

USEFUL CONSTANTS:

R (gas constant) = $8.315 \text{ J}\cdot\text{mol}^{-1}\cdot\text{Kelvin}^{-1} = 8.315 \times 10^{-3} \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{Kelvin}^{-1}$
(25 °C) $T = 298 \text{ K}$; human physiological temperature (37 °C) $T = 310 \text{ K}$.

Ionizable group in peptides and proteins	<i>Approximate</i> ("generic") pK_a in peptides & proteins
α -amino	8.0
α -carboxyl	3.0
ϵ -amino	10.0
guanidino	12.0
thiol	8.5
imidazole	6.5
aromatic hydroxyl	10.0
side chain carboxyl	4.0

1. (10 pts) Skeletal muscle contraction

A. (3 pts) What event is the *immediate* trigger of the "power stroke" in muscle contraction?

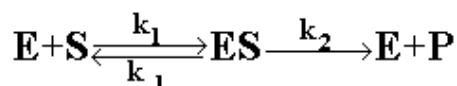
- 1) binding of ATP *3) **release of P_i** 5) binding of Ca²⁺
 2) hydrolysis of ATP 4) release of ADP 6) dissociation of myosin from actin

B. (3 pts) What protein is the "Ca²⁺ sensor" that enables a cytosolic increase in [Ca²⁺] to initiate muscle contraction?" Troponin C

C. (4 pts) A non-hydrolyzable analog of ATP with a methylene group (-CH₂-) instead of an -O- atom as the "bridge" between the terminal phosphate and the ADP component is a potent inhibitor of muscle contraction. Briefly explain what step in the contraction cycle you would expect this compound to inhibit and why you chose that step.

One would expect that the analog could bind, but the cycle would be blocked at the step involving hydrolysis of ATP, because the analog cannot be hydrolyzed. (This is also the step that involves the conformational change of "cocking" of the myosin head.)

2. (5 pts) Write the **equation** describing the *steady state assumption* for the Michaelis-Menten kinetic mechanism (shown below) in terms of the RATE CONSTANTS and the CONCENTRATIONS of the various components.



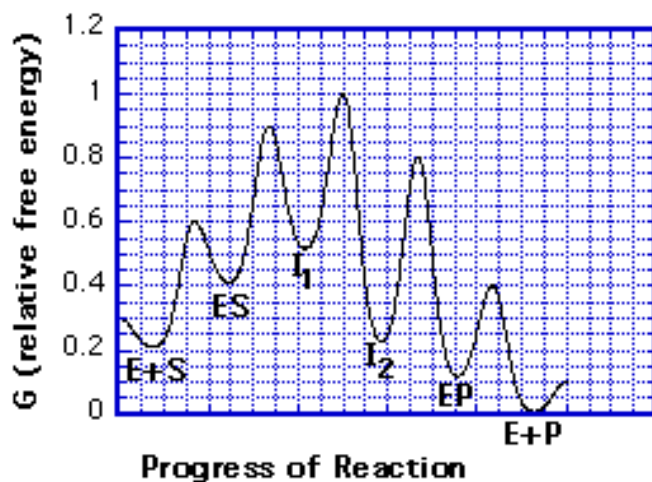
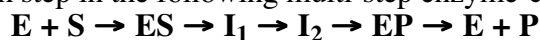
ANSWER:

concentration of [ES] is constant, so rate of formation of [ES] = rate of breakdown of [ES]:

$$k_1[E][S] = k_{-1}[ES] + k_2[ES] \text{ OR}$$

$$k_1[E][S] = (k_{-1} + k_2)[ES]$$

3. (7 pts) Which step in the following multi-step enzyme-catalyzed reaction would be *rate-limiting*?



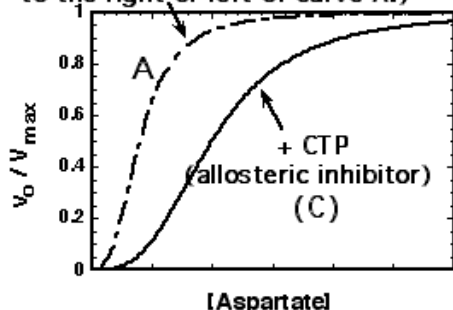
A. (3 pts) 1) E + S → ES 2) ES → I₁ 3) I₁ → I₂ *4) **I₂ → EP** 5) EP → E + P

B. (4 pts) Briefly explain how you decided which step was rate-limiting:

The rate-limiting step is the step with the highest free energy of activation (ΔG[‡]), which is measured from free energy of the preceding trough to the peak (transition state free energy).

