

University of Guelph 3570 Analytical Biochemistry Fall 2004 In-class Quiz #1
Thursday, October 14, 2004 Instructor: Prof. David Josephy
Solutions

Instructions: Please write your answers in pen. No aids may be used, other than a standard “four-function” calculator. *Lengthy answers are not necessary; each of the questions should be answered in a few symbols, words, or drawings. Time allowed = 75 minutes.* Total marks = 50. (No marks will be deducted for incorrect answers.)

1. A deuterium lamp is filled with deuterium gas. What is deuterium gas?

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$^2\text{H}_2$ (D_2); ^2H is the stable heavy isotope of hydrogen.

2. In a standard double-beam spectrophotometer, a *chopper* is placed between the monochromator and the cuvette chamber. What is a chopper?

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A chopper is a spinning, sectored, partially-mirrored disc, which directs the beam alternately through the sample and reference cuvettes.

3. The diffraction of light by a grating is described by the equation: **$m \lambda = d \sin \theta$**

a) What does the symbol **m** represent, in this equation?

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The (integral) order of the diffraction.

b) What does the symbol **d** represent, in this equation?

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The spacing (distance) between grooves of the grating.

4. *Agarose* is commonly used as a matrix for chromatography (and electrophoresis).

What is agarose and where is it obtained?

(Explain briefly in words; no structures are required).

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Agarose is a neutral polysaccharide obtained by processing agar-agar (a type of seaweed).

5. The materials from which a photodiode is constructed are best described as (circle the best answer):
*insulators; conductors; **semiconductors**; superconductors; photoconductive organic polymers.*

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6. Briefly explain why soapy water does not transmit visible light very well, even though soap (e.g., sodium dodecyl sulfate) molecules have no optical absorbance in the visible region of the spectrum.

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Soap forms micelles which scatter light (Tyndall scattering).

7. UV absorbance is routinely used to quantitate proteins and nucleic acids, but is rarely used for quantitating polysaccharides, such as starch or glycogen. Briefly explain why.

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Polysaccharides are composed of sugar subunits; sugars do not have very strong chromophores, since the functional groups are only aliphatic alcohols and carbonyls.

8. DEAE-cellulose is a commonly-used anion exchange resin. The full name of "DEAE" is ...

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Diethylaminoethyl-

9. Write down the chemical formula of ammonium sulfate.

$(\text{NH}_4)_2\text{SO}_4$

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10. Hui *et al.* (A novel neuron-specific aminopeptidase in rat brain synaptosomes, *J. Biol. Chem.* 273, 31053-31060, 1998) described the purification of an aminopeptidase enzyme from rat brain cytosol, and presented the following summary table for their purification protocol.

	Total Protein	Total activity	Specific activity
	<i>mg</i>	<i>μmol/min</i>	<i>μmol/min/mg</i>
1. Rat brain homogenate	7500	107	0.01
2. Centrifugation (30,000 × g supernatant retained)	3045	68.1	0.02
3. (NH ₄) ₂ SO ₄ (40-70% fraction retained)	634	55.7	0.09
4. Chromatography on phenyl-Sepharose	45	42.0	
5. Ion-exchange chromatography on Q-Sepharose	24	28.5	1.19
6. Gel filtration chromatography on Sephadex G-200	10.1	15.5	1.52
7. Mono Q ion-exchange chromatography (run twice)	0.14	9.8	70.3

a) Fill in the missing entry in the last column of the table.

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$$42 \mu\text{mol}/\text{min} / 45 \text{ mg} = 0.93 \mu\text{mol}/\text{min}/ \text{mg}$$

b) Calculate the final % *yield* achieved for this enzyme.

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$$9.8 \text{ u} / 107 \text{ u} \times 100\% = 9.2 \%$$

c) Calculate the final (*-fold*) *purification* achieved for this enzyme.

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$$70.3 \mu\text{mol}/\text{min} / .01 \mu\text{mol}/\text{min} = 7 \times 10^3 \text{ -fold.}$$

d) Although step 6 (G-200 gel filtration chromatography) contributed rather little to the overall purification, the authors commented that inclusion of this step was, nevertheless, important to the success of the procedure. Why would this be the case?

