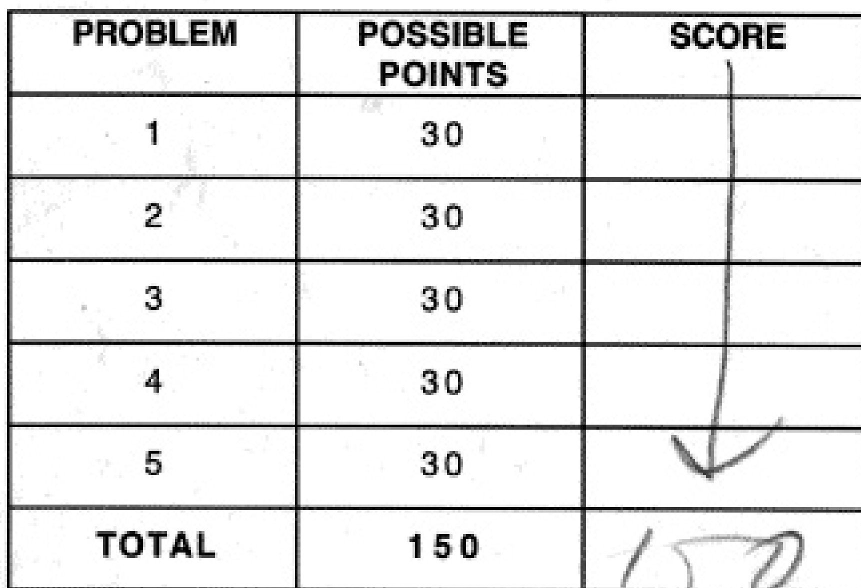
Professor Charles McKenna NAME: KEY
Chemistry 519, Spring 2000
Monday, February 28, 2000 10:00 a.m.

Midterm Exam

GOOD LUCK!



PROBLEM	POSSIBLE POINTS	SCORE
1	30	
2	30	
3	30	
4	30	
5	30	
TOTAL	**150**	150

1 Answer briefly:

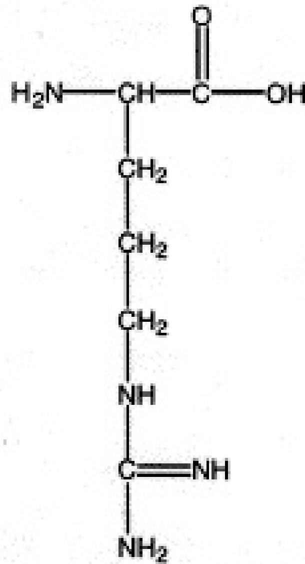
(a) Single-letter equivalent for Ala-Asp-Gly-Ser.

A - D - G - S

3

(b)

3



Name this amino acid: ARG

(c - e) List three types of non-covalent bonding interactions important for maintaining protein structure.

1: H-bonding

2: Van der Waal's

3: Electrostatic

+ Hydrophobic

@3

9

Σ = 6 RSH

(f) If a protein contains three disulfide linkages (Cys-Cys) and can form them randomly, how many different linkages can result?

3 = 5 · 3 · 1 = 15

(g - i) Distinguish primary, secondary and tertiary structure in proteins.

1° - amino acid seq

9 2° - α-helix, β-sheet (periodic)

