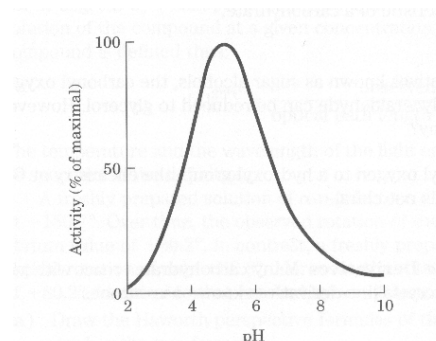


BIOC*4540 Enzymology **Problem Set #3**

1. The active site of lysozyme contains two amino acid residues essential for catalysis: Glu³⁵ and Asp⁵². The pK_a values of the carboxyl side chains of these two reactions are 5.9 and 4.5, respectively. What is the ionisation state (protonated or deprotonated) of each residue at pH 5.2, the pH optimum of lysozyme? How can the ionisation states of these residues explain the pH activity profile of lysozyme shown below?



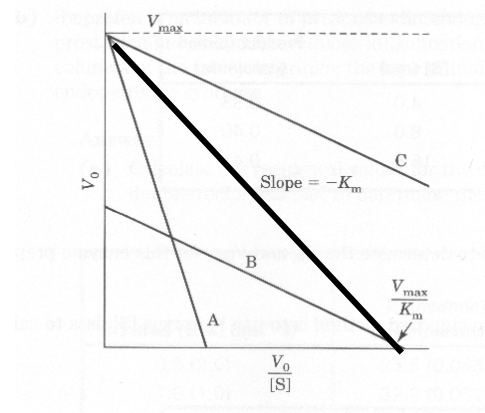
2. Prostaglandins are a class of eicosanoids, fatty acid derivatives with a variety of extremely potent actions on vertebrate tissues. Prostaglandins are responsible for producing fever and inflammation and its associated pain. They are derived from the 20-carbon fatty acid arachidonic acid in a reaction catalyzed by the enzyme prostaglandin endoperoxide synthase. This enzyme, a cyclooxygenase, uses oxygen to convert arachidonic acid to PGG₂, the immediate precursor of many different prostaglandins.

(a) The kinetic data in the table below are for the reaction catalyzed by prostaglandin endoperoxide synthase. From the data in the first two columns determine the V_{max} and K_M of the enzyme.

Arachidonic Acid (mM)	Rate of Formation of PGG ₂ (mM/min)	Rate of Formation of PGG ₂ with 10 mg/mL ibuprofen (mM/min)
0.5	23.5	16.67
1.0	32.2	25.25
1.5	36.9	30.49
2.5	41.8	37.04
3.5	44.0	38.91

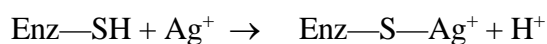
(b) Ibuprofen is an inhibitor of prostaglandin endoperoxide synthase. By inhibiting the synthesis of prostaglandins, ibuprofen reduces inflammation and pain. Using the data in the first and third columns of the table, determine the type of inhibition that ibuprofen exerts on prostaglandin endoperoxide synthase.

3. An Eadie-Hofstee plot for an enzyme-catalyzed reaction is shown below.



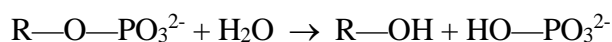
The bold line curve was obtained in the absence of inhibitor. Which of the other curves (A, B, or C) shows the enzyme activity when a competitive inhibitor is added to the reaction mixture?

4. Many enzymes are inhibited irreversibly by heavy-metal ions such as Hg^{2+} , Cu^{2+} , or Ag^+ , which can react with essential sulfhydryl groups to form mercaptides.



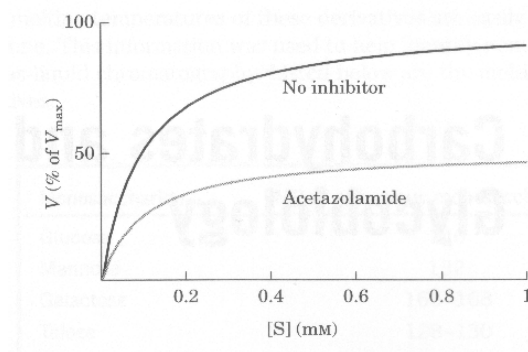
To 10 mL of a solution containing 1.0 mg/mL of a pure enzyme, an investigator added just enough AgNO_3 to completely inactivate the enzyme. A total of 0.342 μmol of AgNO_3 was required. Calculate the *minimum* molecular weight of the enzyme. Why does the value obtained in this way give only the minimum molecular weight?

5. Human blood serum contains a class of enzymes known as acid phosphatases, which hydrolyse biological phosphate esters under slightly acidic conditions (pH 5.0):



Acid phosphatases are produced by erythrocytes, the liver, kidney, spleen, and the prostate gland. The enzyme from the prostate gland is clinically important because its increased activity in the blood is frequently an indication of prostate cancer. The phosphatase from the prostate gland is strongly inhibited by tartrate ion, but the acid phosphatases from other tissues are not. How can this information be used to develop a specific procedure for measuring the activity of the acid phosphatase of the prostate gland in human blood serum?

