

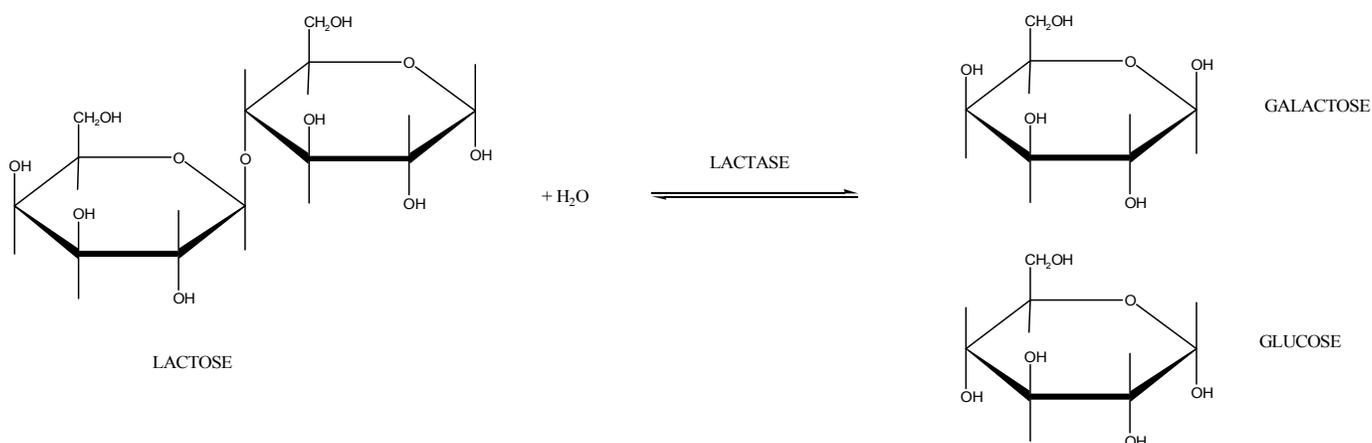
Dusty Carroll

Lesson Plan 4: Getting to know Lactase

Background Information

Lactase is the enzyme responsible for breaking down the lactose in your body. Lactose is a disaccharide that is found in milk. Human babies produce lactase in large quantities, but the production of the enzyme decreases significantly into adulthood. Deficiency of the lactase enzyme causes many people to have trouble digesting the lactose found in milk. This leads to the familiar problem of lactose intolerance. (1)

Lactase can act as a catalyst for several different biological reactions. The lactase enzyme is the only human enzyme that can cleave a β -glycosidic linkage like that found in lactose. The specific reaction that is the focus of this lesson is the breakdown of lactose into the two monosaccharides, galactose and glucose as seen in the reaction below:



Much research has been done on the mechanism of this reaction. In order to understand the mechanism, however, a brief overview of the structure of the enzyme is necessary.

The lactase enzyme is found in humans and other organisms. In the human intestines, lactase is combined with another enzyme called phlorizin hydrolase to form a transmembrane enzyme complex called lactase-phlorizin hydrolase. It has been shown that the lactase portion of this enzyme complex is the only portion active in the breakdown of lactose. (2) The mechanism under study for this lesson comes from the lactase found in the *Escherichia coli* bacteria. It was here that molecular biologists discovered the phenomenon of enzyme induction. Basically, the presence of lactose induced the biosynthesis of an enzyme to split it. (3) The lactase-lactose system became the focus of much research. Essentially, the lactase enzyme is genetically regulated. In other words, the action of the enzyme results from its synthesis which is regulated by the genes that code for it.

The remainder of this lesson will concentrate on the independent lactase enzyme (not that complexed with the phlorizin hydrolase). The structure of lactase is rather complex. Its crystal structure contains four identical subunits. Each subunit contains a chain of 1023 amino acid residues. When this structure was determined, it was the longest polypeptide for which an atomic structure had been obtained. (3) It is a very large enzyme and scientists continue to query about the biological reasons for such a large structure. Since each region of the enzyme seems to have a clear purpose, the common belief is that portions of the molecule were useful in certain ways and it just sort of...happened. The large size does not appear to have any reason other than that resulting from the combination of all of its parts. (3,4)

Primary Structure (5)

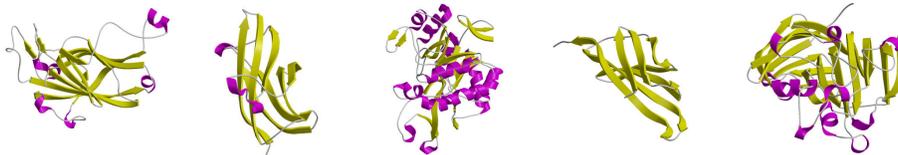
Again, the lactase enzyme consists of four identical subunits or chains. Below is the sequence of amino acids that make up just one of those chains. The sequence is shown using the one-letter abbreviations for the amino acid residues.