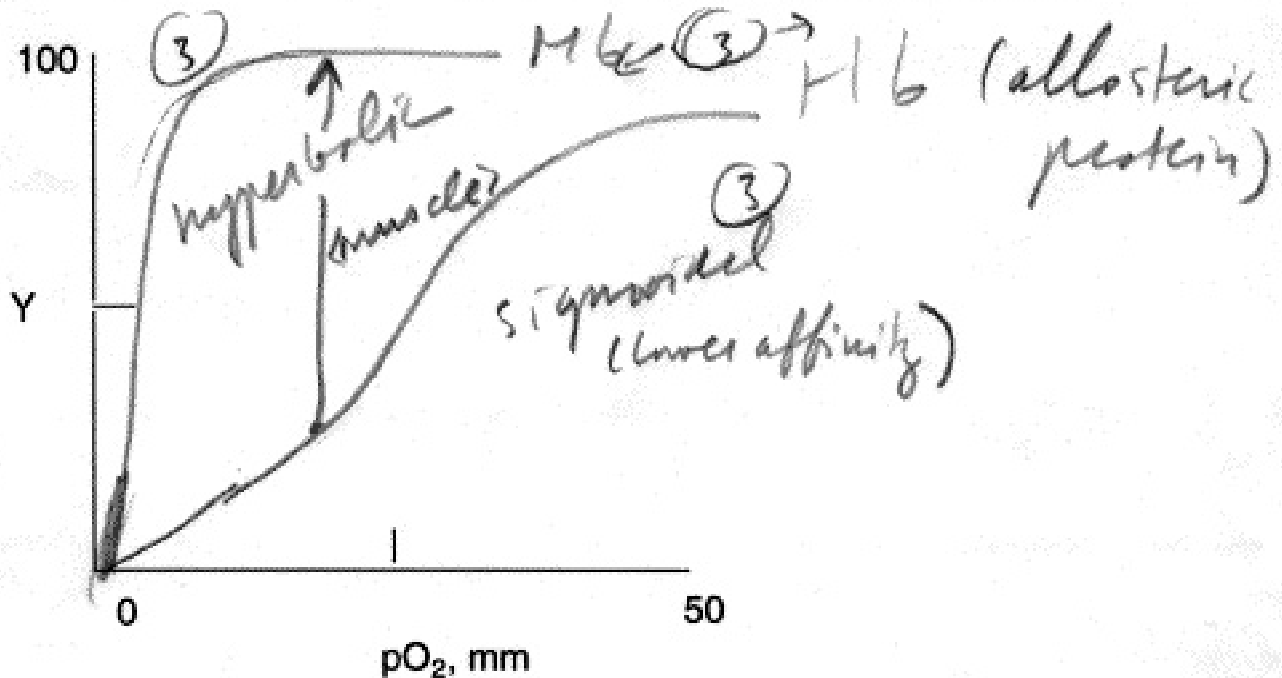
3° - conformation in 3D

(j) List a factor that may contribute to enzyme catalysis, besides strain.

3 orientation
orbital steering
electrostatics
proximity
acid/base / nucleophilic / electrophilic catalysis

2. (30 Points)

(a - c) The O_2 saturation function, $Y = [MbO_2 / (Mb + MbO_2)] \times 100$.
Sketch Y vs. pO_2 for: (i) myoglobin, **Mb**; (ii) hemoglobin, **Hb**



(d -e) Briefly explain the difference between the two curves, given: pO_2 in muscle ~ 20 mm, pO_2 in lungs ~ 100 mm.

(6) Hb - cooperative - dumps O_2 from blood to muscle; loads O_2 in O_2-rich lungs; modulated by H^+ (pH), CO_2 — when 1st O_2 released, 2nd/3rd, 4th O_2 released more easily

f) Given that for O_2 binding by Mb, Keq = 10^{-6} M, where Keq = $[Mb][O_2]/[MbO_2]$, and the "on" rate of O_2 binding, $k_{on} = 2 \times 10^7\ M^{-1} s^{-1}$, find k_{off}.

(3) $K_{eq} = \frac{k_{off}}{k_{on}}$; $k_{off} = (10^{-6}\ M)(2 \times 10^7\ M^{-1} s^{-1})$

$= 20\ s^{-1}$

MW ~16K MW ~64K

g) Suggest a simple method to separate Mb from Hb preparatively (not gel electrophoresis).

