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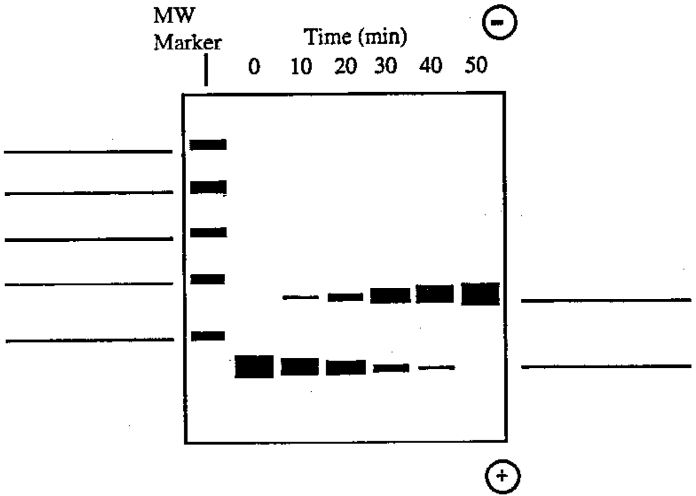
Chemistry 130A Final (Tinoco)

December 13, 1994
Open book three hour exam
(Read the entire exam and do the easiest parts first)

1. (30 points)

Heat Shock Transcription Factor (HSF) is a protein that takes part in protecting cellular functions from extreme temperature increases. A particular sequnce of HSF was purified, and placed in an aqueous solution of high pH. Samples taken over time were observed to have the following behavior on an SDS-PAGE gel.



(a) The bands of the MW marker lane arise from known molecular weights of 14.5, 21.5, 31.0, 45.0, 66.0 kDaltons (kD or kg mol^{-1}). Label the bands appropriately to the left of the figure. Estimate the molecular weights of the HSF bands to the nearest 1.0 kD. Label the bands to the right of the figure.

(b) What process must the HSF protein have undergone? Write a simple, 1 line mechanism.

(c) Considering the type of gel run, what can you say concerning the stability of the product in the 50 min lane?

(d) From the mechanism you proposed in part (b), write a differential equation for the rate of the reaction. What is the kinetic order that you would predict for the reaction?

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(e) Describe how you could use the data from the gel electrophoresis experiment to test your prediction about the order of the reaction, and to obtain the rate constant for the reaction.

(f) A slightly different sequence of HSF was obtained. Mass spectroscopy determined its molecular weight to be 31.0 kD and its isoelctric point, pI, was determined to be 10.6. However, running a sample of this protein on an SDS-PAGE gel (of pH 8.8) showed it to run as a single band of apparent molecular of 40.0 kD. No reaction took place. Explain the result qualitatively.

2. (25 points)

Antibodies normally only bind molecules; catalytic antibodies, co-discovered by Peter Schultz at Berkeley, have been engineered to also catalyze chemical reactions, i.e., they can act as enzymes. An antibody was generated to catalyze the hydrolysis of a carboxylic ester and the kinetics of the reaction were examined [Science **234**, 1566 (1986)]. A plot of the kinetics of the hydrolysis of the ester is shown in Fig. 3, below.

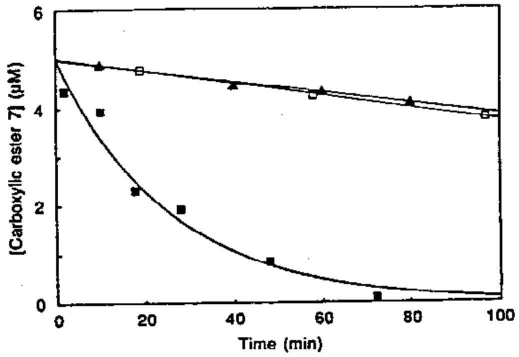

Fig. 3. Rate of hydrolysis of carboxylic ester 7 determined by HPLC under the conditions described in Table 1 (50 m*M* phosphate buffer, *p*H 8.0, 23°C). (▲) Uncatalyzed (background) rate of hydrolysis. (□) Effect of 0.5 μ*M* non-specific monoclonal immunoglobulin G. (■) Monoclonal antibody (0.1 μ*M*) from hybridoma 6D4 against 4 (anti-4). The superimposed curve represents a theoretical exponential decay that fits the data points.

(a) From the shape of the curve denoted by filled squares, what is the order of the reaction? The half-life for hydrolysis is 16 minutes, what is the rate constant for this reaction in min^{-1}?

