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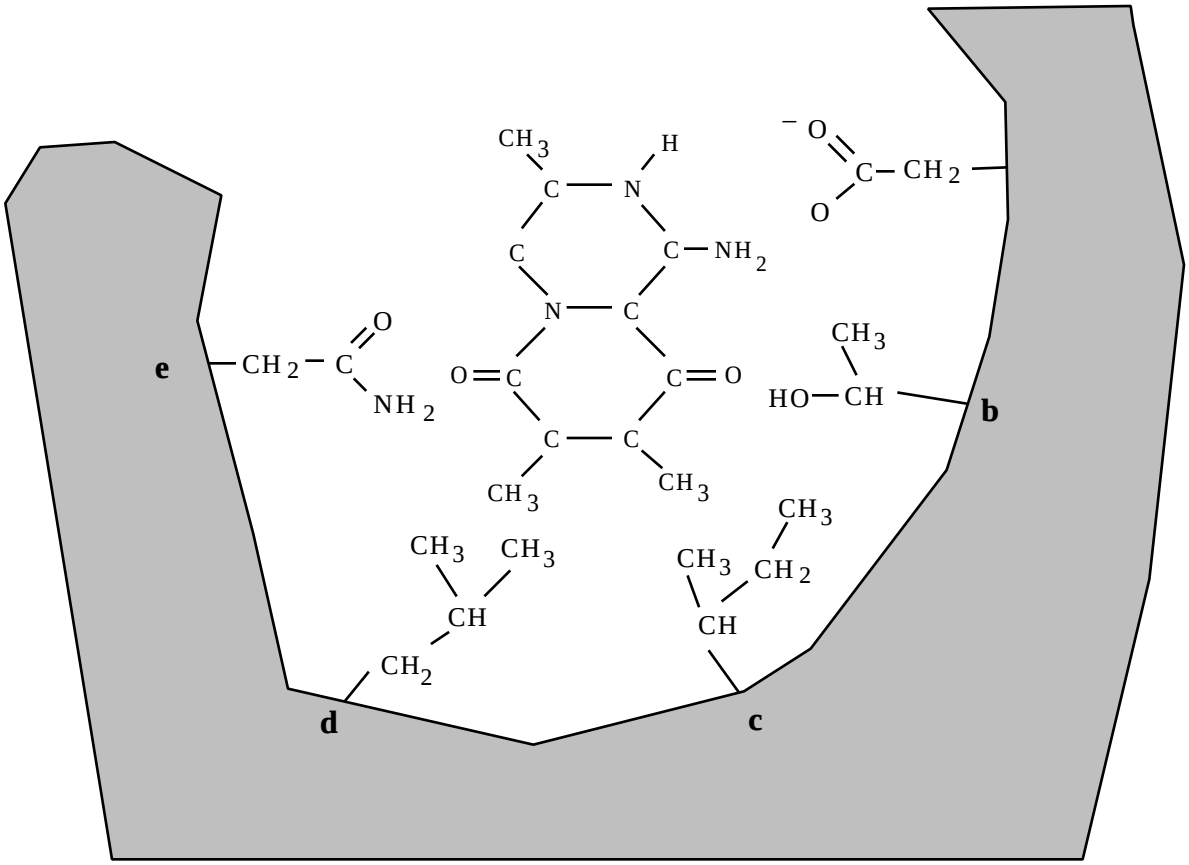
Solutions to 7.014 Problem Set 2

Question 1

A schematic of the active site of an enzyme with its substrate bound is shown below. The side chains of the amino acids important to the function of that active site are shown as a-e.



a) Identify each of the amino acids a-e and indicate the <u>strongest</u> type of non-covalent interaction it makes with the substrate.