(3) By MW – use gel filtration; or diafiltration / 20K MW cut-off membrane

h) The migration velocity of a protein in an ultracentrifuge is

$v = Fc/f = \frac{m(1-v\rho)^{*}\omega^2 r}{f}$ (Fc = force, f= frictional coefficient)

(3) (i) Define the sedimentation coefficient, S. 10^{-13} s

$= \frac{v}{\omega^2 r} = \frac{m(1-v\rho)}{\omega^2 r}$

(3) (ii) Which has a larger "S" value- ribonuclease or an *E.coli* bacterium?

~1-2 ≈ 10^4

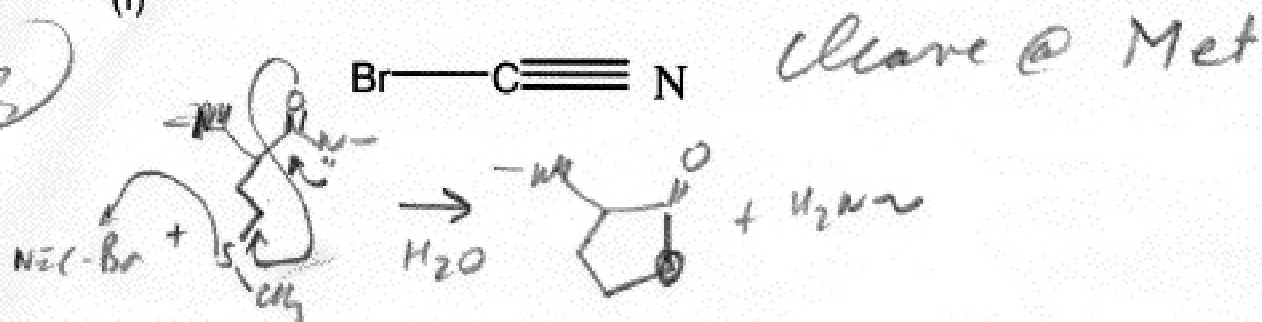
(iii) What is the important advantage of ultracentrifugation over SDS-gel electrophoresis in determining the MW of a protein that has multiple subunits (quaternary structure)?

(3) SDS-gel method denatures protein – get MW of subunit – not MW of entire protein

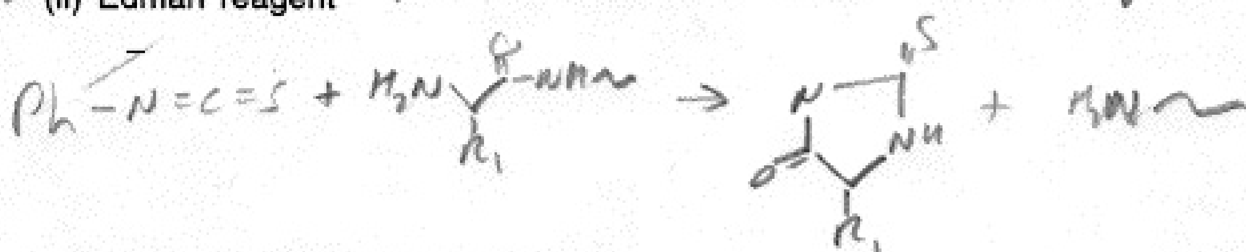
3. (30 pts.)

a) Explain briefly how each of the following is used in peptide analysis:

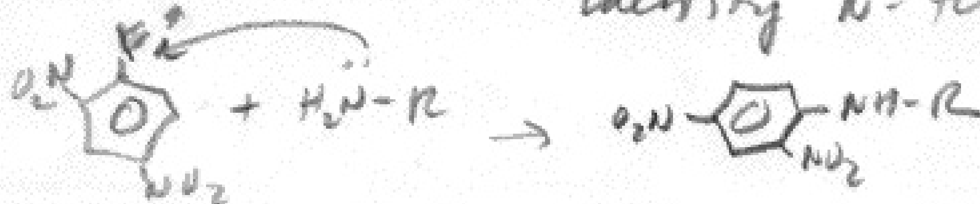
(i)