4. (9 pts) There are 2 curves below showing relative velocity (v_o/V_{max}) for the ATCase reaction as a function of substrate (aspartate) concentration. One of the curves represents ATCase activity in the absence of any heterotropic effectors; the other curve represents ATCase activity in the presence of the pyrimidine nucleotide CTP.

No heterotropic effectors (A)
(Curve B, with CTP + ATP, would be close to curve A, or just barely to the right or left of curve A.)



- A. (5 pts) On the plot at left, label the curve in the **absence of any heterotropic effector** as **A**, and the curve in the **presence of CTP** as **C** (or CTP). Briefly explain the role of CTP in regulation of the activity of ATCase -- why CTP has the effect it has in terms of regulation of a metabolic pathway, and to what part of the structure of ATCase CTP binds.

CTP is the end-product of the pyrimidine nucleotide biosynthetic pathway for which ATCase catalyzes the first committed step, and thus acts as a feedback (allosteric) inhibitor of ATCase. It binds to regulatory subunits of ATCase in T (less active) state, stabilizing/increasing concentration of T state.

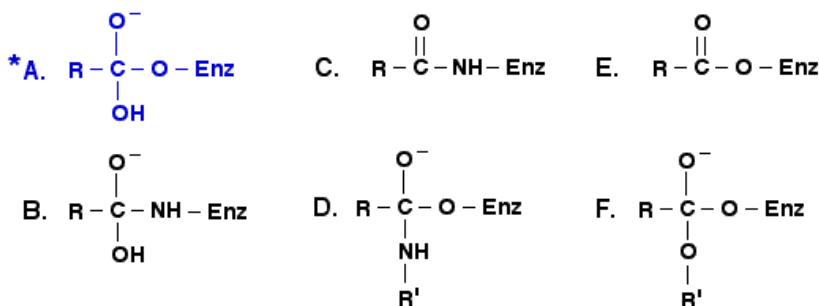
- B. (4 pts) Sketch a new curve on the plot above and label it **B** for results expected if **ATP** were added *in addition to CTP* in the experiment above, i.e. in the **presence of both CTP + ATP**. Briefly explain the role of ATP in regulation of the activity of ATCase -- why ATP has the effect it has in terms of regulation of a metabolic pathway, and to what part of the structure of ATCase ATP binds.

ATP is a purine nucleotide, and is an allosteric activator of ATCase. ATP binds to the regulatory subunits in R (more active) state. Explain with either or both of the following:

- high [ATP] is an indicator that the cell is "energy-rich" so it can grow and divide, which requires pyrimidine nucleotides as well as purine nucleotides, so cell should keep making pyrimidine nucleotides, and/or
- high [ATP] is an indicator that purine nucleotides are plentiful and the cell needs to balance supply of pyrimidine nucleotides with purine nucleotides for nucleic acid synthesis (growth and cell division).

5. (8 pts) Chymotrypsin

- A. (4 pts) Which of the following structures represents the tetrahedral intermediate in the **deacylation** half-reaction of chymotrypsin?



- B. (4 pts) In the chemical mechanism of chymotrypsin-catalyzed hydrolysis of a peptide substrate, what is the species that is actually hydrolyzed (the species with which H_2O reacts)?

- peptide bond in substrate
 - His--Ser bond in catalytic triad
 - 1st tetrahedral intermediate
 - 2nd tetrahedral intermediate
- *5) **acyl-enzyme intermediate**

6. (5 pts) Suppose that a homodimeric enzyme with a **phosphoserine** residue is **less active** than the enzyme with the free Ser-OH. **Briefly** state how the cell would remove the phosphate group if it needed to activate the enzyme. (Be as specific as you can about the mechanism the cell would use, with type of reaction, what type of enzyme (as specific a category as possible), etc.)

Phosphate group removal is a hydrolysis reaction catalyzed by a phosphoprotein phosphatase.

