

CHEM*3570 Analytical Biochemistry Summer 2002

In-class Quiz #1 Solutions

Tues. June 11, 2002 Instructor: D. Josephy

Please write all of your answers in ink. Remember to write your name on this page and on the exam booklet. Time allowed = 75 minutes. This quiz contributes 10% of the final course grade. Total marks for this quiz = 30.

Questions 1-6 are "multiple-choice", and are worth 2 marks each. Circle the best answer. No marks will be deducted for incorrect answers.

1. The net charge on the peptide MWRVLLPHDQNAE at pH 5.5 will be approximately:

- a) -2 b) -1 **c) 0** d) +1 e) +2

+1 amino term. +1 R +1H -1 D -1E -1 carboxy term.

2. An ultrasonic homogenizers can be used to disintegrate cells or to prepare emulsions. In this device, electrical energy is converted to mechanical (acoustic) energy at a frequency of about:

- a) 20 Hz b) 2 kHz **c) 20 kHz** d) 2 MHz e) 2 Ghz

(slightly above the acoustic range of about 15 Hz - 15 kHz, depending on the age of your ears)

3. "Salting-out" of proteins is most often achieved by the addition of:

- a) $(\text{NH}_4)_2\text{SO}_4$** b) $(\text{NH}_4)\text{Cl}$ c) $(\text{NH}_4)_2\text{H}(\text{PO}_4)$
d) NaCl e) guanidinium hydrochloride

4. In the *monoclonal antibody* technique (Kohler and Milstein, 1975) antibody-producing lymphocytes, isolated from mouse spleen, are used to establish cell lines which can be propagated indefinitely in culture. This is usually accomplished by:

- a) transforming the lymphocytes with a retroviral oncogene.
b) transforming the lymphocytes with ethylnitrosourea, a mutagenic chemical.
c) fusing the lymphocytes with myeloma cells, by treatment with polyethylene glycol.
d) transforming the lymphocytes with chromosomal DNA of mouse myeloma cells.
e) any of the above methods can be used successfully.

5. If the recovery of activity at each step in a protein purification procedure is 90%, approximately what fraction of the initial activity will be recovered after 10 steps?

- a) <1% b) 9% **c) 35%** d) 90% e) almost 100%

(= 0.9^{10})

6. The filament of a tungsten-halogen lamp is made out of tungsten (rather than a more common metal, such as copper), because of tungsten's unusually high:

- a) tensile strength **b) melting point** c) conductivity
d) resistance to oxidation e) all of the above

(Tungsten has the highest melting point (3410C) and lowest vapor pressure of all metals. “From 1878, when Swan demonstrated his ... carbon lamps at Newcastle, search was made for a more satisfactory filament material than carbon. ... Von Welsbach produced the first successful metal filament by using osmium; attempts had previously been made to use platinum, but its relatively low melting point of 1774C prevented its successful development. ... Tantalum, with a melting point of 2996C, compared with osmium, 2700C, was extensively used as a drawn wire from 1903 to 1911 ... Developments in the use of tungsten started about 1904, and it has been used exclusively since about 1911. ...”

<http://www.tungsten.com/tunghist.html>)

The following questions require short answers and are worth 3 marks each.

Answer any six (6) of these seven (7) questions. Write your answers in the exam booklet provided.

1. Why are tungsten-halogen lamps filled with halogen gas?

To prevent deposition of tungsten onto the glass bulb; tungsten halide forms and then decomposes on the filament, releasing free tungsten.

2. a) Define the term A (absorbance) in terms of T (% transmittance). b) The accuracy of spectrophotometers declines sharply at high absorbance values ($A > 2$), but this decline is less drastic for instruments with photomultiplier detectors than it is for instruments with photodiode detectors. Briefly explain why this is so.

a) $A = \log(100\%/T)$

b) **At high A, transmitted light intensity is low; photomultipliers are much more sensitive than photodiodes.**

3. With the aid of a simple schematic diagram of the structure of an IgG class antibody, explain the results of treatment of such an antibody with the enzyme *papain*.

See figure in notes; products are two Fab fragments and one Fc fragment.

4. The human hepatic enzyme N-acetyltransferase (NAT) catalyzes the acetylation of the amino group of the substrate 2-aminofluorene (2-AF, molar mass = 181.2) by acetyl CoA (molar mass = 763.5). An aliquot (5 μL ; protein concentration = 0.24 mg per mL) of crude cytosol preparation from *E. coli* cells expressing recombinant human NAT is found to catalyze the acetylation of 23 μg 2-AF per minute, when assayed in a 1 mL incubation volume. What is the specific activity of the enzyme preparation?

Total protein: $5 \mu\text{L} \times 0.24 \text{ mg per mL} = 1.2 \mu\text{g}$

Substrate consumed per min. = $23 \times 10^{-6} \text{ g} \times (1 \text{ mol} / 181 \times 10^2 \text{ g}) = 1.27 \times 10^{-7} \text{ mol}$

Activity = $1.27 \times 10^{-7} \text{ mol} / \text{min.} = 0.127 \text{ units}$ (1 u = 1 μmol substrate consumed per min.)

Specific activity = $0.127 \text{ units} / 1.2 \mu\text{g protein} = .106 \text{ units per } \mu\text{g} \Rightarrow 106 \text{ units per mg}$

(Volume of the incubation and the molar mass of acetyl CoA are “red herrings”.)

5. Name two materials commonly used for preparation of gel-filtration columns. Briefly explain (in words) how each of these materials is synthesized or obtained.

polyacrylamide (polymerization of acrylamide)

Sephadex (chemically cross-linked dextran; dextran is a polysaccharide produced by fermentation)

Agarose (neutral polysaccharide from agar)

6. We have purified the enzyme lactate dehydrogenase (LDH) almost to homogeneity from beef liver cytosol, and we have mg quantities of the protein at hand. We now hope to purify LDH from a *sheep* liver sample. Affinity chromatography on an (anti-beef-liver-LDH) antibody column seems like a good idea, because this method can achieve high purification in a single step, but how should we obtain an antibody? Arthur suggests that we should raise a polyclonal antibody against beef LDH, by immunizing a rabbit with our LDH protein and obtaining an immune serum. But Brenda recommends that we should immunize a mouse, instead, and then develop a monoclonal antibody. Whose approach do you recommend? Justify your choice.

Arthur's approach. It will surely be faster and cheaper to immunize a rabbit and get a polyclonal. There is also a serious potential problem with using a monoclonal. The MAb might recognize an epitope which is present on the beef enzyme but not on the sheep enzyme. The polyclonal will recognize multiple epitopes, many of which are likely to be shared by the two enzymes.

7. Glutathione is the tripeptide γ -glutamylcysteinylglycine. Draw the complete structure of glutathione.