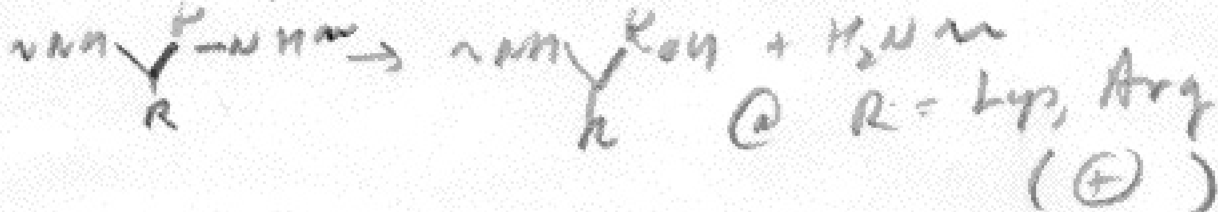
(ii) Edman reagent N-terminal am. acid sequencing



(iii) 2,4-dinitrofluorobenzene identify N-terminal am. acid

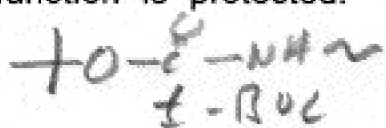


(iv) trypsin endopeptidase → smaller peptides / known cleavage point;

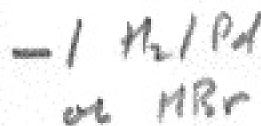


(b-c)

Give an example of a protecting group used in peptide sythesis, including a method to deprotect. Be sure to identify what function is protected.



Bz (amino or ester)



(d - e) Given that $K_m = (k_{-1} + k_2)/k_1$ and that k_{cat}/K_m (or k_2/K_m) is a measure of "enzyme efficiency":

show that if [S] is small,

$V = (k_2/K_m)[S][e_o]$ (e_0= total enzyme concentration). (M-M Eq)

$V = \frac{Vm \cdot S}{(Km + S)}$; $S \ll Km \Rightarrow$ $V = \frac{Vm \cdot S}{Km}$ But $Vm = k_2 \cdot e_0$ so $V = \frac{k_2}{Km} \cdot S \cdot e_0$

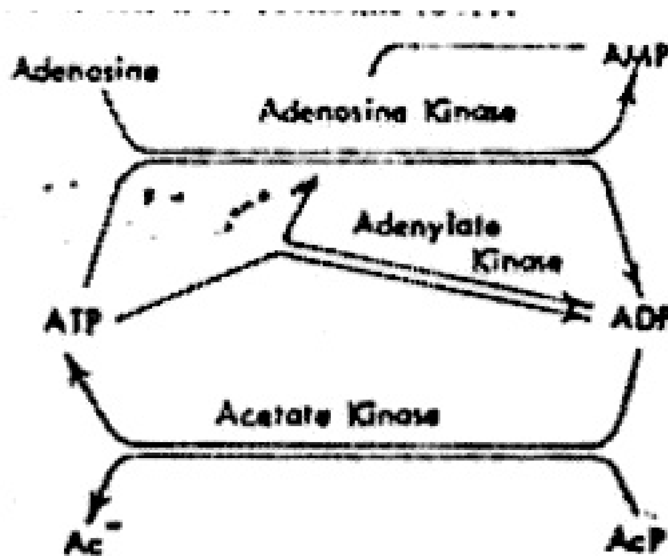
(f - g) Explain the ultimate limit ("diffusion" limit) of 10^8-10^9 M^{-1} s^{-1} imposed on any enzyme-catalyzed reaction. What rate constant does it correspond to, and why?

