

Analytical Biochemistry 3570 Fall 2004 In-class Quiz #2 Thurs. Nov. 11, 2004

Please write all of your answers in ink. Time allowed = 75 minutes. This quiz contributes 10% of the final course grade. Total marks for this quiz = 50.

Questions 1-8 are “multiple-choice” and are worth three (3) marks each. Circle the letter corresponding to the best answer. ~~One-half (1/2) mark will be deducted for each incorrect answer.~~ (Marks were not deducted for incorrect answers).

1. The S.I. unit that is equal to 1 Volt \times 1 Coulomb is the ...

a) Newton; b) kilogram; c) ampere; d) kilopascal; **e) Joule.**

2. In running an isoelectric focusing gel, suitable anode and cathode electrolytes are, *respectively*:

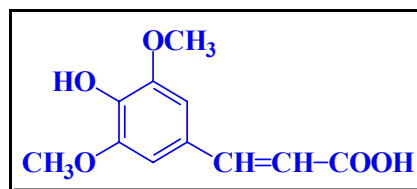
a) 1M phosphoric acid and 1M NaOH; b) 1M NaOH and 1M phosphoric acid;
c) 1M NaCl and 1M phosphoric acid; d) Tris buffer, pH 6.8 and Tris buffer, pH 6.8 + 3M NaCl;
e) 1% SDS and 1M urea.

3. Restriction enzymes that cut DNA at the same site are referred to as:

a) isotopomers; **b) isoschizomers;** c) isotelomers; d) isoligamers; e) isorotamers.

4. The reagent shown at right is used for ...

a) absorbing laser light energy in MALDI m. s.;
b) visualizing the presence of dsDNA on agarose gels;
c) determining the exclusion limits of gel-filtration columns;
d) initiating the polymerization of polyacrylamide gels;
e) conjugating antigens to carrier proteins such as KLH.



5. Heparin is a useful ligand for the affinity purification of ...

a) messenger RNA; b) IgG antibodies; **c) serine proteases;** d) NADH-linked dehydrogenases;
e) glutathione S-transferase fusion proteins.

6. Which of the following sequences is NOT a possible restriction enzyme recognition sequence?
(N represents any base.)

a) AAGCTT; b) AAGCTT; c) GGTNNACC; **d) GAGNNAGA;** e) TGCNNGCA.

7. The pH of the stacking gel in the Ornstein-Davis disc. gel electrophoresis method is ...

a) 4.5; **b) 6.7;** c) 7.4; d) 8.9; e) 9.8.

8. In the earliest mass spectrometers, ionization of neutral molecules was effected by exposing them to:

a) high temperature; b) UV light (254 nm); **c) electrons;** d) neutrons; e) halogen gas.

SOLUTIONS page 2

Answer each of the following questions in a few words:

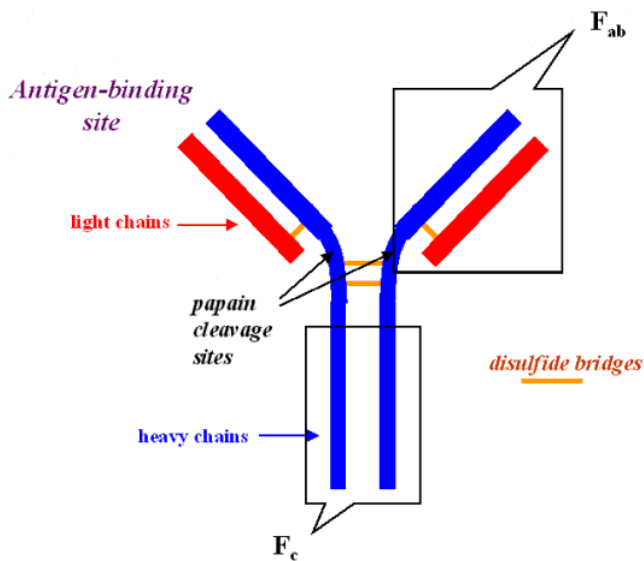
9. In the Southern blotting technique, chromosomal DNA is digested with a restriction enzyme, separated by electrophoresis, denatured to single stranded DNA, and then transferred to a nitrocellulose membrane. How is the DNA denaturation step accomplished? (3 marks)

DNA is denatured by soaking the gel in NaOH solution.

10. Name the two pyrimidine bases that are found in DNA. (4 marks)

Thymine and cytosine

11. Draw and label a schematic diagram of the structure of an IgG antibody. (5 marks)

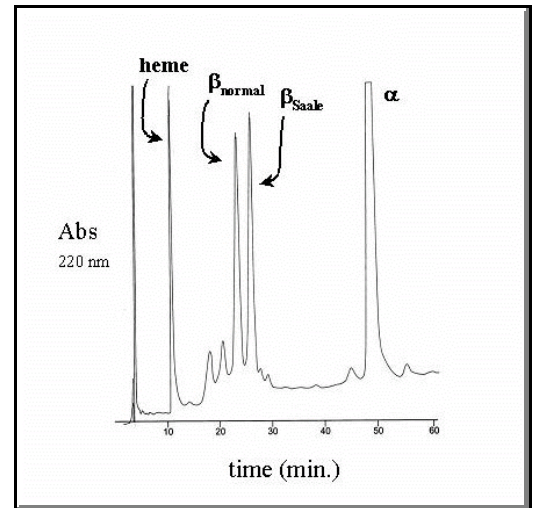


12. Define K_{diss} , the dissociation constant of an antibody-antigen complex.

What is a typical numerical value for K_{diss} ? (4 marks)

$$K_{diss} = \frac{[Ab][Ag]}{[Ab \cdot Ag]} \quad 10^{-5} \text{ to } 10^{-10} \text{ M (note molar units!)}$$