#	Amino acid	Interaction with substrate
a	*aspartate*	*Hydrogen bond*
b	*threonine*	*Hydrogen bond*
c	*isoleucine*	*van der Waals*
d	*leucine*	*van der Waals*
e	*asparagine*	*Hydrogen bond*

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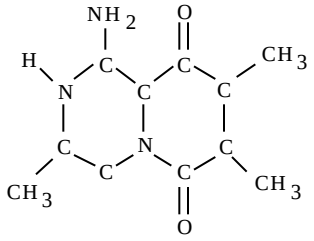
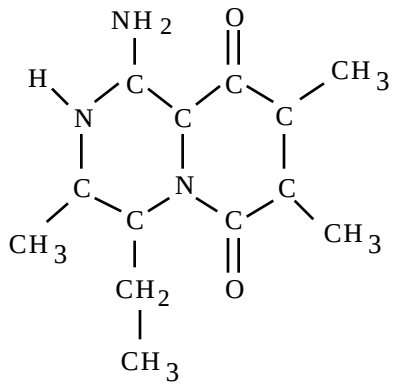
Question 1, continued

The isolated substrate for this enzyme is shown below (rotated 90° to the left). For parts b) and c) below ignore size considerations.



b) For each of the following, indicate if the interaction with the enzyme would be stronger than that seen with the original substrate, the same as that seen with the original substrate, or weaker than that seen with the original substrate. For each, explain your reasoning.

i)

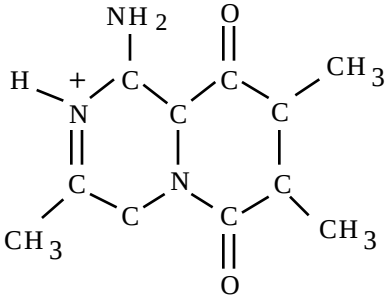


interaction (circle one): stronger weaker (same)

reasoning:

This change is on a region of the substrate that does not bond to the enzyme. It does not change the interaction of enzyme with substrate.

ii)

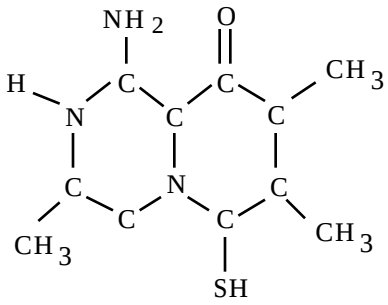


interaction (circle one): (stronger) weaker same

reasoning:

The positive charge on the N can now form an ionic bond with the side chain of amino acid (a) of the enzyme.

iii)



interaction (circle one): stronger (weaker) same

reasoning:

The SH group no longer forms a hydrogen bond with the asparagine (amino acid e) of the enzyme.

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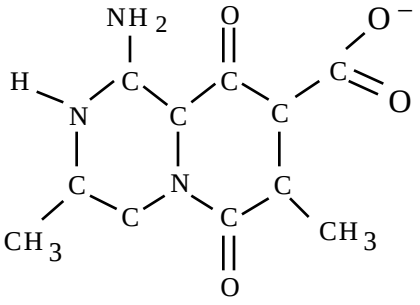
Question 1, continued

c) The following modified substrate does not bind. You want to change one amino acid of the enzyme active site to allow this version of the substrate to bind. Your change should maximize the strength of the interaction between the substrate below and the enzyme.



i) Which amino acid would you change?
You would change amino acid c.

ii) To what would you change it and why?
Change it to something positively charged such as lysine, histidine, or arginine because this would allow the formation of an ionic bond.

Question 2

Speedy, Jerry, and Mickey are three mice that live in your apartment. One night you notice that Speedy, less speedy than usual, is the last mouse to reach the cheese that you dropped on the floor. The next week he approaches your frosted flakes with a swaggering gate and unusual twitching. Two days later you find Speedy dead. You pick him up, gently place him in a plastic sandwich bag and take him to the lab where you are a UROP. The following week Mickey meets the same fate.

Your labmate believes that the mice died from bacterial infections of the fluid in the spinal cord and brain, but you believe that they may have suffered from a prion disease (http://science-education.nih.gov/nihHTML/ose/snapshots/multimedia/ritn/prions/prions1.html). You perform the following experiment independently on each dead mouse.

1. Remove the brain.
2. Suspend brain matter in liquid.
3. Divide the suspended brain matter into three different samples.
4. Label the samples and treat each sample in the following way.

    Sample 1: treat with an agent that destroys nucleic acid
    Sample 2: treat with a protease
    Sample 3: do not treat

5. Inject each of three new mice with a different sample and wait.

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Question 2, continued

a) You observe the mice treated with the samples and find that…

- the mouse treated with sample 1 gets sick and dies.
- the mouse treated with sample 2 stays healthy.
- the mouse treated with sample 3 gets sick and dies

Are these results consistent with death by a bacterial infection, a prion disease or neither? Explain using the results seen with both sample 1 and 2.