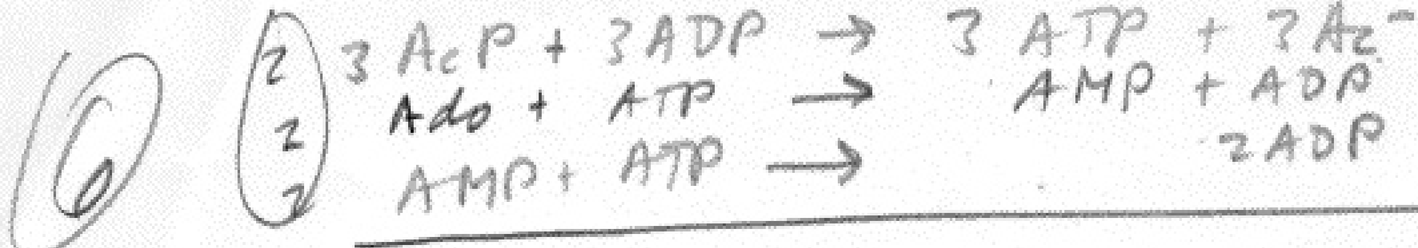
$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$; at the limit, every collision of E + S → P so rate of collision is limiting, controlled by k_1

4. (30 pts.)

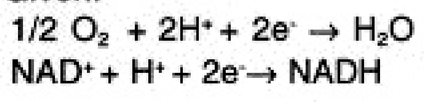
(a - b) Consider the scheme below. Show that the net stoichiometry is:

3AcPi +Adenosine → 3ATP + 3 acetate





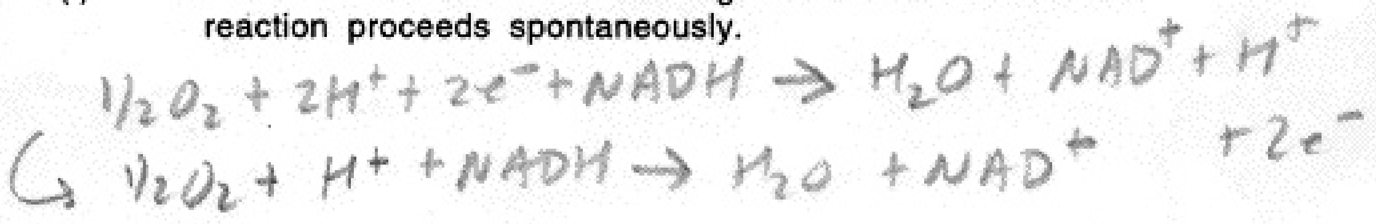
c) Given:



$1/2\ O_2 + 2H^+ + 2e^- \rightarrow H_2O$ $\quad E'_o = +0.82V$

$NAD^+ + H^+ + 2e^- \rightarrow NADH$ $\quad E_o' = -0.32V$

(i) Add these two half reactions together so that the overall reaction proceeds spontaneously.



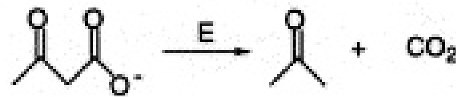
(ii) Calculate $\Delta E_o'$.

(iii) Calculate $\Delta G_o'$.

(iv) About how many ATP could be made from ADP + Pi if coupled to this reaction? (Explain answer)

d) Given: $2H^+ + 2e^- \rightarrow H_2$, $E_o' = -0.42V$ at ph 7; find E_o at pH 14.

e) Acetoacetate decarboxylase (E) catalyzes:



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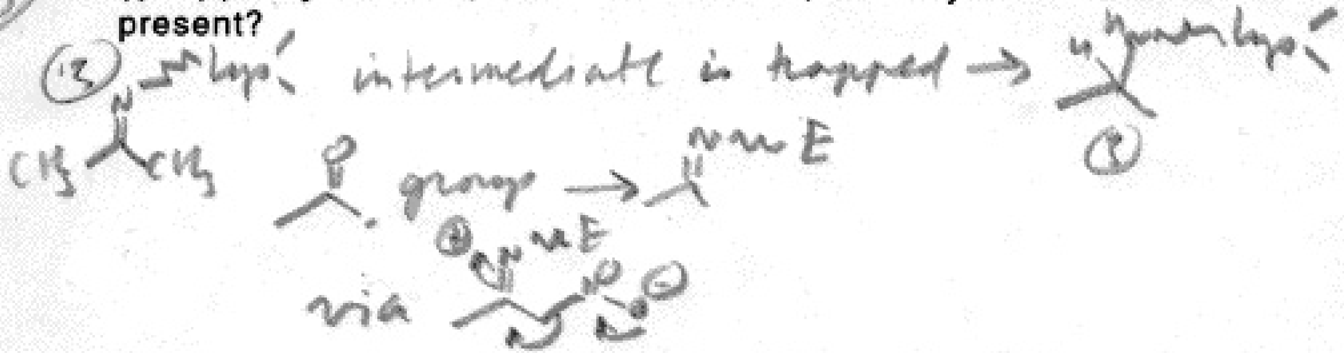
(i) - (ii) Why does BH_4^- inhibit this reaction, but only if the substrate is present?



