BIOS 452/CHEM 452

First Exam

Fall, 2010

Friday, September 24, 2010

Name: Study Points

UIN or SSN: _____

Circle Discussion Section:

Mon	8
Tue	9:30
Wed	9
Thu	9:30
Fri	9
Fri	11

* Do not turn the page until you are told to do so.
* General instruction:
- Fill in the blanks indicated as underlines and/or circle one of the given choices.
- For calculations, show all your work.

1. (1) DNA are polymers composed of monomer units called _____linked by _____ bonds.

(2) Proteins are polymers composed of monomer units called _____linked by _____ bonds.

* How about carbohydrates & lipids ? Afterall ?
* Do you think you have learned something about these 4 classes of biomolecules

2. (1) The sugar found in DNA is (α , β –*circle one*) –_____–deoxy-(**D** , **L** –*circle one*) _____.

* Now time to connect this with more general subject on carbohydrate.

(2) Draw the Haworth perspective of this sugar in the box on the right.

* Don't forget "deoxy"

(3) Circle the atom that is responsible for the D- or L- configuration.

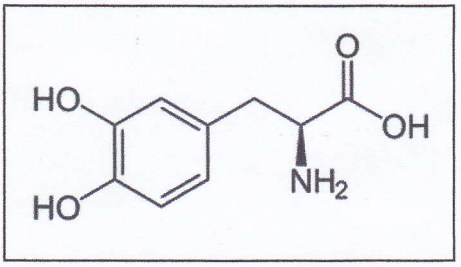
* again D & L ...

(4) Show the numbering of the carbons on the Haworth perspective drawn in (2). The number on the carbon is responsible for the α or β configuration is _____.

* again α & β ...
* Where is the anomeric carbon here? acetal ? ketal ? hemiacetal ? hemiketal?

3. (1) Draw the Fisher projection of the molecule shown on the right.

**Hint: First identify the functional groups and see if it resembles something you already know!*



(2) The configuration of this molecule is (**D** , **L** , **cannot be determined** –*circle one*).

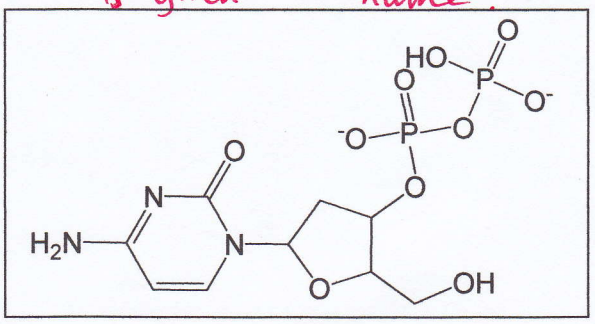
(3) Therefore, it has the (**same** , **opposite** , **cannot be determined** –*circle one*) configuration as the amino acids found in naturally made proteins.

Natural types :

- D - ribose
- L - amino acid
- L - like phospho glycero lipids
- D - glucose / sugar except arabinose (?)

* or can you draw correct structure if given a name?

4. **(1)** The molecule shown on the right is called



_____.

Do not abbreviate.

Don't forget to denote the location of phosphates.

* Many variation is possible. ← Practice !!!

(2) The base in *(1)* is called _____, which pairs with _____(*base name*) in DNA. ***Do not abbreviate.***

5. **(1)** Draw the amino- and imino- tautomers of adenine in the given squares below.

* How about keto ⇌ enol of a base?

Amino

Imino

(2) Draw the complete structure of the base pair involving the major tautomeric form of adenine found in DNA double helix. Indicate the hydrogen bonds with dotted lines. It is not necessary to show the sugar phosphate backbone, but indicate where the backbone is attached.

* Should, Must Be able to draw W-C base pairs. Try not to make regrettable mistakes !! like mixing >=< or omitting H's.

(3) Circle the atoms that can accept a hydrogen bond in the major groove side of the base pair drawn in (2).

* Should know what is major & minor grooves & how to distinguish in a structure.

* Should know hydrogen bond donors & acceptors exposed in these major & minor groove sides.

6. Draw the chemical structure of the peptide PVNRW. For amino acids that are charged, draw them in the charged form. **Note the sequence is read from the N-terminus to C-terminus – ALWAYS!*

* No doubt you cannot get good grade W/o being able to do this type of question! So please practice. Make sure you can do this kind of Q.
* For advanced students: Can you draw structures according to a give pH value? (of course, will (change) affect only ionizable side chains)

7. The T_m of human DNA is 84 °C in 0.11 N NaCl.

(1) Draw a plot showing the change in absorbance (A) at 260 nm as a function of temperature for melting of human DNA under 0.11 N NaCl. Label the axes and indicate T_m on the plot.