```
GSHMLEDPVVLQRRDWENPGVTQLNRLAAHPPFASWRNSEEARTDRPSQQLRSLNGEWRFAWF
PAPEAVPESWLECDLPEADTVVPSNWQMHGYDAPIYTNVTYPITVNPFFVPTENPTGCYSLTFN
VDESWLQEGQTRIIDGVNSAFHLWCNGRWVGYGQDSRLPSEFDLSAFLRAGENRLAVMVLRW
SDGSYLEDQDMWRMSGIFRDVSL LHKPTTQISDFHVA TRFNDDFSRAVLEAEVQMCGELRDYLR
VTVSLWQGETQVASGTAPFGGEIIDERGGYADRVTLRNLNVENPKLWSAEIPNLYRAVVELHTAD
GTLIEAEACDVGFREVRIENGLLLLNGKPLLIRGVNRHEHHLHGQVMDEQTMVQDILLMKQNN
FNAVRC SHYPNHPLWYTLCDRYGLYVVDEANIETHGMVPMNRLTDDPRWLPAMSERVTRMVQ
RDRNHPSVIIWSLGNESGHGANHDALYRWIKSVDPSPRVQYEGGGADTTATDIICPMYARVEDD
QPFPAPVKWSIKKWLSLPGETRPLILCEYAHAMGNSLGGFAKYWQAFRQYPRLQGGFVWDWV
DQSLIKYDENGNPWSAYGGDFGDTPNDRQFCMNGLVFADRTPHPALTEAKHQQQFFQFRLSGQ
TIEVTSEYLF RHSDNELLHWMVALDGKPLASGEVPLDVAPQ GKQLIELPELPQESAGQLWLTVR
VVQPNATAWSEAGHISAWQQWRLAENLSVTLP AASHAIPHLTTSEMDFCIELGNKRWQFNRS
GFLSQMWIGDKKQLLTPLRDQFTRAPLDNDIGVSEATRDPNAWVERWKAAGHYQAE AALLQC
TADTLADAVLITTAHAWQHQGKTLFISRKTYRIDGSGQMAITVDVEVASDTPH PARIGLNCQLA
QVAERNWLGLGPQENYPDRLTAACFDRWDLPLSDMYTPYVFPSENGLR CGTRELNYGPHQW
RGDFQFNISRYSQQLMETSHRHLLHAE EGTWLNIDGFHMGIGGDDSWSPSVSAEFQLSAGRYH
YQLVWCQK
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From this list it is easy to see why scientists are curious about the size of the enzyme. The four chains together amount to 4092 amino acid residues!

Secondary Structure

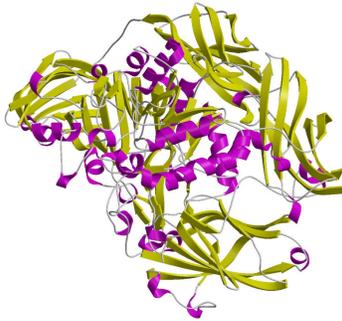
Each of the four chains is analyzed as having five separate domains. Each domain serves a different purpose in the enzyme. Some act to help the polypeptide chain attain its tertiary structure. Some act to hold one chain to another to aid in the formation of the quaternary structure. Some join with a domain on another chain to form the active site for the molecule. Below is a picture of the five domains found on chain A of lactase. These images are taken from the CATH Protein Structure Classification website. (6)



