**BIOS 452/CHEM 452**

Third Exam

Fall, 2010

12:00-12:55pm, Monday, November 22, 2010

Name: Study Points

UIN: _____

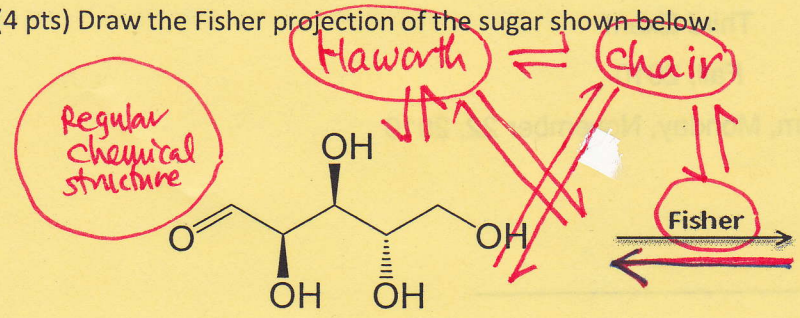
Circle Discussion Section:

| | |
|---|---|
| Mon | 8 |
| Tue | 9:30 |
| Wed | 9 |
| Thu | 9:30 |
| Fri | 9 |
| Fri | 11 |

General Instruction

* Do not turn the page until you are told to do so.
* You may take the exam with you only after 12:35pm.
* No calculators allowed. **For calculations and graphing, show all your work!!!**
* The exam is total 9 pages (including cover page), ~30 questions grouped into 15 for 55 minutes.
* Read the question carefully to the end.
* Pay attention to units.

**1.** (4 pts) Draw the Fisher projection of the sugar shown below.




* Practice w/ sugars on the monosaccharides on the handout (Table).
* should know the names & structures of sugars on the summary table.

**2.** (1-3) Choose from following:

| | | | | |
|---|---|---|---|---|
| (a) Glucose | (b) Galactose | (c) Ribose | (d) Sucrose | (e) Fructose |

**(1)** (2 pts) Which one is enriched in nerve tissues and thus called "brain sugar"? _____

**(2)** (2 pts) Which has the highest molecular weight? _____

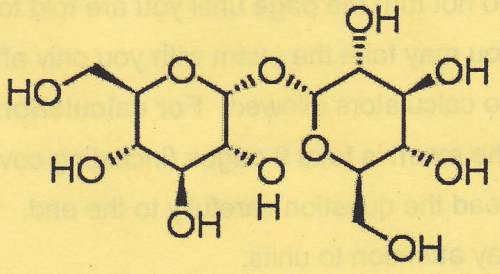
* Distinguish mono, di, & Poly/oligo saccharides.

**(3)** (2 pts) Which ones are diastereomers of one another? Choose all that apply. _____

* What is diastereomer? What is enantiomer?

* Read Table 20-1.
Understand characteristics of various sugars
e.g. sucrose = cane sugar ; fructose = enriched in fruits etc. etc.

**3.** Shown on the right is a disaccharide called Trehalose.

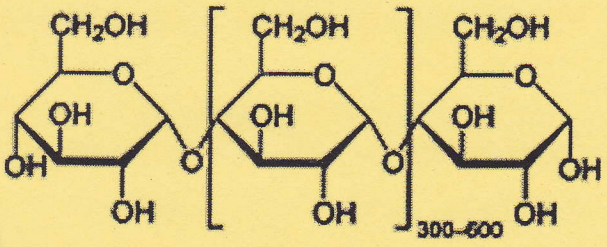



**(1)** (3 pts) Circle all the anomeric carbons.

**(2)** (3 pts) Trehalose as shown on the right is an (acetal, ketal, hemiacetal, hemiketal –*Circle one*)

* Understand what is glycosidic bond.
- Formed by anomeric carbons. Know how to name the bond configuration.
- Acetal vs Ketal
- Don't forget "glycosidic bond" in DNA or RNA !!! – same questions apply to them.

**4.** (5 pts) Shown on the right is Amylose. The repeating DISACCHARIDE unit of this sugar is _____, which has (_____; *Choose either α or β*) anomers linked via (_____-->



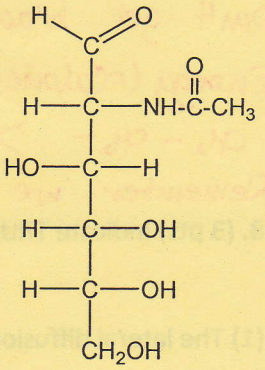
_____ ; *Write in numbers*) glycosidic bond.