(b) The reaction was also run in the presence of an inhibitor; a plot of the data is shown below (Fig. 4). From the plot of the data with no inhibitor present (filled squares) calculate K_M in units of nM and V_{max} in nM sec^{-1}. The y-intercept is 0.454 nM^{-1} sec and the slope is 8.64 x 10^{-4} sec.

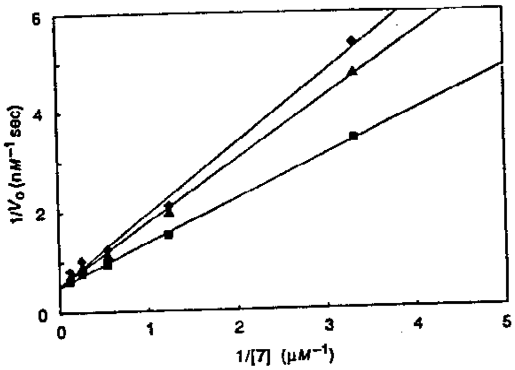

Fig. 4. Lineweaver-Burk plot for hydrolysis of substrate **7** by anti-**4**. Velocities were determined spectrophotometrically by measuring initial rates during the first linear portion of the reaction as described in Table 2. The substrate concentrations were corrected for amounts consumed during initial equilibration. (■) No inhibitor present. (▲) Inhibited by 50 n*M* phosphonate **3**. (◆) Inhibited by 100 n*M* **3**.

(c) What type of inhibition does the graph in Fig. 4 illustrate? Write the expression you would use to calculate the value of K_I for the inhibitor from the data in Fig 4. Define the terms in your equation.

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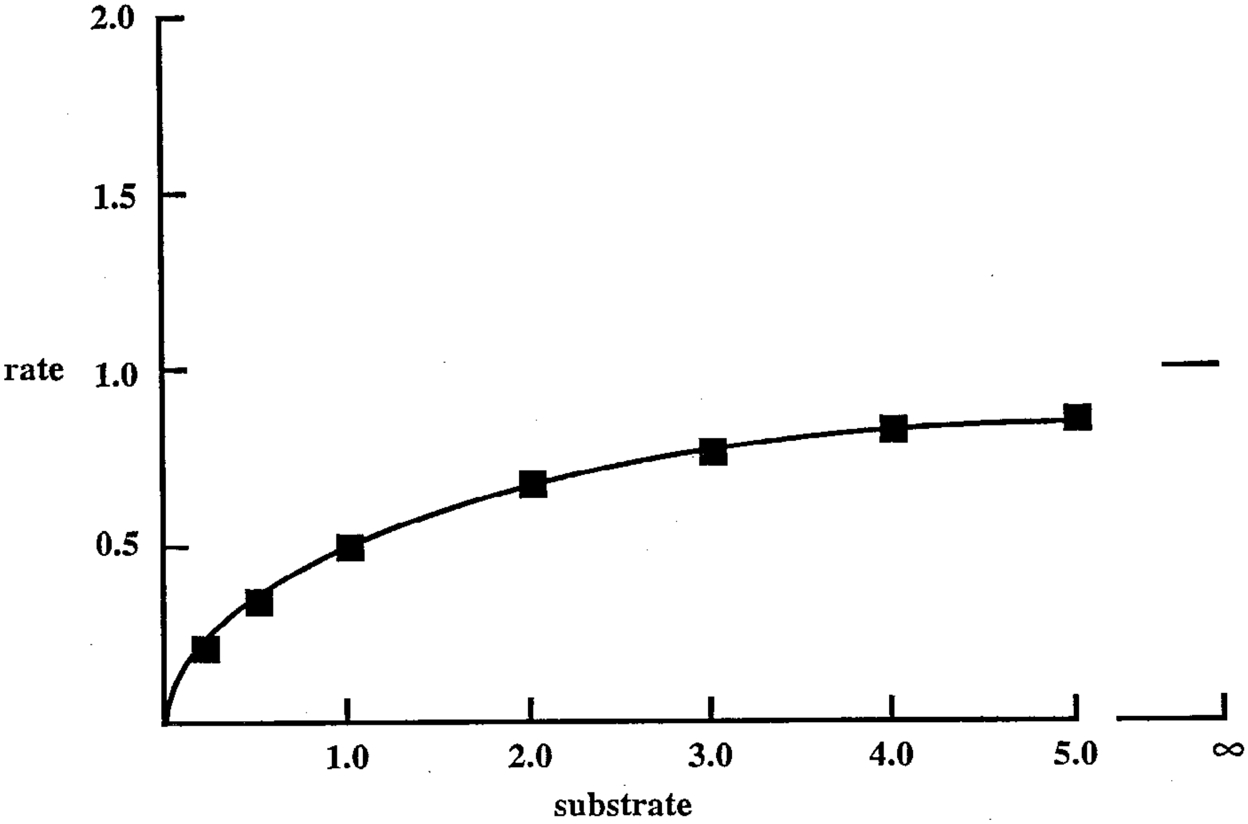
(d) The plot below is obtained for $K_M = 1$ and $V_{max} = 1$ for Michaelis-Menten enzyme kinetics. Sketch in the curve you would obtain for $K_M = 1$ and $V_{max} = 2$. Sketch in the curve you would obtain for $K_M = 2$ and $V_{max} = 1$. Label both curves.