(iii) An E lysine is involved in catalysis but at a pK of ~ 6 (normal pKa is ~10.5). Why does the enzyme need to change this pk from 10.5 to 6?

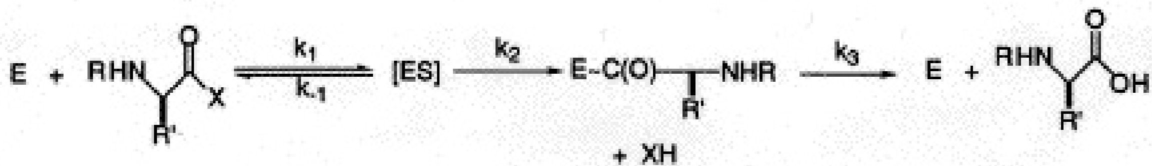
3

lys ~NH$_2$ is normally in ~NH$_3^{\oplus}$ form, but need ~NH$_2$ form as nucleophile.

Note that need ⊕ at N atom of imine to catalyze the decarboxylation

5. (30 pts.)

a) Chymotrypsin (E) catalyzes



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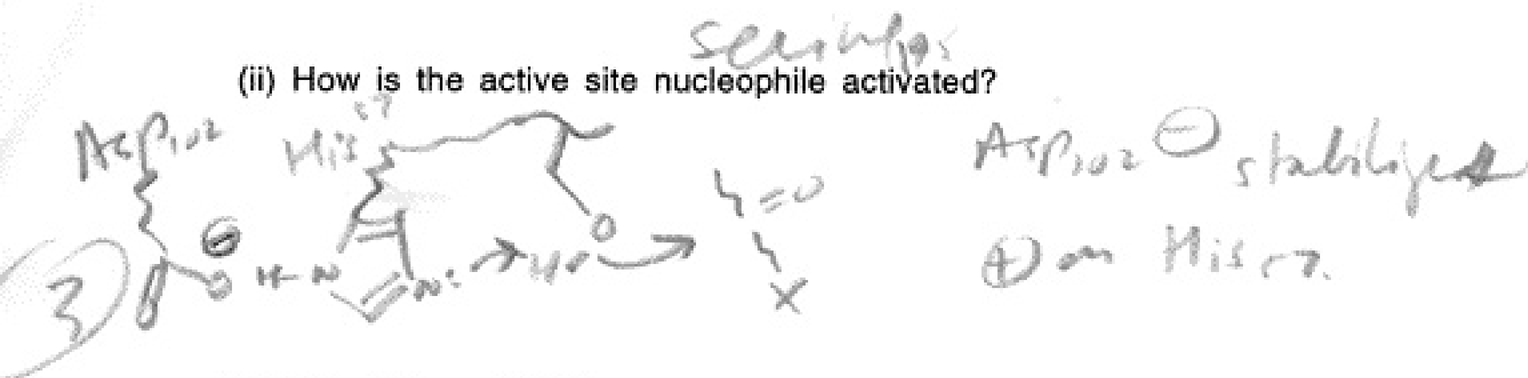
(i) Give one R′ preferred by E.

or Isoleucine

(ii) How is the active site nucleophile activated?