Note that each domain appears to have different secondary structures. The 3rd domain is the one where most of the helices appear. The 4th domain has no helices and consists of only beta sheets. The other domains contain mostly beta sheets and a few sections of helices. The seemingly individual nature of each domain may be necessary to facilitate the folding of the entire chain. (4)

Tertiary Structure

Together, these five domains form just one chain of the enzyme with a molecular weight of 116,570.8 D:

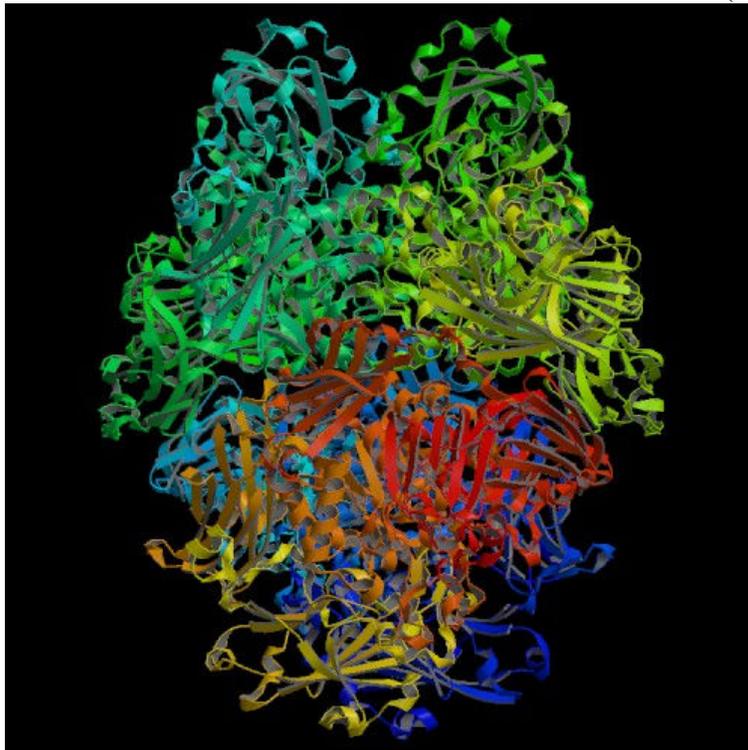


Jacobsen, Zhang, DuBose & Matthews, in their *Nature* article (4) show a similar structure of the one chain with the domains labeled. The picture in the article is a stereo view of the chain.

Quaternary Structure

Many pictures of the full enzyme are available. (3,4,5,7) The overall structure is a homotetramer consisting of four identical chains. It is thought that the individual monomers form first, followed by formation of dimers, then dimerization of the dimers to form the tetramer. (7) Each domain has a hydrophobic core, consistent with the notion that the monomers formed individually before joining. Some of the domains then interact with each other through polar networks on their exterior surfaces. There are three major regions of interface formed from complementary regions of the monomers. There are four active sites in each tetramer. Each site is formed from the interaction of two of the monomers. Each site is also marked by two metal ligands, Na^+ and Mg^{2+} . In the image from the Juers, *et.al.* article, the domains on each monomer are colored such that each domain on a given monomer is a different shade of the same color.

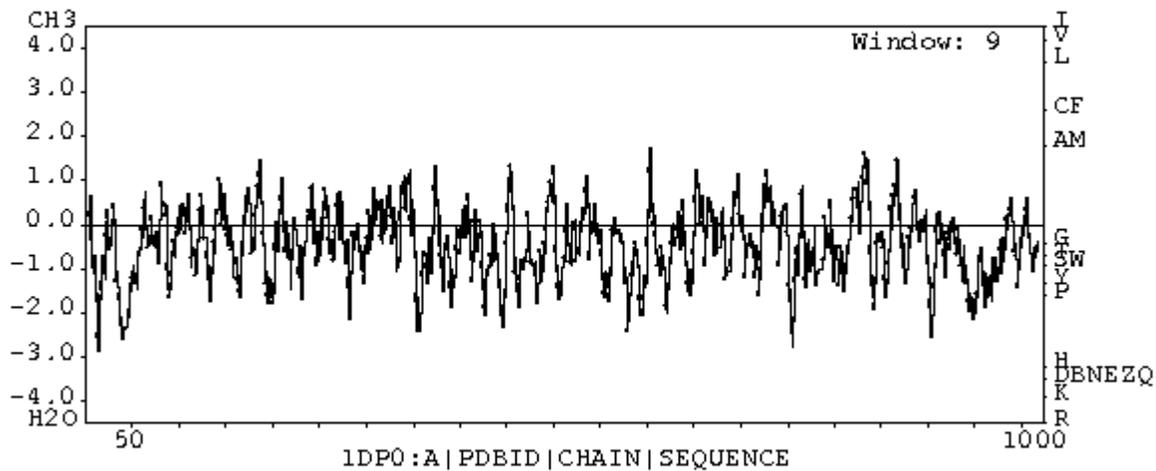