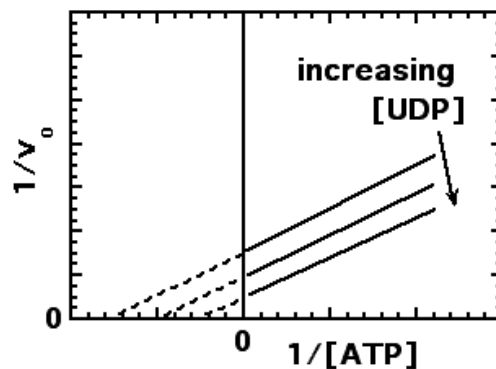
7. (4 pts) The enzyme *nucleoside diphosphate kinase* catalyzes a bisubstrate reaction, transfer of a terminal phosphate from ATP to an acceptor nucleoside diphosphate, as in the following reaction:



Lineweaver-Burk plots of $1/v_0$ vs. $1/[\text{ATP}]$ are shown at right for 3 different fixed concentrations of $[\text{UDP}]$.

Does this reaction have a kinetic mechanism that is **sequential (single displacement)** or **ping-pong (double displacement)**? On what basis did you make that choice?

ping-pong (double displacement), because parallel lines on a double reciprocal plot for different fixed concentrations of 2nd substrate are diagnostic of a ping-pong kinetic mechanism.



8. (6 pts) In the context of a bisubstrate reaction (a reaction with 2 substrates),
A. (3 pts) What is a **ternary complex**?

Ternary complex is a complex of ENZYME WITH BOTH SUBSTRATES BOUND AT THE SAME TIME ($E \cdot S_1 \cdot S_2$)

- B. (3 pts) A ternary complex occurs during the reaction catalyzed by an enzyme with a
*1) **sequential kinetic mechanism** 2) ping-pong kinetic mechanism 3) both 4) neither

9. (14 pts) The activity of an enzyme was assayed as a function of pH, and the results below were obtained.

- A. (5 pts) What is the apparent pK_a for the functional group affecting enzyme activity, (show how you got your answer) and what **functional group** on what type of **amino acid** residue is the likeliest cause of the observed pH dependence?

apparent $pK_a = 6.0$ (pH at which activity is 50% of maximum, so 50% of the ionizable functional group is in active form) -- His imidazole

- B. (4 pts) Does the group need to be in its protonated or unprotonated form for the enzyme to be active? **PROTONATED**

Why did you give that answer? **Because the activity decreases as the pH increases, so the conjugate acid is the active form. (Base form = inactive form)**

- C. (5 pts) **CALCULATE** (don't read off the graph) what **per cent** of the enzyme molecules would have that functional group in the **INACTIVE** form at pH 5.5. **SHOW YOUR WORK.**

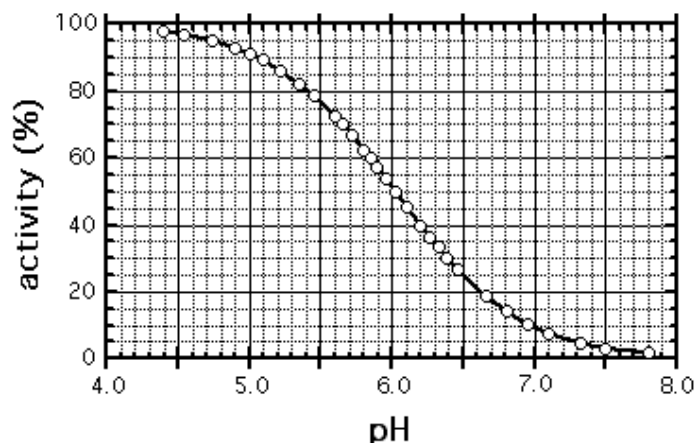
$$\begin{aligned} \text{pH} &= \text{p}K_a + \log([\text{base}]/[\text{acid}]); \text{pH} - \text{p}K_a = \log([\text{Im}^0] / [\text{Im}^+]) \\ \log([\text{Im}^0] / [\text{Im}^+]) &= 5.5 - 6.0 = -0.5; [\text{Im}^0] / [\text{Im}^+] = 10^{-0.5} = \underline{0.316 / 1} \\ & \text{(= base/acid RATIO.)} \end{aligned}$$

INACTIVE FORM IN THIS CASE IS CONJUGATE BASE, Im^0 .

