

Final Examination — 115:413 Experimental Biochemistry

Part A - multiple choice; answer each question by circling the letter of the correct answer.

- Statistical treatments of plotted data seek to minimize
 - the intercept of the best line through the points.
 - the variation of slope of lines from the origin through the points.
 - the sum of deviations of actual points from the best line through the points.
 - the sum of squares of deviations of actual points from the best line through them.
- In pipetting with a micropipetter, you should
 - hold the pipetter horizontal as much as possible.
 - deliver the sample into the solution to which you are adding it.
 - push the plunger down to the first stop when sucking up, beyond it when delivering.
 - suck up into the tip as quickly as possible.
- The pK_1 of H_2CO_3 is 6.1, the pK_2 is 10.6, and the pK_a of NH_4^+ is 9.3. The pH of 1 M NH_4HCO_3 is approximately
 - 6.1
 - 7.7
 - 9.3
 - 10.0
- To take 50 ml of water to pH 12.0 will require how much 0.5 M NaOH?
 - 0.5 ml
 - 1.0 ml
 - 2.5 ml
 - 5.0 ml
- Which of the following is not an advantage of the Coomassie Blue method?
 - lack of interference
 - sensitivity
 - speed
 - low background
- For a protein standard curve you wish samples (0.25 ml) to contain a maximum of 50 μ g of protein. You have a stock solution of ovalbumin, 10 mg/ml. How much should you dilute this to make a working solution, samples of which you will use for the standard curve?
 - 1:100
 - 1:50
 - 1:25
 - 1:10
- Solutions of a protein appear much higher in concentration when measured by the Coomassie Blue method than when measured by the Lowry method, and have very little UV absorbance. This suggests that the protein contains
 - very few tyrosine and tryptophan residues, a lot of arginine.
 - a lot of leucine and isoleucine, very few polar residues.
 - a lot of proline and hydroxyproline residues, very few charged side chains.
 - a lot of tyrosine and tryptophan residues.
- In which protein determination method would polyglycine give a higher absorbance per mg than polytryptophan?
 - UV absorbance
 - Lowry
 - biuret
 - Coomassie Blue
- In polarimetry, the symbols + and - indicate
 - whether the solution rotates the plane of polarization to the right or left of zero.
 - whether the optical rotation is greater or less than zero.
 - whether the sugar is of the D or the L series (related to D- or L-glyceraldehyde).
 - whether or not the sugar is a reducing sugar.
- Colorimetric methods for carbohydrates in strong acid depend on
 - reaction of furfurals with other molecules to produce color.
 - depolymerization of polysaccharides by acid.
 - dehydration of the carbohydrates to furfurals.
 - all of the above.
- The glycosidic oxygen in a sugar (through which glycosides are formed) may be identified as the one which in the open-chain version of the structure
 - is at the top.
 - is at the bottom.
 - is next to the bottom.
 - is a carbonyl.