Another image of the overall structure is found on the RSCB Protein Data Base (5) as seen below:



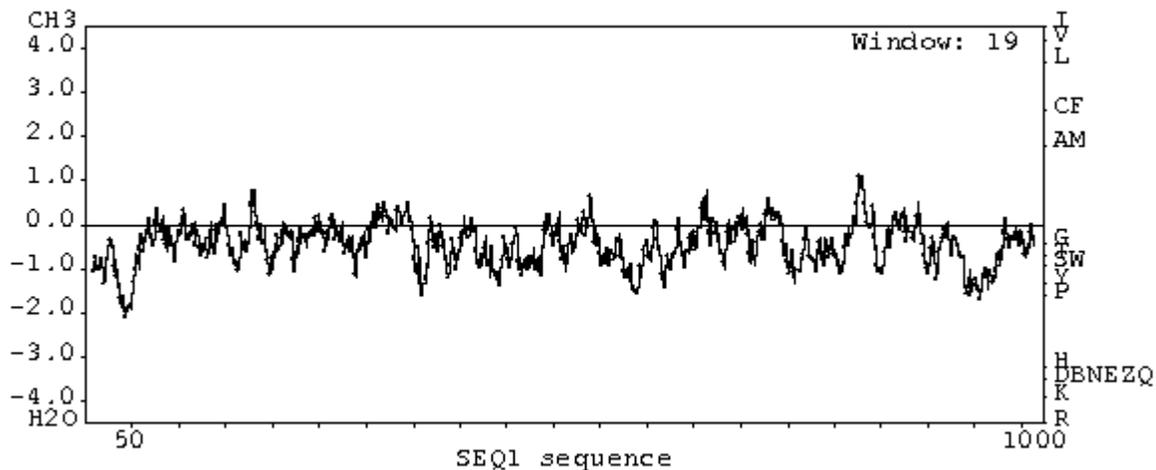
Here you can see how the interactions of the four monomers form into a single tetramer.

Kyte-Doolittle Hydropathy Plot (8)

This plot is calculated for a single 1023 residue chain of the lactase enzyme.



With a window size of 9, this plot shows many peaks below the midline. This corresponds to surface regions of the globular protein. With so many peaks, it appears that there is a large surface area which is hydrophilic. (Hydrophilic regions are given negative values.)



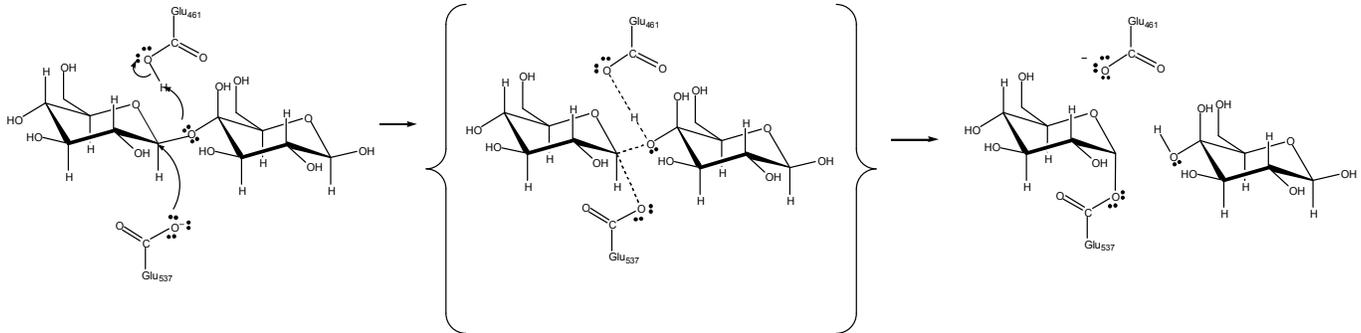
With a window size of 19, possible transmembrane regions show as peaks above 1.8. This lactase chain shows no peaks and therefore is unlikely to have any transmembrane regions. The lactase-phlorizin hydrolase enzyme noted in the introduction is a transmembrane enzyme. According to this plot, the lactase portion of that enzyme would not be the portion spanning the interior and exterior of the membrane.

Reaction Mechanism

The Enzyme Commission code for lactase is 3.2.1.23.

- 3 – Hydrolases
 - 2 – Glycosylases
 - 1 – Glycosidases (enzymes hydrolyzing O- and S-glycosyl compounds)
 - 23 – lactase or beta-galactosidase

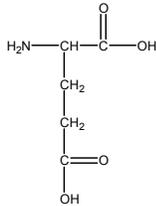
In other words, lactase acts as a catalyst for the hydrolysis of the O-galactosidic bond in the sugar, lactose. The exact mechanism of the reaction has been studied and Juers, Heightman, *et.al.* have summarized previous work and given clarification to the proposed mechanism. (9) Lactase, aka β -galactosidase, hydrolyzes its substrate (lactose) while allowing the constituent monosaccharides to keep their stereochemistry. The reaction is a two step reaction. The first step is cleavage of the glycosidic bond.