* We learned:
  - Tm of DNA, (double-stranded)
  - Tm of Lipids

How are these measured?

↑ Sharp transition in physical state w/ temperature change.

(2) Human DNA is be expected to have a T_m (**lower than** , **the same as** , **higher than** – *circle one*) 84 °C under 0.01 N NaCl.

8. *The label on a Diet Coke says "PHENYLKETONUNIRCS: CONTAINS PHENYLALANINE."*

Phenylketourea is a genetic disorder which can result from a point mutation that leads to the substitution of a tryptophan in normal phenylalanine hydroxylase (PAH) for an arginine in a patient's PAH. This point mutation makes the enzyme inactive and hence the disease. Based on the standard genetic codes shown on page 9, indicate changes in mRNA *(not DNA!)* sequence that are consistent with this mutation. If there is more than one possibility, write all the possible cases.

* What is point mutation?
* What if I ask about a DNA sequence change rather than RNA?
* Codon?

9. One strand of a double-helical DNA has the sequence 5'-GCATCTCATGC-3'.

(1) Write the base sequence of the complementary strand.

* So basic easy (A-T, G-C).

5'-_____-3'

(2) Does the DNA have the potential to form any alternative structures? structure ?

→ dideoxy nucleotide ddxtp.

* [DNA sequencing - Sanger ≠ Sanger reagent in N-terminal residue determination
  Protein sequencing - Edman
  ↳ phenyl isothiocyanate - structure ? mechanism ? product ?

10. The following DNA fragment was sequenced by the Sanger method. The asterisk indicates a fluorescent label primer with a sequence of 5'-GCCG-3'.

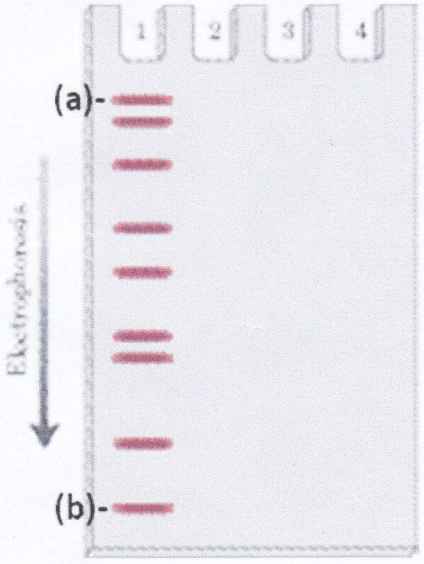
*5'——3'-OH

3'——ATTACGCAAGGACATTAGAC -5'

A sample of the DNA was reacted with DNA polymerase and each of the nucleotide mixtures (in an appropriate buffer) listed below. Dideoxynucleotides (ddNTPs) were added in relatively small amounts.

1. dATP, dTTP, dCTP, dGTP, ddTTP
2. dATP, dTTP, dCTP, dGTP, ddTDP
3. dATP, dTTP, , dGTP, ddATP

The resulting DNA was separated by electrophoresis on an agarose gel, and the fluorescent bands on the gel were located. The band pattern resulting from nucleotide mixture 1 is shown on the right.



(1) What is the sequence of the DNA of the following bands in lane 1? **Pay attention that there are 9 bands (not 8!). Assume the template DNA was in excess of the primer and again, note that the fluorescent label is on the primer.*

Band **(a)** _____ why ?

Band **(b)**_____ why ?

(2) Assuming that all mixtures were run on the same gel, what would the lane 2 of the gel look like? Draw directly on the gel picture. why so ?

(3) What would the lane 3 of the gel look like? Draw directly on the gel picture. why ?

* etc. then how big is a human cell? (Answer: tens of μm)

0.2 μm

11. Bacteriophage T2 has a DNA of molecular weight 120×10^6 Da contained in a head about 200 nm long.

(1) Calculate the length of the DNA (assume the molecular weight of a nucleotide pair is 600 Da). Assume that the DNA is in B-form and that there is 10 bp per turn.

* Dimensions of protein 2° structure & DNA B-helix
* Molecular weights are important.

(2) Compared with the length of the T2 head, the total length of the DNA is 340 times (**longer** , **shorter** – *circle one*).

(really long DNA) (tiny virus)

* This means ____ fit into ! but the thickness / width of B-DNA is much thinner than width of virus. (0.2 μm = 200 nm)
(2.4 nm)
so, it is possible in principle.