(e) The Michaelis constant is a combination of three rate constants. Describe what kinetic experiments you would have to make to determine all three rate constants.

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3. (25 points)

According to the chemiosmotic theory, an electrochemical proton gradient is used to synthesize ATP in mitochondria. The enzyme that synthesizes ATP is located on the inside of the mitochondrial membrane. The oxidation of carbohydrates and fats is used to pump protons from the inside of the membrane to the outside of the membrane until the steady state membrane potential is –140 mV and the pH gradient is 1.5 pH units. At 37 °C the concentrations inside a mitochondrion are shown below:

pH = 7.0
[ATP] = 1.0 mM
[ADP] = 1.0 mM
[Pi] = 3.0 mM
Φin = –140 mV

The standard free energy change for the reaction ATP + $H_2O \rightarrow$ ADP + Pi at 37 °C is $\Delta G^{\circ\prime} = -31.0$ kJ mol^{-1}. The biochemist's standard state is used. You may approximate activities by concentrations in the following calculations.

(a) How much free energy is required to synthesize ATP inside the mitochondrion?

(b) How much free energy is made available by moving one mole of protons from the outside to the inside of the mitochondrion as favored by the pH gradient and potential difference? Is this enough to drive ATP synthesis?

(c) What is the minimum number of protons that must be translocated to provide enough free enregy to synthesize one molecule of ATP?

(d) In living biological cells the concentration of Na^+ inside a cell is kept at a lower concentration than the concentration outside the cell by active transport of Na^+ ions from the inside to the outside of the cell. Calculate ΔG for the transport of one mol of Na^+ ion by the following process at 37°C; you may ignore any effects of potential gradients.

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$$Na^+ \text{ (0.020 M inside)} \rightarrow Na^+ \text{ (0.10 M outside)}$$

(e) If there is a concentration difference for a molecule on the inside and outside of a living cell, does this mean that there must be active transport of the molecule? Describe how you could test whether there was, or there was not, active transport.

4. (25 points)

A DNA oligonucleotide was synthesized to use as a PCR probe and its melting temperature was measured with its complementary oligonucleotide. The equilibrium is shown below.

$$S_A + S_B \rightleftharpoons$$

(a) The fraction of duplex formed depends on the initial concentrations of single strands, and on the temperature. The "melting temperature" (T_m) is defined as the temperature where the equilibrium concentrations of single strands are half their initial values. For initial concentrations of $[S_A]_0 = [S_B]_0 = c/2$, derive the equation for the equilibrium constant at the T_m. Your equation will be only a function of c—the total strand concentration.

(b) The standard enthalpy change for the reaction above was found to be $\Delta H^\circ = -200.0 \text{ kJ mol}^{-1}$ and $\Delta S^\circ = -650.2 \text{ J K}^{-1} \text{ mol}^{-1}$. Assume these values are independent of temperature and calculate the equilibrium constant at 40° C.

(c) Calculate the T_m in °C for this duplex for total strand concentration of c = 5.0 mM.

(d) You want the T_m to be larger so you decide to add another G•C base pair to the right hand end of the duplex above. This adds a

5'CC
3'GG

nearest neighbor stacking interaction to the duplex. Calculate the values of $\Delta H°$ and $\Delta S°$ for forming your new duplex.

(e) Will adding a base pair increase, decrease or leave the T_m unchanged?
Will increasing the strand concentration increase, decrease or leave the T_m unchanged?
Will increasing the ionic strength increase, decrease or leave the T_m unchanged?

5. (25 points)

An enzyme binds a substrate S, then binds a cofactor G by reversible equilibria. A chemical step produces product with rate constant k_C, then there is release of product with rate constant k_P. The mechanism is given below.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \qquad ES + G \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} GES$$

$$GES \xrightarrow{k_c} EP$$

$$EP \xrightarrow{k_P} E + P$$

(a) Write the differential equation—the rate equation—for the production of ES: d[ES]/dt.