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This step removes the salt used to elute the protein from the ion-exchange column in the previous step; note that ion-exchange is also used in the following step.

11. De Felice and colleagues (*J. Biol. Chem.* 278: 46424-46431, 2003) reported on the “Biochemical characterization of a CDC6-like protein from the crenarchaeon *Sulfolobus solfataricus*”. The native molecular mass of the protein referred to in the title was estimated by gel filtration on a Superose 6 column. The figure (bottom of page) shows the elution profile of the partially purified protein from the chromatographic column, measured as A_{280} ; the arrows indicate the elution positions of the MW marker proteins which were used to calibrate the column. The marker proteins were ribonuclease A (18.6 ml, 13.7 kDa), BSA (16.1 ml, 69 kDa), ferritin (14.1 ml, 440 kDa), and tyroglobulin (12.1 ml, 669 kDa).

What is the approximate value of V_0 for this column?

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The 440 and 669 kDa standards were well-separated, eluting around 14 mL and 12 mL, respectively; so V_0 will be significantly lower than 12 mL, probably around 8-9 mL.

What is the approximate value of V_t for this column?

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The smallest standard eluted around 18.5 mL, and another peak is seen around 20 mL, so V_t must be somewhat more than 21 mL. We expect $V_0 = 0.35 V_t$, approximately, so this answer is consistent with the previous one ($0.35 \times 22 = 7.7$).

What is the approximate value of V_e for the CDC6-like protein?

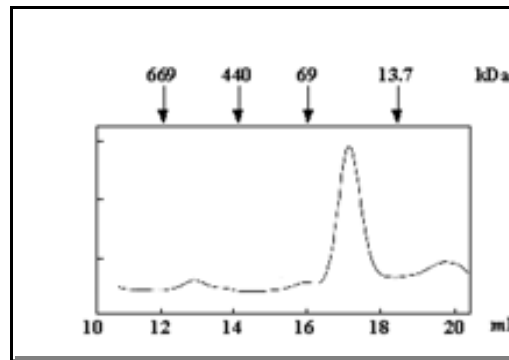
2

The largest peak (presumably the purified protein) eluted at $V_e = 17$ mL.

What is the approximate value of K (the retention index) for the CDC6-like protein?

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$$(V_e - V_0) / (V_t - V_0) = 7 \text{ mL} / 9 \text{ mL} = 0.8$$



12. Answer either one of the following two questions (A or B).

Option A) The primary sequence shown below (which was deduced from the DNA sequence of a cloned gene) is that of a 122 residue protein from the purple sea urchin (Swiss-Prot: database entry #P16889).

**PAKAQAAGKKGSKKAKAPKPSGDKKRRRKRKESYGIYIYKVLKQVHPDTGISSRAMSIMNSFVNDVFERI
AAEASRLAHYNKKSTITTSREVQTAVRLLLLPGELAKHAVSEGTKAVTKYTTSK**

The composition of this protein is:

A 15; D 3; E 6; F 2; G 7; H 3; I 6; K 19; L 6; M 2; N 3; P 5; Q 3;
R 9; S 12; T 8; V 8; Y 5.

a) Two of the 20 amino acids usually found in proteins are not found in this sequence.
Name them.

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Cys and Trp.

b) What would be the approximate net charge on this protein, at pH 5.5? (*An integral approximate answer is sufficient; you do not need to apply the Michaelis equation to determine fractional charges.*)

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**At pH 5.5, the bases (H, K, R) are +1 and the acids (D, E) are -1.
H + K + R = 31 D + E = 9 Net charge = 22.**
(The terminal amine and carboxylate give a net charge of zero.)

c) The protein has been cloned and expressed in *E. coli* cells, and we wish to purify it by ion-exchange chromatography. Suggest what conditions we might use (type of column, starting pH, type of gradient for elution).

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This is a strikingly basic protein (actually, it is a histone DNA-binding protein); so the best approach should be to perform cation-exchange at neutral (or even slightly basic) pH, and then elute with a NaCl gradient or increasing pH gradient.

OR

Option B) On the back of this page, draw the complete structure (indicating every atom) of the dipeptide leucylthreonine. (7 marks).