12. Bacteriophage λ infects E. coli by integrating its double-stranded DNA into the bacterial chromosome. The success of this recombination depends on the topology of the E. coli DNA. When the superhelical density (σ) of the E. coli DNA is greater than -0.045, the probability of integration is <20%; when σ is less than -0.06, the probability is > 70%. Plasmid DNA isolated from an E. coli culture is found to have a length of 13,800 bp and an Linking number (L) of 1,200. Assume that the DNA is in B-form and that there is 10 bp per turn.

(1) Calculate W for this DNA when the DNA is allowed to supercoil.

(2) The likelihood that bacteriophage λ will be able to infect this culture is

(**< 20%** , **20-70%** , **> 70%** , **cannot be determined** –*circle one*) .

** Hint: Superhelical density $\sigma = W/L_0$ and $L_0 = T$ when DNA is linearized)*

$\sigma = -0.13 < -0.06$

↑ more negative s.c → more compact!

(3) The plasmid DNA obtaine above has (**right-handed** , **left-handed** –*circle one*) supercoils.

Negative supercoil = Negative Writhe = Underwound → Need to wind more.
RH → RH s.c

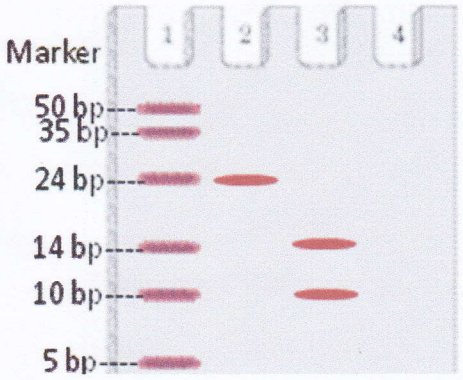
Positive S.C = Positive W = Overwound → Opposite direction than L.
RH → LH s.c

13. Your supervisor asks you to perform PCR on the following piece of double stranded DNA (X)

**By convention, only one strand of the two is shown* :

```
          |    10    |    20    |    30    |
5′- GTTAATGAAATTTAAATTGATATAACGTCCCGTGAG -3′
```

Each primer is 6 nucleotide-long and pairs with the template DNA without any mismatch. However, the sequences of the primers are lost because the computer crashed (*duh!*). Luckily, the PCR reactions worked. In order to identify the primers used in the successful PCR, you treated the PCR product with DraI restriction enzyme of which the restriction site is TTT ↓ AAA. The picture on the right shows the polyacrylamide gel of the following samples: a size marker (*Lane 1*), the PCR product before DraI treatment (*Lane 2*), and the PCR product after DraI treatment (*Lane 3*).



(1) PCR stands for _____.

* This is a must!

(2) Deduce the sequences of the two primers were used in the PCR reaction.

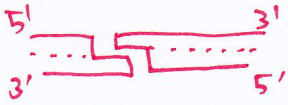
Primer 1 5′-_____-3′

Primer 2 5′-_____-3′

***

* Please Thoroughly understand PCR! Will be a question about PCR.

** What if EcoRI or some other enzymes that generate overhang structure were used in this experiment?



* Please study how DNA polymerase can "polymerize" DNA.

14. The pK_a's of the amino-, carboxy- and the imidazole groups in amino acid *X* are 9.0, 2.0 and 6.0, respectively.

(1) Amino acid *X* is _____ (full name). ***Do not abbreviate.***

(2) What is the ratio of the base to conjugate acid forms (i.e., [base]/[acid]) of the following groups in *X* at pH 7.0?

- Amino-
- Carboxy-
- Side chain

(3) Based on the ratios obtained above, calculate the average charge on each functional groups. Calculate to the first decimal place.

- Amino-
- Carboxy-
- Side chain

(4) What is the net charge on this amino acid at pH 7.0?

**Hint: Sum up the three average charges obtained in (2).*

(5) Calculate the pI of this amino acid *X*.

** Note: A molecule is expected to have a net negative charge when pH > pI and a net positive charge when pH < pI. Does your answer in (4) make sense?*

* 14 (1) → (5) was to show how pI (⇒ pH where overall charge = 0) was making sense.... pH > pI → (−) / pH < pI → (+) by considering charges from each ionizable group.

* Can you modify this question using a different amino acid or at a different pH?

Again, { pI will be there at the Final. Please don't miss it ↳ Will allocate ~5min time for this Q.
pKa
charge, protonation problems