These results are consistent with death from a prion disease. Prions are infectious proteins, and as such, they would be unaffected by an agent that destroys DNA, hence mice injected with sample 1 would get sick. However, if the infectious agent was bacterial, then treatment with an agent that destroys DNA would kill the bacteria and mice injected with sample 1 would stay healthy.

b) You observe the mice treated with samples from Mickey's brain and find that…

- the mouse treated with sample 1 stays healthy.
- the mouse treated with sample 2 stays healthy.
- the mouse treated with sample 3 gets sick and dies

Are these results consistent with death by a bacterial infection, a prion disease or neither? Explain using the results seen with both sample 1 and 2.

These results are consistent with death from a bacterial infection disease. If the infectious agent is bacterial, then treatment with an agent that destroys DNA would kill the bacteria and mice injected with sample 1 would stay healthy. In addition, treatment with an agent that destroys protein would also kill the bacteria so mice injected with sample 2 would stay healthy.

c) You think that the causative agent of a prion disease may be abnormal cheeseheadin (CHN), a protein that localizes to synapses in the brain.

i) Explain what would happen if you mixed abnormal CHN protein with normal CHN protein under conditions that are identical to cellular conditions.

The abnormal CHN protein (the prion) would convert the normal protein to the abnormal form.

ii) Do you expect both versions of CHN to have the same tertiary structure? Explain why or why not.

No, The abnormal CHN protein (the prion) has an altered shape, and thus has different tertiary structure.

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Question 3

Ignatius Maloy Thursdy, a longtime employee of a large bourbon manufacturer, decides to open up a bourbon microbrewery of his own. To perfect the recipe, he sets up several fermentations in his basement, identical in every way to the open-vat fermentations carried out in the brewery where he works, except his "vats" are 250 mL, rather than the several thousand liters as at the brewery.

a) Iggy worries that something is wrong when he observes yeast growing in the samples much faster than at the brewery. When the yeast stops growing, he finds that no ethanol has been produced, hence no bourbon. Why did Iggy's fermentations fail?
In the small vessels, O_2 was readily available for the growing yeast, and rather than ferment glucose to ethanol, they used the pyruvate for respiration.

b) Dismayed by his failure, Iggy decides to go back to school to study the biochemistry of the enzymes involved in glycolysis. He's fascinated by the enzyme triose phosphate isomerase, because the reaction it catalyzes (DHAP ←→ G3P) proceeds so quickly in the cell. He reasons that since this reaction occurs so quickly in the cell, it would probably occur very quickly in the absence of enzyme as well.

i) Is his reasoning sound? Explain.
No, Even if the reaction is thermodynamically favorable, it may not occur due to the activation energy ($\Delta G‡$). Without enzyme, the ($\Delta G‡$) can prevent the reaction.

ii) This reaction is truly reversible and the reverse reaction is equally as likely as the forward reaction. Explain why the reverse reaction, G3P → DHAP seldom occurs in the cell.
G3P is rapidly removed in the next step of glycolysis. This drives the reactions in the forward (DHAP ← G3P) direction.

c) Iggy needs your help. For both i) and ii) fill in the blank with the appropriate response and explain.

i) The Gibbs free energy of Glucose would be _____ Glucose-6-phosphate.

higher than ***lower than*** **the same as**

Explain.
Adding phosphate groups to glucose to form Glucose-6-phosphate requires ATP.

ii) The Gibbs free energy of Phosphoenolpyruvate would be _____ Pyruvate.

higher than **lower than** **the same as**

Explain.
We know from glycolysis that this reaction produces ATP.