6. Carbonic anhydrase is strongly inhibited by the drug acetazolamide, which is used as a diuretic (to increase the production of urine) and to treat glaucoma (to reduce excessively high pressure in the eye due to accumulation of intraocular fluid). Carbonic anhydrase plays an important role in these and other secretory processes because it participates in regulating the pH and bicarbonate content of a number of body fluids. The experimental curve of initial reaction velocity (as percentage of V_{max}) versus $[\text{S}]$ for the carbonic anhydrase reaction is illustrated below (upper curve). When the experiment is repeated in the presence of acetazolamide, the lower curve is obtained. From an inspection of the curves and your



knowledge of the kinetic properties of enzyme inhibitors, determine the nature of the inhibition by acetazolamide. Explain.

7. An enzyme with a single substrate ($K_m = 2 \times 10^{-4} \text{ M}$) was assayed in the presence of $2 \times 10^{-4} \text{ M}$ substrate and $2.5 \times 10^{-3} \text{ M}$ competitive inhibitor ($K_i = 2.5 \times 10^{-3} \text{ M}$). The V_{\max} (uninhibited) is $55 \mu\text{moles/L-min}$. Calculate the initial velocity in the presence of the competitive inhibitor.
8. Calculate K_i for a competitive inhibitor given the following information: $K_m = 6.7 \times 10^{-4} \text{ M}$, V_{\max} (minus inhibitor) = $300 \mu\text{moles/L-min}$, and v_i (in the presence of 10^{-5} M [I]) at $2 \times 10^{-5} \text{ M}$ [S] = $1.5 \mu\text{moles/L-min}$.
9. Calculate the degree of inhibition of the enzyme-catalyzed reaction described in Problem 2 (above) under the following conditions: (a) [S] = $2 \times 10^{-5} \text{ M}$ and [I] = 10^{-5} M , (b) [S] is increased 10-fold while [I] remains the same, (c) [S] is increased 100-fold while [I] remains the same, (d) [S] remains at $2 \times 10^{-5} \text{ M}$ and [I] is decreased to 10^{-6} M , (e) [S] and [I] remain at $2 \times 10^{-5} \text{ M}$ and 10^{-5} M , respectively, but I is a poorer inhibitor (K_i is 20 fold higher than calculated in Problem 2 (above)), and (f) the ratio of [S]/[I] remains the same but both [S] and [I] are increased 10 fold (and $K_i = 2.02 \times 10^{-6} \text{ M}$).
10. Calculate (a) the velocity and (b) the degree of inhibition of an enzyme-catalyzed reaction in the presence of $3.5 \times 10^{-5} \text{ M}$ substrate ($K_m = 2 \times 10^{-4} \text{ M}$) and $4 \times 10^{-5} \text{ M}$ noncompetitive inhibitor ($K_i = 2 \times 10^{-5} \text{ M}$). The velocity observed at 0.03 M [S] in the absence of inhibitor is $295 \mu\text{moles/L-min}$.
11. What concentration of a noncompetitive inhibitor ($K_i = 4 \times 10^{-6} \text{ M}$) is required to yield 65% inhibition of an enzyme-catalyzed reaction?

12. You are out walking your dog one evening when you come across a young child laying on the front yard of a home and the child is having convulsions. Beside the child is a nearly empty bottle of methyl hydrate (absolute methanol solution used as a de-icer of automobile gasoline). Immediately you call 911 and the paramedic arrives within a few min of your phone call. Unfortunately, the paramedic has forgotten his biochemistry and doesn't know what to do for the child. Miraculously, you recall the metabolism BIOC4520 notes concerning dehydrogenases. You recall that ethanol in the body is oxidized to acetaldehyde by liver alcohol dehydrogenase (LADH). You also recall that other alcohols are also oxidized by LADH. You remember that methanol, which is mildly intoxicating, is oxidized by LADH to the quite toxic product, formaldehyde. You also recall that administering ethanol can reduce the toxic effects of ingesting methanol. The ethanol acts as a competitive inhibitor of the methanol by displacing it from LADH. This provides sufficient time for the methanol to be harmlessly excreted by the kidneys. Instantly and with the confidence of a surgeon, you remind the paramedic that he should dose the child with ethanol. Your acute senses tell you that nearby there is a drunk laying on the side of the road with a nearly full bottle of Molson Canadian (5% ethanol by volume) resting at his side in his left hand. You race over to the hapless drunk and snatch the bottle of beer from him and you hand the bottle to the awe-struck paramedic. A quick check of the bottle of methyl hydrate indicates that the child may have ingested 1 mL of the methanol solution (a potentially lethal dose).

How much beer must the paramedic administer to the child to reduce the activity of the child's LADH towards methanol to 4% of its original value? The child's body contains approx. 6 L of aqueous fluids throughout which ingested alcohols are rapidly and uniformly mixed. In your hand-held calculator, which you carry with you at all times, you have the following data in tabular form: the densities of ethanol (MW = 46 g/mol) and methanol (MW = 32 g/mol) are both $0.79 \text{ g}\cdot\text{cm}^{-3}$ and the K_m values of LADH for ethanol and methanol are $1.3 \times 10^{-3} \text{ M}$ and $1.7 \times 10^{-2} \text{ M}$, respectively, and that $K_i = 0.2K_m$ for ethanol. Will the child be able to perform the Canadian Rant after recovering from the intoxication incident?