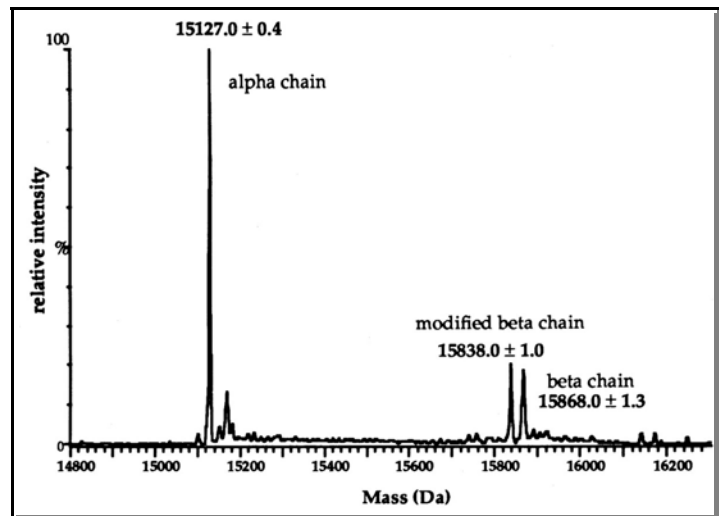
13. Bissé *et al.* (*J. Biol. Chem.* 275: 21380-21384, 2000) describe the case of a 3-year-old German girl who was anemic, but otherwise showed normal physical and intellectual development. They examined the patient's hemoglobin (Hb). Hb is a tetramer consisting of two alpha-chain and two beta-chain globin subunits. Analysis of the patient's Hb by electrophoresis and isoelectric focussing did not reveal anything unusual. Reversed-phase HPLC, which separates molecules according to their hydrophobicity, was then performed (see chromatogram at right). The expected alpha-chain peak was observed, but the beta-chain peak was split into two peaks of similar height, one of which had the same retention time as normal Hb beta-chain. The variant peak was named Hb beta "Saale" (for the town in Germany where the patient lives).



a) What can we deduce from the fact that the amounts of the normal and variant beta-chain proteins present in the blood cells are similar? (2 marks).

The patient is a heterozygote for the beta-chain gene mutation.

b) An electrospray mass spectrum of the patient's Hb was then obtained (right). Protein sequencing of peptides from a proteolytic digest of Hb beta "Saale" revealed an amino-acid substitution at position 84 (threonine, in the normal Hb beta-chain). Suggest which amino acid may be substituted for threonine-84 in the patient's Hb beta-chain. (3 marks)



The variant protein is smaller by 30 mass units, compared to threonine (-CH(OH)CH₃). This is probably made up of -14 (loss of CH₂) plus -16 (loss of O), leaving CH₃. This corresponds to alanine (R = CH₃). Substitution of alanine for threonine 84 might be consistent with the variant protein's near-normal structure and function. The substitution causes no charge difference, consistent with the observed unchanged behaviour on electrophoresis and isoelectric focussing. A slight decrease in polarity (thr→ala) is also consistent with the reversed-phase HPLC findings.

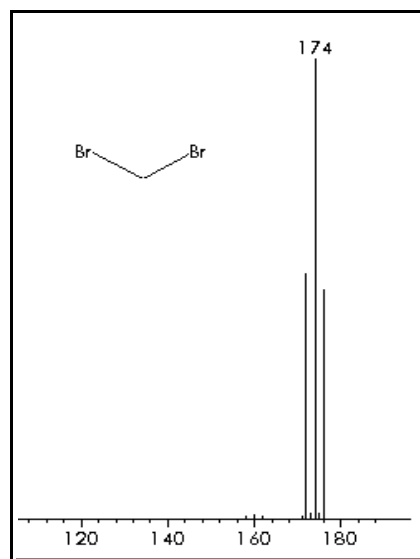
14. Sketch the expected appearance of the mass spectrum (the base peak only; ignore fragmentation) of dibromomethane (CH_2Br_2). Approximate abundances of stable isotopes are as follows:

H		C		Br	
^1H	99.99 %	^{12}C	99 %	^{79}Br	51 %
^2H	0.015 %	^{13}C	1 %	^{81}Br	49 %

(3 marks)

Each CH_2Br_2 molecules has two Br atoms, and the distribution of bromine isotopomers will follow $(a+b)^2 = a^2 + b^2 + 2ab$, where a and b are the abundances of the two isotopes. Br isotopes 79 and 81 are of almost equal abundance, so the distribution of Br isotopes will be almost exactly 1:2:1 ($^{79}/^{79}$; $^{79}/^{81}$; $^{81}/^{81}$). So, the highest peak will be $m/z = 12 + 2 + 79 + 81 = 174$, with peaks of about half this intensity at 172 and 176.

Each of these three peaks will be accompanied by a peak of about 1% relative intensity, at $m/z = 173, 175, \text{ and } 177$, due to ^{13}C . The ^2H contribution is negligible.



15. Name and draw the chemical structure of SDS, the detergent used in SDS-PAGE protein electrophoresis (2 marks).

Sodium dodecyl sulfate. (1)