**Make sure you filled out ALL of the 4 BLANKS.*

**5. (1)** (2 pts) Write the name of an amino acid that can form N-linked glycosidic bonds with carbohydrates.

_____ - *Choose from the 20 amino acids.*

**(2)** (6 pts) Show the structure of the amino acid you chose (2) covalently attached to the monosaccharide on the right in its β-conformation, as it may appear in a glycoprotein.




* Glycoprotein – How can carbs attached to a protein ?
  - O-linked
  - N-linked
  - structure ?
  - which amino acid ?
  - How ?
  - α, β ?

**6. (1)** (8 pts + 2 Bonus pts with correct stereochemistry.) Mild hydrolysis of a naturally occurring lipid with dilute NaOH generated **L**-glycerol 3-phophoserine and the sodium salts of a hexadecanoate and a $\Delta^9$-octadecenoate. Draw a chemical structure of the parent lipid.

* can ask what are the products after hydrolysis of a lipid >
  - of whole molecule
  - of individual components
* Don't get confused on : Please distinguish →
  - Fatty acid
  - Triacylglycerol
  - Phosphoglycerolipid
  - Sphingolipid
  - Name
  - structure
  - charge
  - Melting point
  - occurrence e.g. bilayer, micelle, oil droplet
* Know sn-1, sn-2, sn-3 ?
* Understand if I say "1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphocholine"?

btw: this is = "1-palmitoyl-2-arachidonyl-sn-phosphatidylcholine"
- indicates glycerophospholipid
- + "phosphocholine" by itself.
- this is just headgroup.

**(2)** (4 pts) What are the common names of the constituent fatty acids indicated in (1)?

_____ AND _____

* Common name ≠ IUPAC name of fatty acid chains.

**(3)** (3 pts) What is the net charge of the parent lipid in (a) at a neutral pH?

(Positive, Negative, Neutral –*Circle one*)

**7.** (3 pts) Which of the following INCREASE(S) during the hydrogenation process used in making traditional margarine?

*Circle all that apply.*

(a) Number of saturated bonds (b) Trans-fat content (c) Food calories per molecule (d) Melting point of the fat

* Don't get biased that there would be only 3 answers b/c there are 3 points.
* Energy (=Calories) per molecule ↑ w/ more reduced carbons.

$-CH_2-CH_2- > -CH=CH \cdots > O=C=O$

Remember we get energy through burning carbons: [lipid, carb, protein] $+ O_2 \xrightarrow{\text{Metabolism}} CO_2 + H_2O$

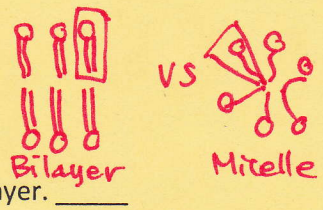
(High energy) → (Low energy)

**8.** (3 pts) Indicate TRUE (T) or FALSE (F) for each of the following statements about the plasma membrane of a cell.

**(1)** The lateral diffusion of molecules is much easier than the transverse diffusion. _____

* **(2)** Free fatty acids are a major component. _____

→ tend to form micelles rather than bilayer




**(3)** Glycoproteins and glycolipids expose their carbohydrate groups on the outer leaflet of the bilayer. _____

**9.** (3 pts) Arrange the following compounds (a)-(e) in an increasing order of permeability across a pure synthetic lipid bilayer.

| (a) $Cl^-$ | (b) $O_2$ | (c) Water | (d) Glycerol | (e) Glucose |
|---|---|---|---|---|

_____ < _____ < _____ < _____ < _____

* If glucose is not that permeable through cell membrane, how can cell get glucose to use it for energy source (e.g. Glycolysis)

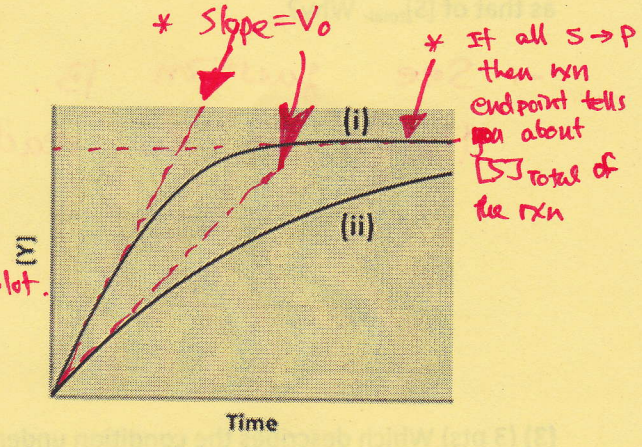
Answer: There are channels & transporters made of proteins that can transport molecules not permeable to pure lipid membrane.