12. In the Nelson-Somogyi method, the product actually measured is
 a. a furfural complex
 b. reduced arsenomolybdate
 c. Cu^+
 d. a carboxylic acid
13. A carbohydrate shows as much color per mg in the orcinol reaction as L-arabinose, but half as much per mg in the Nelson-Somogyi reaction. Its calculated $[\alpha]_D$ is $+27^\circ$, but after hydrolysis it shows essentially no optical rotation. Relevant $[\alpha]_D$ are: L-arabinose, $+104^\circ$; D-xylose, $+18.1^\circ$; D-mannose, $+14.1^\circ$. A possible structure is
 a. L-xylosyl-(1 \rightarrow 5)- D-xylose.
 b. L-xylosyl-(1 \rightarrow 4)- L-arabinose.
 c. L-xylosyl-(1 \rightarrow 1)- D-xylose.
 d. L-xylosyl-(1 \rightarrow 4)- D-mannose.
14. In our purification of D-amino acid oxidase, the largest amount of protein is removed by
 a. $(\text{NH}_4)_2\text{SO}_4$ precipitation
 b. acidification + heating
 c. DEAE-Sepharose chromatography
 d. phenyl-Sepharose chromatography
15. Phenyl-Sepharose chromatography is an example of
 a. hydrophobic chromatography
 b. gel filtration
 c. ion exchange chromatography
 d. hydroxylapatite chromatography
16. We use crotonate, not benzoate, during the DEAE-Sepharose chromatography because
 a. benzoate absorbs strongly at 280 nm.
 b. crotonate buffers better at pH 6.8.
 c. crotonate keeps FAD on the enzyme better.
 d. crotonate interferes less with the assay.
17. An advantage of $(\text{NH}_4)_2\text{SO}_4$ for protein precipitation is that
 a. its ions associate with proteins
 b. very high concentrations can be made
 c. it doesn't shift the pH of neutral solutions
 d. all of the above
18. To determine the μmoles pyruvate formed in an assay tube,
 a. divide the A_{560} by the slope of the standard curve ($A/\mu\text{mole}$).
 b. multiply the A_{560} by the slope of the standard curve.
 c. divide the A_{560} by the molar extinction coefficient.
 d. divide the A_{560} by the ml dilute enzyme used.
19. The peroxidase assay would be inhibited by presence of
 a. $(\text{NH}_4)_2\text{SO}_4$
 b. L-alanine
 c. catalase
 d. oxygen
20. In the polarographic assay, the curvature of the chart trace is due to
 a. loss of activity of the enzyme
 b. $[\text{O}_2]$ decreasing below the K_m
 c. accumulation of pyruvate
 d. bubble formation
21. In the spectrum of the purified enzyme, a peak at 405 nm is due to
 a. FAD bound to the D-amino acid oxidase
 b. free FAD
 c. denatured hemoglobin
 d. benzoate
22. In high speed centrifugation, it is important to
 a. fill the bottles no more than 3/4 full.
 b. balance bottles to achieve equal mass.
 c. fasten the lid to both rotor and shaft.
 d. wash out the rotor if solution has spilled.
 e. all of the above.
23. The mobility of proteins in SDS polyacrylamide gel electrophoresis is determined by
 a. charge density
 b. molecular weight
 c. molecular shape
 d. all of the above
24. The key property of the stacking gel is
 a. low (4%) acrylamide concentration
 b. presence of a zwitterionic buffer
 c. pH several units lower than pK_a of glycine
 d. all of the above
25. The key ingredient for forming a gel, rather than a viscous solution, by polymerization of acrylamide is
 a. methylenebisacrylamide
 b. tetramethylethylenediamine
 c. ammonium persulfate
 d. triethanolamine

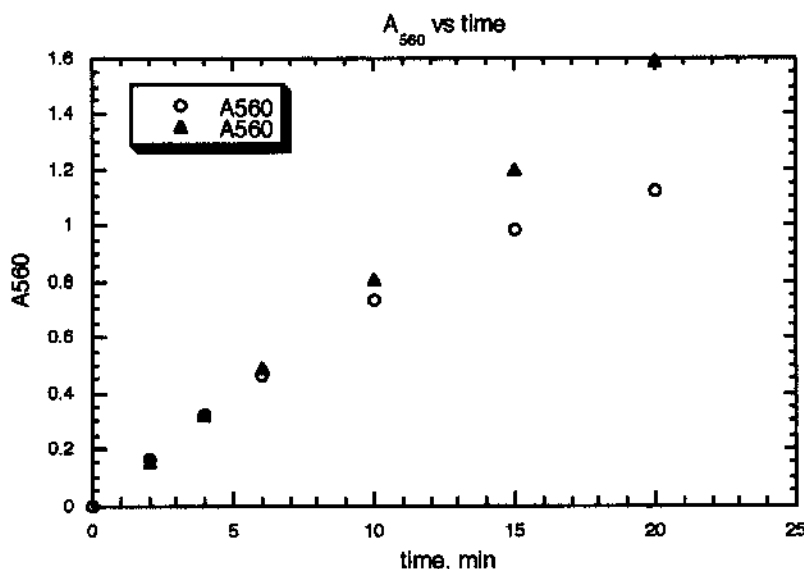
26. What is the function of the 'blocking agent' in which the nitrocellulose is soaked after transfer?
- to keep transferred proteins on the membrane.
 - to block all other sites on the membrane so that they do not bind antibody directly.
 - to block any proteins other than D-amino acid oxidase from reacting with antibody.
 - to inhibit the growth of bacteria.
27. What is the function of the 'second antibody' (goat anti-rabbit IgG alkaline phosphatase conjugate) in western blot visualization?
- to react with D-amino acid oxidase.
 - to reduce Nitro Blue Tetrazolium, which precipitates where the antibody has bound.
 - to catalyze hydrolysis of 5-chloro-4-bromoindole-3-phosphate.
 - to react with rabbit antibody which has bound to D-amino acid oxidase.
28. Isoelectric focusing separates proteins on the basis of
- isoelectric point.
 - size.
 - SDS binding.
 - charge density.