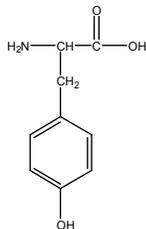
It is believed that this process takes place with a mechanism somewhere between that of an S_N1 and that of an S_N2 . The Glu537 from the active site of the enzyme acts as a nucleophile toward the anomeric carbon of the galactosyl group. This forms an intermediate with enzyme Glu537 in the alpha-glycosidic orientation. As seen in the above reaction, this is facilitated by a concerted protonation of the glycosidic oxygen. This particular step is not well-proven, yet, but it is one explanation. The acid responsible for protonation may be the Glu461 from the active site of the enzyme.

Functional Amino Acid Residues within Lactase

Glutamic acid: In its anionic state (like Glu537), this acts as a nucleophile. In its neutral state (like Glu461), it acts as an acid and donates a proton as described in the text.



Tyrosine: The $-OH$ group on the side chain participates in hydrogen bonding in order to stabilize the transition state.



The second step is the transfer of the galactosyl product from the nucleophile of the enzyme (Glu537) to an acceptor molecule. This step is believed to occur in an S_N1 release of the nucleophile. During this process, the carbocation (oxocarbenium ion) transition state is thought to be stabilized by interactions between Glu537, Tyr503 and the oxygen on the galactosyl ring. Glu461 abstracts a proton from the acceptor molecule, allowing the acceptor molecule to act as a nucleophile toward the oxocarbenium ion.

There is some debate as to whether the metal ligands in the active site play a role in the catalysis. They appear to facilitate the catalysis, but the exact mechanism for this is not yet determined. The major metal ion ligands found in the enzyme are magnesium and either sodium or potassium ions. An alternate theory of mechanism for the first step

of this reaction involves magnesium forming a complex by direct electrophilic attack on the glycosidic oxygen. This reaction will not be further discussed, but it is important to realize that there are still several possibilities for showing the actual reaction mechanism for this catalysis.

Catalytic Function

Catalytic efficiency values are altered by pH and also by the absence of magnesium for the lactase enzyme. (9) Though the mechanism through which magnesium ion works is not clear, it appears that its

presence is necessary for optimal catalysis by lactase. This may be some sort of enzyme regulation by the metal ions, but the exact parameters are still unknown. The efficiency values are listed by Juers, Heightman, *et.al.* as:

- $K_{\text{cat}} \approx 60 \text{ s}^{-1}$
- $K_m \approx 1 \text{ mM}$ or $1 \times 10^{-3} \text{ M}$ (This value is reported elsewhere as 4 mM) (10, 11)

These values are used in a ratio to determine the catalytic efficiency of the enzyme as follows (*I*):

$$k_{\text{cat}} / K_m = 60 \text{ s}^{-1} / 1 \text{ mM} = 60,000 \text{ s}^{-1} \text{ M}^{-1}$$

Enzymes whose ratios are in the range of $10^9 \text{ s}^{-1} \text{ M}^{-1}$ are considered to be the most efficient. This shows that lactase has a pretty good efficiency.

Target Audience for Lesson

This is an interdisciplinary lesson intended for a combination AP chemistry/AP biology class. The school in which I teach puts great value on interdisciplinary lessons, so it is quite realistic to combine these two classes. It will be necessary for the chemistry teacher and the biology teacher to collaborate on this lesson. In addition to the background information in this lesson, both teachers should have a working knowledge of basic chemical kinetics.

The AP biology students will have a strong background in the structure and function of enzymes. The AP chemistry students will have a strong background in the kinetics of chemical reactions.

Objectives

- Students will gain a basic overview of the structure and function of the lactase enzyme.
- Students will describe the differences between a unimolecular process and a bimolecular process.
- Students will use given data to determine the kinetic aspects of a given reaction.

Lesson

Using the classroom projector, a structure of lactase will be on the screen (from the Protein Data Base website⁵). Students will be broken into groups of four with approximately equal numbers of chemistry and biology students per group. Students will complete the “Introductory Worksheet” together for 5 minutes.