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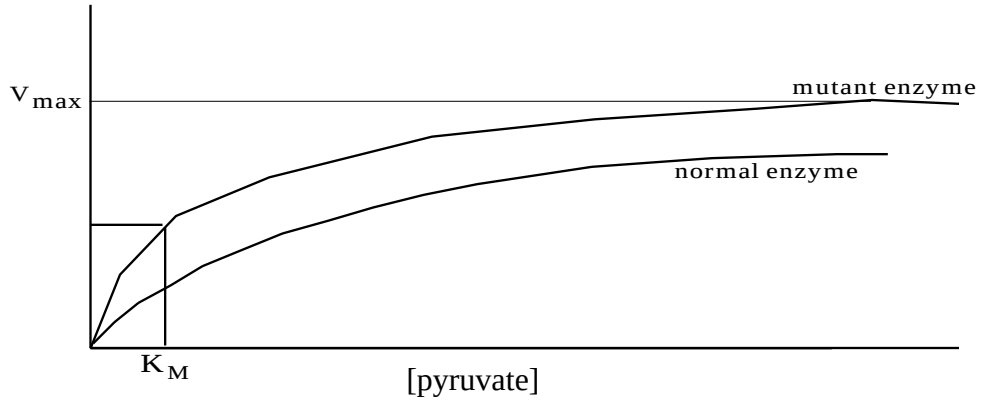
Question 3, continued

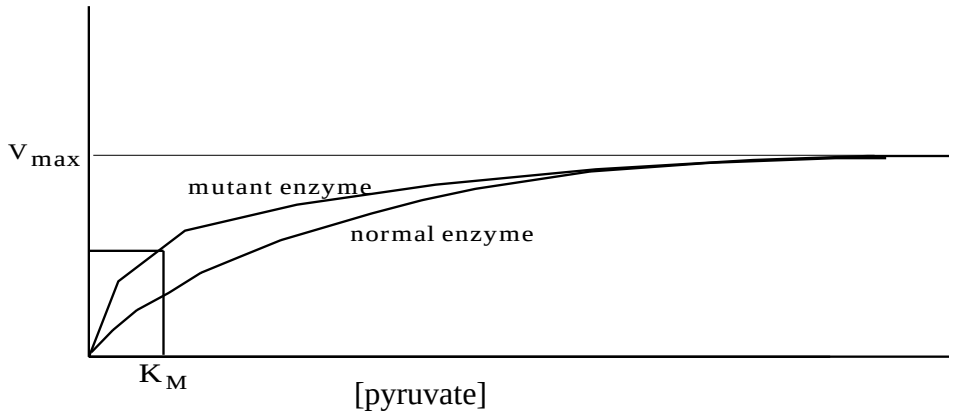
d) After completing his dissertation, Dr. I.M. Thursdy founds a biotech company with the aim of improving the fermentation process. He isolates 2 strains of mutant yeast that produce ethanol faster than normal yeast. Both have mutations in the enzyme that catalyzes the reaction pyruvate → acetaldehyde + carbon dioxide. He examines the kinetics of both the mutant and normal enzymes.

He finds that at low [pyruvate], the initial velocity of the reaction for both mutant enzymes is higher than that of the normal enzyme. At high [pyruvate], the initial velocity of the reaction for mutant enzyme A is higher than the normal enzyme, while for mutant enzyme B the reaction is the same as that of the normal enzyme.

i) The data for the normal enzyme is shown below. On the same graph, draw the curve of [substrate] vs. V_i for mutant A and label the V_{max} and K_m for each mutant enzyme.



ii) The data for the normal enzyme is shown below. On the same graph, draw the curve of [substrate] vs. V_i for mutant B and label the V_{max} and K_m for both.



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Question 4

In both cyclic and non-cyclic photophosphorylation, energy from the sun can drive the conversion of CO_2 into glucose

a) Explain why respiration is sometimes referred to as the opposite of photosynthesis.
Photosynthesis = CO_2 ← glucose and Respiration = glucose ← CO_2
Photosynthesis produces O_2 and Respiration use it.

b) Even though respiration is sometimes referred to as the opposite of photosynthesis, there are many similarities in these two processes. ATP is generated by a similar mechanism in respiration, cyclic and non-cyclic photophosphorylation. Explain how the movement of electrons from one protein complex to another results in the formation of ATP in these processes.
Electrons are passed from one protein to another more electronegative one. In doing so, H^+ ions are passed across a membrane creating a charge and concentration gradient. The H^+ ions flow down this gradient with the concomitant formation of ATP from ADP.

c) Explain why the evolution of non-cyclic photophosphorylation was a prerequisite to the evolution of respiration.
Until the evolution of Non-cyclic photophosphorylation, there was no oxygen in the atmosphere. Non-cyclic photophosphorylation obtains electrons from H_2O forming O_2. Respiration requires O_2 to act as the final electron acceptor.