Part B – Short Answers.

1. (3 pts) Write the balanced chemical reaction for the reaction catalyzed by D-amino acid oxidase.

1a (3 pts EXTRA CREDIT) Write the balanced chemical reaction for the net reaction catalyzed by D-amino acid oxidase + catalase.

2. (5 pts) In the graph below, the circles represent A_{560} data obtained using FAD without catalase, incubated without shaking. The triangles represent data obtained using FAD with catalase and shaking the tubes during incubation. Explain the difference between the results under the two conditions.



Part C. Problems.

1. The following rather involved pH problem has been broken into sections to assist you in proceeding to the final answer - if I just asked for the final answer, I expect most would be daunted. Try to do as many parts as you can.

a. (4 pts) What is the molarity of free acetic acid (CH_3COOH) in 10 M acetate buffer pH 4.0?

What is the molarity of free acetic acid in 10 M acetate buffer pH 5.3? The difference between these may be used later in the problem as the effective acetic acid concentration available to adjust pH to 5.3, since appreciable acetic acid must remain to fulfill the Henderson-Hasselbalch equation at this pH. (Calculation same as the first part, different pH.)

b. (2 pts) If the molecular weight of Na benzoate is 144, and the molecular weight of Ammediol (2-amino-2-methyl-propane-1,3-diol) is 105, what are their respective concentrations in 0.1% Na benzoate - 0.25% Ammediol?

c. (2 pts) If the pK_a of benzoic acid is 5.0, what will be the concentrations of benzoic acid and benzoate if the pH of 0.1% Na benzoate is adjusted to 5.3?

d. (2 pts) Therefore, how many millmoles of acid will have to be added to titrate 500 ml 0.1% Na benzoate - 0.25% Ammediol to pH 5.3? (The Ammediol will be fully protonated at this pH.)

e. (2 pts) Using your results from a and d, how many ml 10 M acetate buffer pH 4.0 will have to be added to titrate 400 ml 0.1% Na benzoate - 0.25% Ammediol to pH 5.3? (You actually added more, because you were also titrating proteins and other cellular constituents.)

2. You are given 15 ml of a solution of pure carbohydrate, and told its $[\alpha]_D$ is $+27^\circ$.

a. (3 pts) You determine the optical rotation in a 2 dm cell to be $+4.3^\circ$. What is the concentration of the stock solution in g/100 ml? In mg/ml?

b.(1 pt) You dilute this stock 1:100, and then dilute 2.5 ml of the dilute solution to 20 ml. What is the concentration, in mg/ml?

c. (3 pts) You carry out the Nelson-Somogyi reaction on this second diluted solution and a glucose standard, 0.1 mg/ml. The results are:

sugar	0.05	0.1	0.2	0.3	0.4
glucose	0.21	0.41	0.83	1.27	1.68
unknown		0.151	0.298	0.456	0.664

What is the apparent concentration(mg/ml) of the dilute unknown sugar?



d. (3 pts)What is a likely reason that the result from c does not agree with that from b?

3. a) The following rates of enzymatic oxidation of samples of 0.04 M DL-methionine are observed in the pyruvate assay (assay volume 1.0 ml, slope of standard curve 3.0 A/ μ mole):