Discussion

- Review the correct answers to the worksheet
- Using the structure on the projector, point out the various aspects of structure that can be seen
 - The four chains are colored differently
 - Helices and beta sheets as symbolized
 - Strands that are not helices or beta sheets
 - Show the regions where the active sites are
 - Discuss the meaning of “active site” (the specific portion of the enzyme that interacts with the substrate)
- Connect the idea of an enzyme to that of a catalyst (small group discussions)
 - Write the following on the board:
 - Speeds up reaction
 - May interact with substrate but is not used up
 - Lowers activation energy for reaction

- Tell groups to discuss what this means. Chemistry and biology students should have a different perspective to share with each other. Highlights should be:
 - Speeds a reaction
 - Necessary in the body because without enzymes, reactions would be too slow
 - May interact with substrate but is not used up in the reaction
 - Substrate is the reactant that the enzyme works on
 - Lowers the activation energy
 - Provides a lower energy path for the reaction to begin, yet overall free energy of the reaction is not affected
- Have groups write the overall reaction for the following:
 - “lactose, a disaccharide, is broken into galactose and glucose by hydrolysis when the enzyme lactase is present”
 - Student reactions should match the reaction in the introduction. All of these structures are in any AP biology textbook and several AP chemistry textbooks. Students just need to make an equation out of it.
 - “lactase” should be written above the arrow; water and lactose are the reactants; galactose and glucose are the products; students should recognize the 1:1 stoichiometry throughout the reaction.
 - Ask whether the reaction mechanism is obvious from this reaction (no).
 - Ask how a mechanism might be determined.
 - Only by experimentation
 - Determining the rate equations and rate constants
- The exact mechanism for this process is under debate.
 - May be unimolecular or bimolecular
 - Chemistry students: explain to the bio students how this relates to the reaction order
 - For elementary steps, molecularity is the same as order (uni = 1st order, etc)
 - Biology students explain to the chem. students how this relates to the enzyme and substrate
 - For a unimolecular reaction the rate depends only on the concentration of the substrate
 - For a bimolecular reaction the rate depends on the concentration of the enzyme and of the substrate
- Summarize: More experimentation is necessary to determine the exact reaction mechanism for the lactase enzyme. This may be accomplished through kinetic studies similar to the basic kinetics we study at this level.

Follow-up

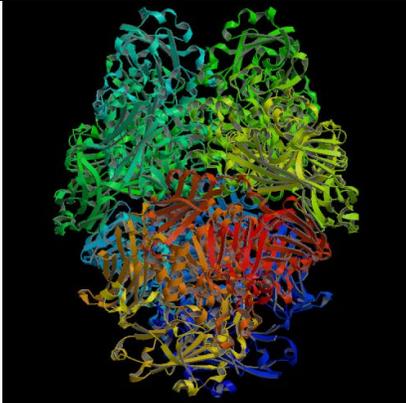
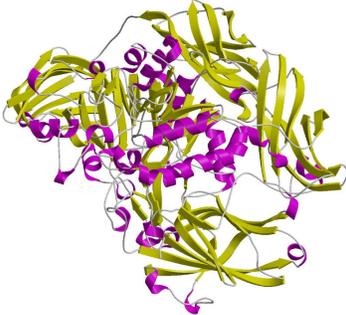
Complete “Putting it all together” sheet as a group. Each student should have the answers. Turn in the most legible copy.

AP Chem/Bio Enzyme Lesson Introductory Worksheet

Try to answer the following questions without the help of those in your group.

1. Next to each of the following descriptions or pictures, write one of the following:

- Primary Structure
- Secondary Structure
- Tertiary Structure
- Quaternary Structure

 <p>Several chains of amino acids can come together to form a large 3-dimensional structure which is made up of several units held together by covalent or intermolecular forces.</p>	 <p>The flat arrows represent beta sheets</p> <p>The spirals represent alpha helices</p> <p>This shows how sections of amino acids interact with each other in long chains.</p>
<p>The sequence of amino acids that make up the enzyme protein. Below are a few amino acids that may be included in the sequence:</p> <div style="display: flex; justify-content: space-around; align-items: center;"> <div data-bbox="232 1304 386 1514"> $\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{C}(=\text{O})-\text{OH} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{C}=\text{O} \\ \\ \text{OH} \end{array}$ </div> <div data-bbox="553 1283 699 1514"> $\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{C}(=\text{O})-\text{OH} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_4 \\ \\ \text{OH} \end{array}$ </div> </div>	 <p>The alpha helices and beta sheets can interact with each other to form a larger 3-dimensional structure that has a particular shape.</p>

2. What is a catalyst? _____

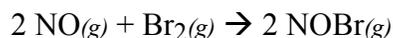
3. What is an enzyme? _____

Putting It All Together

Work together in your chem/bio groups to answer the following questions. Make sure that EACH of you knows how to answer the questions and is able to understand what you write down. This may NOT be the last time you see these questions!!!

1. You have learned how the study of enzymes can depend on some chemistry principles. Using this simpler example, answer the following questions.