**10.** (3 pts) A spontaneous conversion of a compound S to another compound P has a forward reaction rate constant, $k_f$ of 100 hour$^{-1}$ and a reverse reaction rate contstant, $k_r$ of 1 hour$^{-1}$. In an enzyme-catalyzed reaction, the same conversion takes place with a different $k_f$ ($k_{f,\ catalyzed}$) that is 2 sec$^{-1}$, what would be the $k_{r,\ catalyzed}$ of this reaction? *Make sure to write a unit to your answer.*

$$S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} P$$

* Understand what is the role (meaning) & "catalysis" of a chemical reaction?
* Can you describe Equilibrium constants in terms of rate constants?
* Can you sketch Free energy vs Reaction coordinate for uncatalyzed vs catalyzed?
* How does the enzyme lower the activation energy barrier?

**11.** (6 pts) An enzyme (E) catalyzes the conversion of substrate X to product Y. The plot on the right shows the concentrations of Y ([Y]) versus time of reactions. Can the following change in the reaction condition shift the curve from (i) to (ii)? ~~Answer Yes(Y) or No(N).~~ or Indicate the change on plot.




**(1)** Add an uncompetitive inhibitor. _____

**(2)** Replace the substrate X with X' that has a lower $K_m$ but the same $V_{max}$. _____

**(3)** Decrease the total reaction volume without changing the concentrations of reaction components. _____

(4) Decrease temperature of reaction

(5) Use less enzyme.

(6) Use an enzyme w/ higher $k_{cat}$ but same $K_m$

(7) " w/ lower $K_M$ but same $k_{cat}$



For (1) ~ (7). Draw / indicate the change in $V_o$ vs [S] plot.

**12.** Below is a kinetic scheme of a simple enzyme-catalyzed reaction.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Michaelis & Menten used a steady state assumption to express the reaction velocity ($= \frac{d[P]}{dt}$) as a function of quantities that can be easily measured such as $[E]_{total}$ and $[S]_{total}$: $V=k_2*[E]_{total}*[S]/(K_m + [S])$ *(eq. 1)*

**(1)** (4 pts) Write a rate equation, i.e., a differential equation, that describes the steady state assumption.

* Do not get confused about $\frac{d[P]}{dt}$ which is $\frac{0}{T}$ when it is still $[S] \gg [P]$ : initial velocity.

$\frac{d[ES]}{dt}$ which should be 0 when steady-state is reached.

** **(2)** (3 pts) In the *eq. 1,* [S]is the concentration of free S, NOT total S ($[S]_{total}$). Nevertheless, we often take the value of [S] as that of $[S]_{total}$. Why?

→ See question 13.

Make sure the reaction condition meets the requirement there.

$[S]_T \gg [E]_T$

↓ μM vs. nM !

**(3)** (3 pts) Which describes the condition under which $K_m$ can be regarded as the dissociation constant of the binding equilibrium between E and S to form ES complex?

( $k_1 >> k_{-1}$ , $k_1 >> k_2$ , $k_{-1} >> k_2$

$k_1 << k_{-1}$ , $k_1 << k_2$ , $k_{-1} << k_2$ - *Circle one.*)

* What is a Kd for $E + S \rightleftharpoons ES$ ?

What is a Kd in terms of reaction rate constants ?