(iii) If X = OMe or OPNP,

the overall rate (kcat) is the same (even though OPNP is a much better leaving group), but not if X = NH_2 (10^3 x slower)

<scheme>

Explain.

For the esters, $k_2 > k_3$ so no leaving group effect.
For the amide, $k_3 > k_2$ so k_2 is rate-determining

(3)

(iv) For which X group(s) will Km = Ks, the dissociation constant for ES, and why?

(3) For the amide, k_2 is slow (k_1 should be ~same) so $K_m \approx \frac{k_{-1}}{k_1} = K_s$

b) If an enzyme is inhibited irreversibly,

(i) Does this effect the observed V_m, K_m, on both?

(3) V_m

(ii) What type of inhibition would you expect?

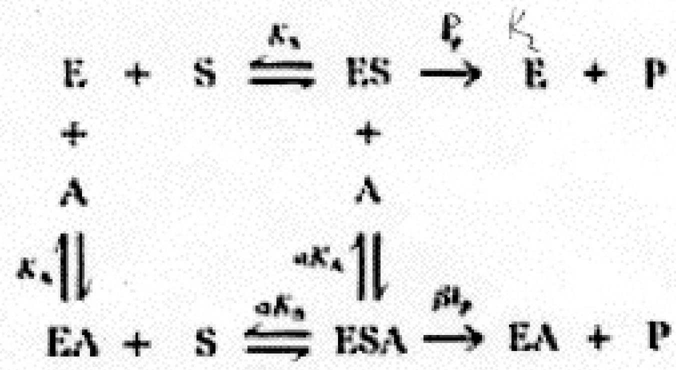
(3) non-competitive

(3)

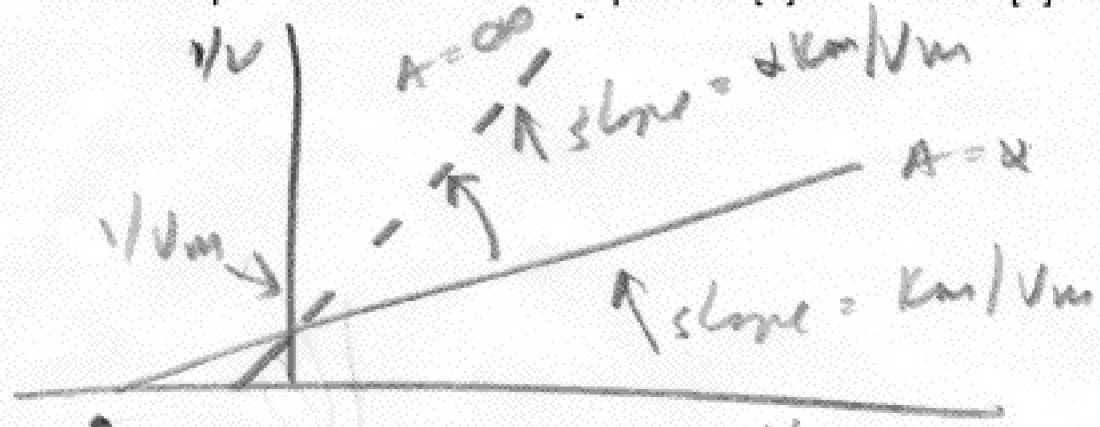
(iii) Given that the same type of inhibition can be caused by a reversible inhibitor, suggest a simple method to distinguish the two cases, based on either diafiltration or dialysis.

Wash away excess inhibitor + re-assay enzyme –

(c - e) Consider the scheme (A is an inhibitor):



If $\alpha>1$ and $\beta = 1.0$ sketch the L-B plots for $[A] = \infty$ and for $[A] = 0$.



(5)

$-1/K_m$

$1/s$

Type of inhibition? ?Why?

(2) Partial "C"

at I = ∞, no effect on Vm;

(2) cannot drive V to zero

(if α → ∞, ⇒ pure "C")