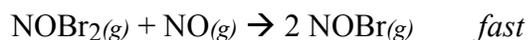
From the 1999 AP Chemistry Test



A rate study of the reaction represented above was conducted at 25°C. The data that were obtained are shown in the table below.

Experiment	Initial [NO] (mol L ⁻¹)	Initial [Br ₂] (mol L ⁻¹)	Initial Rate of Appearance of NOBr (mol L ⁻¹ s ⁻¹)
1	0.0160	0.0120	3.24x10 ⁻⁴
2	0.0160	0.0240	6.38x10 ⁻⁴
3	0.0320	0.0060	6.42x10 ⁻⁴

- (a) Calculate the initial rate of disappearance of Br₂(g) in experiment 1.
- (b) Determine the order of the reaction with respect to each reactant, Br₂(g) and NO(g). In each case, explain your reasoning.
- (c) For the reaction,
- write the rate law that is consistent with the data, and
 - calculate the value of the specific rate constant, *k*, and specify units.
- (d) The following mechanism was proposed for the reaction:



Is this mechanism consistent with the given experimental observations? Justify your answer.

2. You have learned that the rate of enzyme-catalyzed reactions may depend on various factors. Think about the conditions inside your body and list several other factors which may influence the rate of a reaction inside your body.

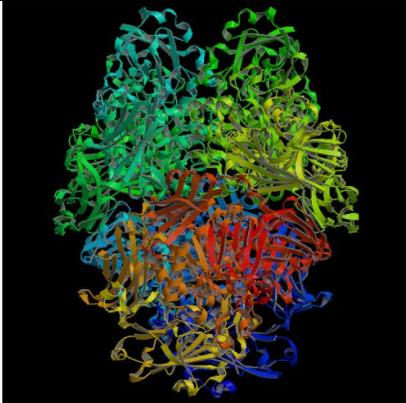
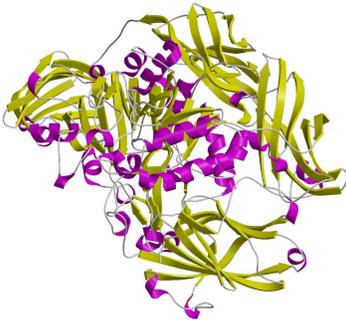
3. It was mentioned that in the quaternary structure of an enzyme, the individual chains may be held together by covalent or intermolecular forces. What are the intermolecular forces we have discussed previously in this class?

AP Chem/Bio Enzyme Lesson Introductory Worksheet

Try to answer the following questions without the help of those in your group.

1. Next to each of the following descriptions or pictures, write one of the following:

- Primary Structure
- Secondary Structure
- Tertiary Structure
- Quaternary Structure

 <p>Several chains of amino acids can come together to form a large 3-dimensional structure which is made up of several units held together by covalent or intermolecular forces.</p> <p style="text-align: center;">Quaternary</p>	 <p>The flat arrows represent beta sheets</p> <p>The spirals represent alpha helices</p> <p>This shows how sections of amino acids interact with each other in long chains.</p> <p style="text-align: center;">Secondary</p>
<p>The sequence of amino acids that make up the enzyme protein. Below are a few amino acids that may be included in the sequence:</p> <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\begin{array}{c} \text{O} \\ \parallel \\ \text{H}_2\text{N}-\text{CH}-\text{C}-\text{OH} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{C}=\text{O} \\ \\ \text{OH} \end{array}$ </div> <div style="text-align: center;"> $\begin{array}{c} \text{O} \\ \parallel \\ \text{H}_2\text{N}-\text{CH}-\text{C}-\text{OH} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_4 \\ \\ \text{OH} \end{array}$ </div> </div> <p style="text-align: center;">Primary</p>	 <p>The alpha helices and beta sheets can interact with each other to form a larger 3-dimensional structure that has a particular shape.</p> <p style="text-align: center;">Tertiary</p>

2. What is a catalyst? Something that speeds up a chemical reaction without being used up in the process.

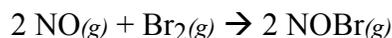
3. What is an enzyme? A biological catalyst. An enzyme is a protein in the body that helps important biochemical reactions occur at an acceptable rate.

Putting It All Together

Work together in your chem/bio groups to answer the following questions. Make sure that EACH of you knows how to answer the questions and is able to understand what you write down. This may NOT be the last time you see these questions!!!

1. You have learned how the study of enzymes can depend on some chemistry principles. Using this simpler example, answer the following questions.