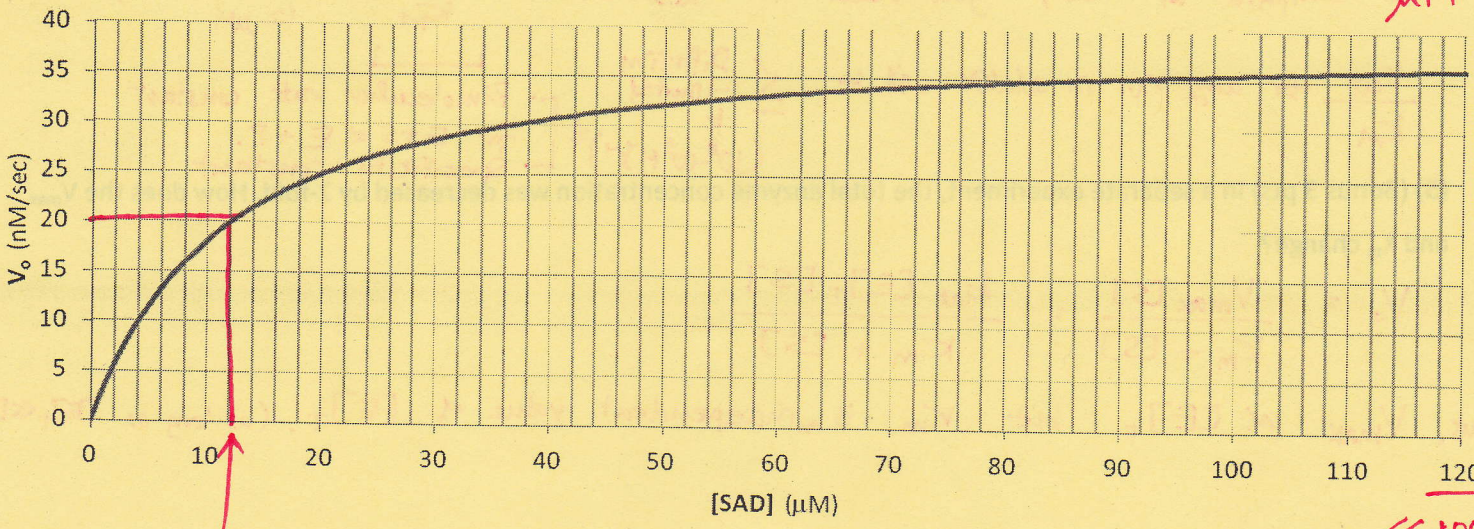
* $K_M$ is sort of like a Kd. ...Just includes another way ($k_2$) that contributes to the disappearance of ES.

$K_M = \frac{k_{-1} + k_2}{k_1}$ (vs) $K_d = \frac{k_{-1}}{k_1}$

**13.** An enzyme called "happyase" catalyzes the following reaction with a $k_{cat}$ of 100 $sec^{-1}$: SAD $\rightleftharpoons$ HAPPY

Below is a plot of the initial velocity versus the substrate concentration for happyase. The initial velocity at 1 mM [SAD] was measured to be 40 nM/sec.

= 1000 μM




≪ 1000 μM

**(1)** (3 pts) What is the total happyase concentration used above? Indicate unit.

$V_{max} = k_{cat}[E]_T$

$V_{max}$: ↑ given in question

$k_{cat}$: ↑ given in question

$[E]_T$: ↑ (?)

**(2)** (4 pts) What is the $K_m$ of happyase for SAD? Indicate unit.

$K_m$ is $[S]$ when $V_o = \frac{V_{max}}{2}$.

(B/C When you use $[S] \gg K_m$, you can't get a good approx on $K_m$.)

* Using equation is ok, but graph is faster.

When using $V_o = \ldots$ equation, make sure to plug in a $[S]$ not too big

**(3)** (4 pts) What is the SAD concentration at time = 0 when 480 pmole of HAPPPY is formed during the first minute in a 1-mL reaction?

* Understand what initial velocity ($V_o$) actually means?

" how $V_o$ is " calculated?

**(4)** (3 pts) Considering the diffusion-limited bimolecular rate constant is $1 \times 10^8\ M^{-1} s^{-1}$, how much slower is the reaction catalyzed by happyase than a diffusion-limited reaction? ( 1, (10,) 100, 1000, 10,000 – *Circle one* ) – times.

* To compare w/ this, you need to calculate $\frac{k_{cat}}{K_M} = \frac{100\ sec^{-1}}{12\ \mu M} \sim 10^7\ M^{-1} s^{-1}$

* $\frac{k_{cat}}{K_M}$ for any rxn in solution will be $\leq$ Diffusion-limited k $(10^8\text{-}10^9\ M^{-1}s^{-1})$ [ Bimolecular rate constant for $E+S \rightleftharpoons E+P$. Specificity constant ]

**(5)** (Bonus 3 pts) In a separate experiment, the total enzyme concentration was decreased by 2-fold. How does the $V_{max}$ and $K_m$ change?

* $V_o = \frac{V_{max}[S]}{K_m + [S]} = \frac{k_{cat}[E]_T[S]}{K_m + [S]}$

Thus, $V_{max} \propto [E]_T$ but $K_m$ is an independent value of $[E]_T$. (as long as $[E]_T \ll [S]_T$)

**(6)** (Bonus 2 pts) Thus the effect of reducing total enzyme concentration is similar to the effect of adding a ( Competitive, Uncompetitive, (Noncompetitive) – *Circle one*) inhibitor.