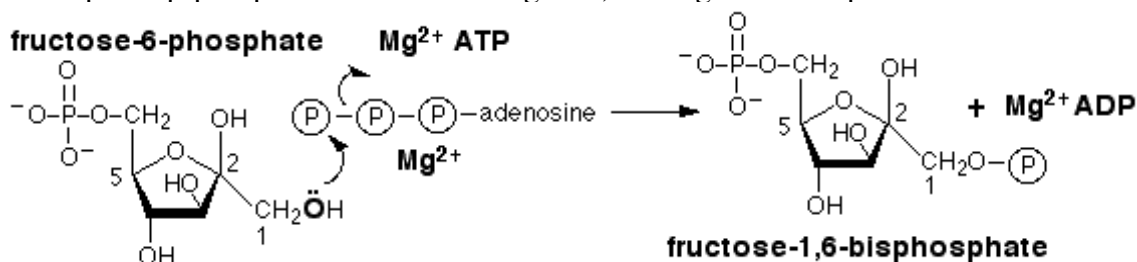
FRACTION of total that's INACTIVE = $[\text{Im}^0] / \text{total} = [\text{Im}^0] / ([\text{Im}^+] + [\text{Im}^0])$

FRACTION of total that's INACTIVE = $0.316 / (1 + 0.316) = 0.24$

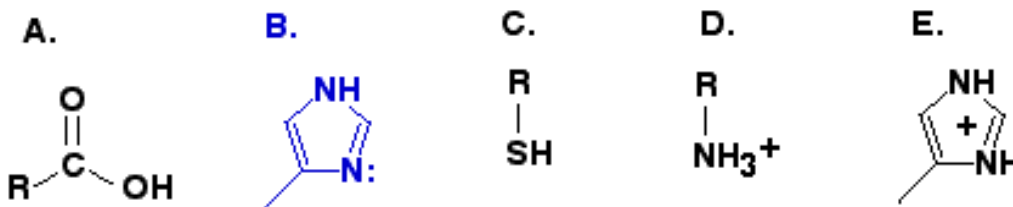
(24% of total imidazole is neutral, so 24% of enzyme molecules are inactive.)



10. (7 pts) In the chemical mechanism of the enzyme phosphofructokinase-1, the two substrates are both bound in the active site, with the C-1 hydroxyl of the fructose-6-phosphate aligned with the γ -phosphoryl group (the terminal phosphate group) on MgATP. The O of the C-1 OH group attacks the electrophilic γ -phosphorus atom of the MgATP, and MgADP is displaced:



- A. (3 pts) Which of the following functional groups in the enzyme active site close to the substrate's C-1 hydroxyl might be expected to facilitate the attack of the C-1 hydroxyl group on the ATP?

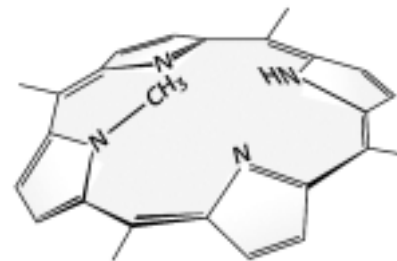


- B. (4 pts) Briefly explain what catalytic role the group in part A would play in the chemical mechanism (how the group would facilitate the attack of the C-1 hydroxyl group on the ATP).

The only conjugate BASE answer above (B, His imidazole) would act as a GENERAL BASE, abstracting a proton from the C-1 hydroxyl group and thus assisting in the nucleophilic attack of the C-1 hydroxyl on the ATP.

11. (5 pts) The enzyme **ferrochelatase** catalyzes the final step in the biosynthetic pathway for production of heme, insertion of the Fe^{2+} into protoporphyrin IX, the organic portion of the heme structure. The nearly planar porphyrin structure has to be **bent** for the iron to enter, and the enzyme indeed bends one of the pyrrole rings of the substrate during catalysis, distorting it 36° , so the enzyme permits the iron to enter 2.5×10^4 -fold faster than the uncatalyzed insertion of iron.

An N-methylporphyrin (structure at right) that resembles the bent porphyrin was used to generate an antibody, and the resulting antibody catalyzes iron insertion into protoporphyrin IX 2.5×10^3 -fold faster than the uncatalyzed reaction, i.e., only 10 times slower than the rate catalyzed by ferrochelatase itself.



Explain why an antibody that binds to the N-methylporphyrin would catalyze the metal insertion in protoporphyrin IX.