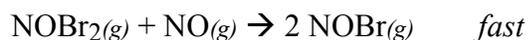
From the 1999 AP Chemistry Test



A rate study of the reaction represented above was conducted at 25°C. The data that were obtained are shown in the table below.

Experiment	Initial [NO] (mol L ⁻¹)	Initial [Br ₂] (mol L ⁻¹)	Initial Rate of Appearance of NOBr (mol L ⁻¹ s ⁻¹)
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2	0.0160	0.0240	6.38x10 ⁻⁴
3	0.0320	0.0060	6.42x10 ⁻⁴

- (a) Calculate the initial rate of disappearance of Br₂(g) in experiment 1.
- (b) Determine the order of the reaction with respect to each reactant, Br₂(g) and NO(g). In each case, explain your reasoning.
- (c) For the reaction,
- write the rate law that is consistent with the data, and
 - calculate the value of the specific rate constant, *k*, and specify units.
- (d) The following mechanism was proposed for the reaction:



Is this mechanism consistent with the given experimental observations? Justify your answer.

Answer

Note: Some of the equations show the infinity sign instead of the multiplication sign. My apologies...I'll have to reload my equation editor.

(a) Since the disappearance of 1 Br₂ produces 2 NOBr, then the rate would be half as much or rate = -1.62x10⁻⁴.

(b) With respect to Br₂: rate = *k* [NO]^{*m*}[Br₂]^{*n*}

In expt. 2 the [Br₂] is twice the concentration in expt. 1, as well, the initial rate of expt. 2 is twice the initial rate of expt. 1, while [NO] remains constant. Therefore, it is 1st order with respect to [Br₂], *n* = 1.

With respect to NO: rate = *k*[NO]^{*m*}[Br₂]¹

expt. 2: 6.38x10⁻⁴ = *k* (0.0160)^{*m*}(0.024)

$$k = \frac{(0.0160)^m(0.0240)}{6.38 \times 10^{-4}}$$

$$\text{expt 3: } 6.42 \times 10^{-4} = k (0.0320)^m(0.0060)$$

$$k = \frac{(0.0320)^m(0.0060)}{6.42 \times 10^{-4}}$$

$$\frac{(0.0160)^m(0.0240)}{6.38 \times 10^{-4}} = \frac{(0.0320)^m(0.0060)}{6.42 \times 10^{-4}}$$

solving: $m = 1.97$ or $m = 2$

therefore, 2nd order with respect to [NO].

(c) (i) $\text{rate} = k[\text{NO}]^2[\text{Br}_2]$

(ii) $k = \frac{\text{rate}}{[\text{NO}]^2[\text{Br}_2]}$

$$= \frac{3.24 \times 10^{-4} \text{ mol L}^{-1} \text{ s}^{-1}}{(0.0160 \text{ mol L}^{-1})^2(0.0120 \text{ mol L}^{-1})}$$

$$= 105 \text{ L}^2 \text{ mol}^{-2} \text{ s}^{-1}$$

(d) No; since the rate determining step is the slowest step (and in this case, the first step), then the rate for this proposed mechanism depends only on the concentration of the reactants in the first step and would be: $\text{rate} = k[\text{NO}][\text{Br}_2]$

2. You have learned that the rate of enzyme-catalyzed reactions may depend on various factors. Think about the conditions inside your body and list several other factors which may influence the rate of a reaction inside your body.

Body temperature is fairly regular, but the reactions may be affected when the body has a fever or changes temperature. pH values may affect reaction rates. Depending on where the reactions are occurring, different concentrations of salts or other molecules from the diet may affect the reaction rates.

3. It was mentioned that in the quaternary structure of an enzyme, the individual chains may be held together by covalent or intermolecular forces. What are the intermolecular forces we have discussed previously in this class?

Intermolecular forces (IMF) are the forces resulting from molecules interacting with each other.

***Hydrogen Bonds – the strongest of the IMF; the attraction for a very electropositive hydrogen for the lone pairs of an electronegative atom. Results with H bonds to N, O, or F.**

***Dipole-Dipole Forces – the attraction of polar molecules for each other (opposite ends, of course)**

***Dipole-Induced dipole – a polar molecule induces a dipole moment in a nonpolar molecule, therefore causing an attraction**

***London Dispersion Forces – Temporary fluctuations in the electron clouds surrounding molecules cause temporary dipole moments in molecules. These are then attracted.**

(Those forces involving ions are studied under a separate classification.)

Literature Cited

1. Garrett, R.H. and Grisham, C.M., *Principles of Biochemistry With a Human Focus*, Brooks/Cole and Thomson Learning, 2002.
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