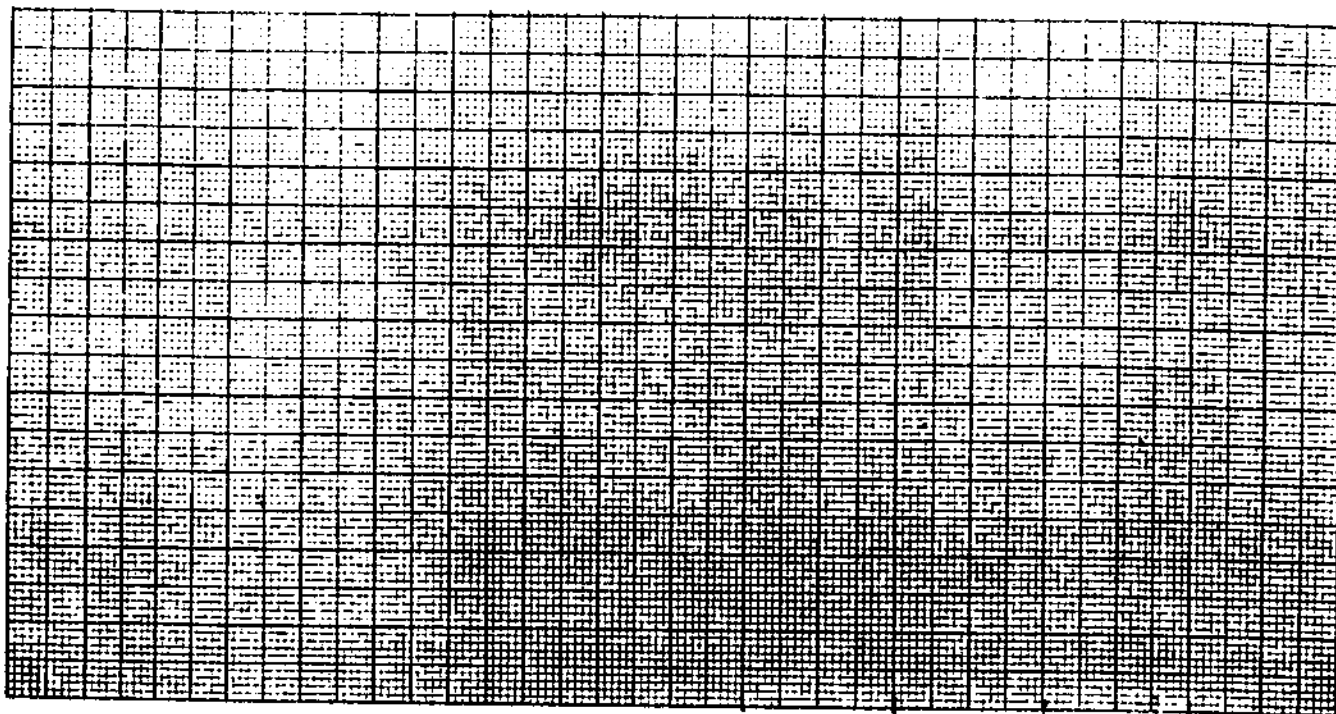
ml DL-met/PP _i	0.015	0.03	0.06	0.1	0.2	0.3	0.5
[D-met], mM:							
A ₅₆₀	0.178	0.294	0.437	0.543	0.662	0.715	0.763

a. (4 pts) Calculate D- DL-methionine concentration (above), and either [S]/v (Woolf plot) or 1/[S] and 1/v (Lineweaver-Burk plot) and plot on the graph below. It is simplest to plot v as A₅₆₀ and calculate μ moles/min etc. only for V_{max}. Label the axes clearly

1/[S]

1/v

[S]/v



- b. (4 pts) Determine V_{max} (in A₅₆₀) and K_m (mM) from the data above.

- c. (1 pt) If the enzyme used was 0.1 ml of a 1:100 dilution, what is the V_{max} in μ moles/min·stock enzyme?

d. (1 pt) If the stock enzyme had a protein concentration of 1.0 mg/ml, and the molecular weight (per subunit) is 39,000, what is the turnover number?

5. (4 points) Observed R_m s and molecular weights of the standard proteins for molecular weight determination by SDS gel electrophoresis are as follows:

Protein	R_m	mol. wt.	Protein	R_m	mol. wt.
lysozyme	0.85	14,300	ovalbumin	0.375	45,000
trypsin inhibitor	0.71	20,000	albumin, bovine serum	0.22	66,000
carbonic anhydrase	0.56	29,000	phosphorylase b	0.075	93,000

An unknown protein has an R_m of 0.50 on the same gel. Calculate its molecular weight (use one of the graphs below, or fit the standard molecular weights to an appropriate equation).