The N-methylporphyrin must be a transition state analog; antibodies against transition state analogs bind specifically to the transition state and thus stabilize (reduce the free energy of) the transition state, thus lowering the free energy of activation (ΔG^\ddagger) and increasing the rate constant for the reaction.

12. (21 pts) Glutamate dehydrogenase catalyzes the reaction

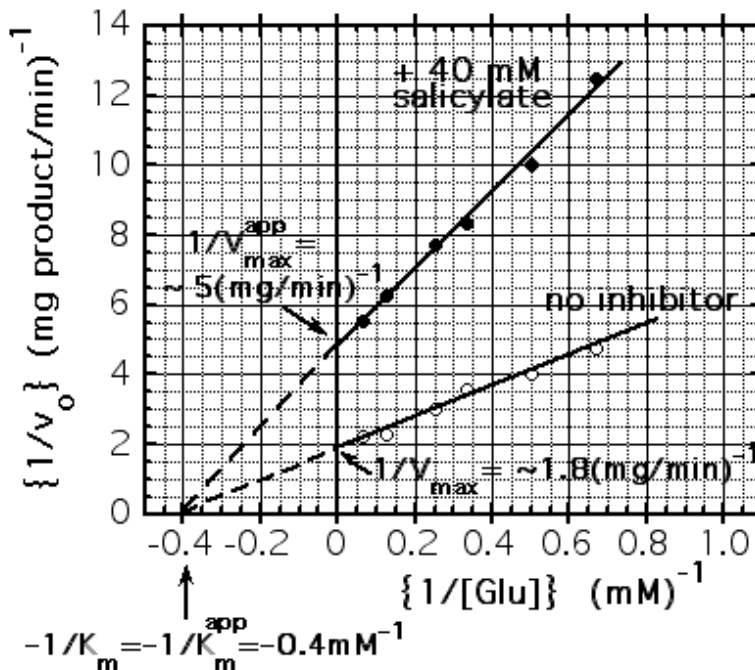


Salicylate is a *reversible inhibitor* of the catalytic activity of the enzyme .

The activity of the enzyme (v_0 in *mg product produced/min*) was measured at different concentrations of the substrate glutamate in the absence and in the presence of **40 mM salicylate** (I), and the data were plotted on the double reciprocal plot on the right, $1/v_0$ vs. $1/[\text{glutamate}]$.

Answer the questions below.

Show clearly how/where you obtained your numerical answers. State units -- just use the units used by the experimentalist, even if they're odd!



A. (3 pts) What type of inhibitor is salicylate? PURE NONCOMPETITIVE
This type of inhibitor binds to: 1) the *free E* 2) the ES complex ***3) both E and ES.**

B. (3 pts) What is K_m for Glu in the absence of salicylate? 2.5 mM
 $-1/K_m = -0.4 \text{ mM}^{-1}$, so $K_m = (+) 2.5 \text{ mM}$

C. (3 pts) What is K_m^{app} for Glu in the presence of 40 mM salicylate? 2.5 mM
 $-1/K_m^{\text{app}} = -1/K_m = -0.4 \text{ mM}^{-1}$, so $K_m^{\text{app}} = (+) 2.5 \text{ mM}$

D. (3 pts) What is V_{max} in the absence of salicylate? ~0.56 mg/min
 $1/V_{\text{max}} = \sim 1.8 \text{ (mg/min)}^{-1}$, so $V_{\text{max}} = \sim 0.56 \text{ mg/min}$

E. (3 pts) What is $V_{\text{max}}^{\text{app}}$ in the presence of 40 mM salicylate? ~0.20 mg/min
 $1/V_{\text{max}}^{\text{app}} = 5.0 \text{ (mg/min)}^{-1}$, so $V_{\text{max}}^{\text{app}} = \sim 0.20 \text{ mg/min}$

F. (5 pts) What is K_I (the dissociation equilibrium constant) for salicylate? ~22.2 mM

$$V_{\text{max}}^{\text{app}} = V_{\text{max}} / \{1 + ([I]/K_I)\}$$

$$1 + ([40\text{mM}]/K_I) = V_{\text{max}} / V_{\text{max}}^{\text{app}} = 0.56/0.20$$

$$([40\text{mM}]/K_I) = 2.8 - 1 = 1.8$$

$$K_I = [40\text{mM}] / 1.8 = \sim 22.2 \text{ mM}$$

/20