(b) Write the equation which describes a steady state approximation for EP.

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(c) Assume that the formation of ES and GES are both fast to equilibrium, and that EP is in a steady state. This allows you to write a rate expression for the formation of P, d[P]/dt, in terms of rate constants, equilibrium constants, and concentrations of reactants only: [E], [S], [G].

(d) If $k_1 = 9 \times 10^7$ M^{-1} min^{-1} and $k_{-1} = 0.2$ min^{-1} at 25 °C, calculate the equilibrium *dissociation* constant (with units of M) for ES at 25 °C.

(e) The transition state enthalpy for k_1 is $\Delta H^{\dagger} = 50$ kJ mol^{-1}; for k_{-1} $\Delta H^{\dagger} = 200$ kJ mol^{-1}. Calculate the ratio of the equilibrium dissociation constant for ES at 50 °C relative to that at 25 °C.

6. (25 points)

The solubilities of two amino acids in two solvents at 25 °C are given below; they are the concentrations present in saturated solutions.

	in water	in ethanol
glycine	3.09 *M*	0.404 *M*
valine	0.60 *M*	0.132 *M*

(a) Calculate the standard free energy of transfer, $\Delta\mu°$, of 1 mol of glycine from the solid to the aqueous solution at 25 °C. The standard state for the solid is the pure solid; the standard state in the solution corresponds to a = 1 extrapolated from a dilute solution; the molarity concentration scale is used. You may consider the solutions to be ideal.

(b) Calculate the standard free energy of transfer, $\Delta\mu°$, of 1 mol of glycine from ethanol to the aqueous solution at 25 °C.

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(c) Assume that the effects of the backbone and sidechain are simply additive for valine (glycine essentially has no side chain), and calculate the standard free energy of transfer of 1 mol of the valine *side chain* from water to ethanol at 25 °C.

(d) Ethanol can be considered to mimic the interior of a protein. Will the mutation of a glycine to a valine in the interior of a protein favor the folding of the protein if interaction with the solvent is the dominant effect? Explain.

(e) Assume that the protein folding equilibrium is a two-state transition: folded or unfolded.

$$F \rightarrow U$$

What hydrodynamic measurement could you make to measure the equilibrium constant for the reaction? Describe what you would measure and how it is related to the unfolding reaction.

7. (45 points) Answer the each of the following questions by just one word: true or false.

(a) The energy of an isolated system never increases.

(b) The entropy of an isolated system always increases.

(c) The free energy of a closed system always decreases.

(d) A developing hen egg can be approximated by a closed system.

(e) The rate of any chemical reaction always increases with increasing temperature.

(f) The average kinetic energy of molecules always increases with increasing temperature.

(g) For a zero-order reaction a plot of ln c vs. time is linear.

(h) The half-life for a zero-order reaction is 60 s, therefore its rate constant $k = 0.0116\ s^{-1}$.

(i) The stoichiometry is $A + B \rightarrow P$: the reaction has second order kinetics.

(j) In enzyme kinetics a plot of rate^{-1} vs. substrate^{-1} is a Michaelis-Menten plot.

(k) In enzyme kinetics doubling the substrate concentration should double the rate.

(l) In enzyme kinetics doubling the enzyme concentration should double the rate.

(m) The mechanism is $A^+ + B^+ \rightarrow P$; increasing ionic strength increases the rate.

(n) In photochemistry doubling the light intensity should double the rate.

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(o) In photochemistry doubling the light frequency (the light energy) should double the rate.

(p) Protein A has twice the molecular weight of B; its sedimentation constant is two times as large.

(q) Protein A has twice the frictional coefficient of B; its diffusion constant is half as large.

(r) Water saturated with liquid mercury has the same mercury vapor pressure as the pure mercury, if the mercury and water are at the same temperature.Water does not dissolve in mercury.

(s) A red blood cell is placed in an aqueous solution with twice the osmotic pressure of the cell contents; the cell will therfore expand and probably break.

(t) The activity of a molecule in solution is always less than its concentration.

(u) The standard entropy change for a reaction is positive, therefore the equilibrium constant must increase with increasing temperature.

(v) The oxidation of β-hydroxybutyrate to acetoacetate by O_2 in aqueous solution at standard conditions at pH 7 is spontaneous at 25 °C. Useful data are on p. 145.

(w) Work can not be converted with 100% efficiency into heat; some work is always lost in the form of entropy.

(x) My name is on each page of this exam!