lowered, much less energy is required to reach it, so more molecules will be able to do so, and the rate of the reaction will increase.

Question 4 (14 points)

A popular line of research in developing environmentally cleaner and cheaper energy sources focuses on biomass fuel, or using ethanol produced from organic sources as fuel. In order for this approach to be economical, sources such as corn stover, wheat straw, wood chips and waste paper would need to be converted to ethanol. All these potential sources are rich in cellulose, molecule depicted in the figure below.



A class of enzymes called cellulases that break down cellulose would be required to start the process of converting these materials into ethanol.

a) Starting with cellulose, briefly outline the pathway needed to produce ethanol.

The pathway will be

Cellulose \rightarrow glucose \rightarrow pyruvate \rightarrow ethanol.

Question 4, continued

In the 1990's a group of researchers, called bioprospectors, set out to find bacteria that could help in the production of biomass fuel. For biomass fuel production, cellulases would have to remain functional in the harsh conditions used by industry (high temperature and acidity).

To isolate thermo- and pH-stable cellulases, bacteria from several sources were examined.

b) Among the possible sources of bacteria listed below, choose the one you believe to be the most likely to produce cellulases stable at high temperature and low pH. For your choice, list the strong and weak points.

- Cow rumen (stomach)
- Human stomach
- Tree bark
- Acidic springs near active volcanoes

Acidic springs near active volcanoes would provide high temperature and low pH environments. Thus, if these bacteria express cellulases, these cellulases should be stable in conditions used by the industry. The weak point of this choice is that we do not a priori know whether bacteria living in this environment would have evolved to produce cellulases. If their environment is not rich in cellulose, but is rich in other organic sugars, these bacteria might not express cellulases.

The bioprospectors eventually isolated the bacteria *Acidothermus cellulolyticus* in Yellowstone National Park. These bacteria secrete cellulases that function optimally at 80°C and pH 5, conditions that denature most proteins.

c) What type of bond might help stabilize the protein tertiary structure under such conditions?

We can expect that disulfide bonds would stabilize proteins under these harsh conditions.

Bacteria do not produce enough cellulases for industrial applications. Scientists are now attempting to make high levels of the cellulases in plants for more efficient production.

However, active cellulases can not be produced in plants without destroying the plant. Cellulases have multiple subunits, and when each subunit is manufactured in a separate plant, the plants are not destroyed.

d) Explain why manufacturing the entire enzyme in plants destroys the plants, whereas manufacturing separate subunits does not.

Because plants are rich in cellulose, expressing massive quantities of the enzyme in a plant would cause the active enzyme to break down cellulose in the cell, and eventually destroy the plant.

Each subunit separately does not have catalytic activity, so none of them separately would destroy their host plant.

Once all subunits are purified separately and assembled together in the test tube, the entire enzyme should demonstrate catalytic activity.