{ $V_{max}$ ↓
$K_m$ no change

**14. (1)** (3 pts) STRESS is a competitive inhibitor of happyase described in **16**. Draw a kinetic scheme for competitive inhibition.

$E + S \rightleftharpoons ES \rightarrow E + P$
$+$
$I$
$\Updownarrow$
$EI$.

where:
E : Happyase (≠ SAD)
S : SAD
P : HAPPY
I : STRESS

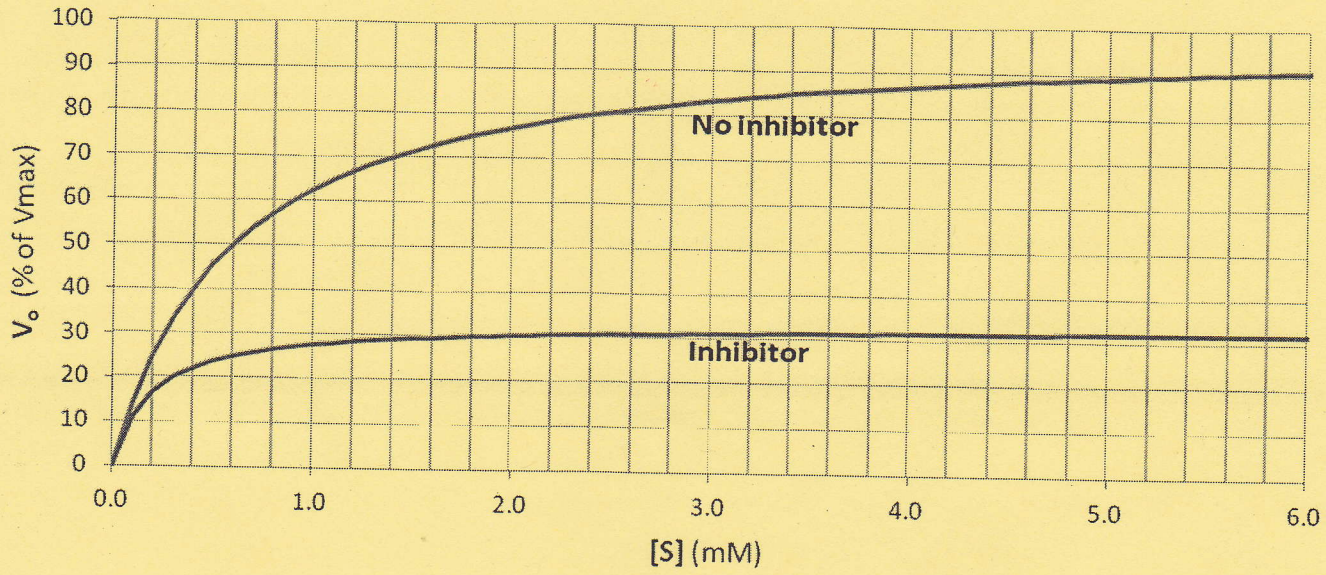
* Do this for Uncompetitive inhibition.
Mixed
Non competitive

**(2)** (4 pts) Ki of STRESS is 10 nM. Calculate the apparent $K_m$ and the apparent $k_{cat}$ when 10 nM STRESS is added to a happyase-catalyzed reaction which shows $K_m$ of 9 uM and $k_{cat}$ of 120 $sec^{-1}$ in the absence of any inhibitor.

* How does { $V_{max} = k_{cat}[E]_T$ / $K_m$ } is affected by inhibitors?

* Do this assuming { uncompetitive inhibition. / Mixed w/ different Ki for binding to E & ES. $K_i = 10\ nM$ $K_i' = 5\ nM$. / Non competitive }

**15.** The experimental curve of initial reaction velocity versus [S] with and without inhibitor (I) is shown for an enzyme and a substrate.




**(1)** (4 pts) What is a possible mechanism of inhibition?

( Competitive, Uncompetitive, Noncompetitive - *Circle one.* )

* Can you see how Vmax & Km changed after adding inhibitor? What inhibition mechanism can match those changes?

* Can you convert $V_o$ vs [S] into $\frac{1}{V_o}$ vs $\frac{1}{[S]}$ plots? (Lineweaver-Burk plot)

**(2)** (3 pts) Which best describes the Lineweaver-Burke plot of the enzyme with the inhibitor (I) at [I]=Ki. Circle one among (i) to (vi)