Fall '13
Hackert

CH370
Exam I

Name __KEY__
UTeID _____

1. Identify each **amino acid** by its **three and one letter codes** (e.g. **Ala / A**, etc.) and each **N base, nucleoside** or **nucleotide** by its **full name** in the blanks above or beside the structures **(26 pts)**.
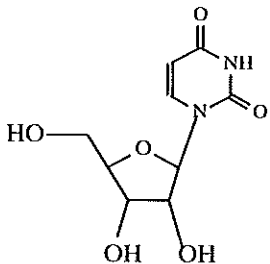
A) __Ser / S__
H_3N^+—CH—COO⁻
|
CH_2
|
HO

@ 1 pt each

B) __Gly / G__
H_3N^+—CH—COO⁻
|
H
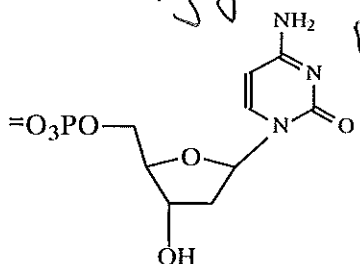
C) __Ile / I__
H_3N^+—CH—COO⁻
|
CH
H_3C-CH_2 CH_3

D) __Val / V__
H_3N^+—CH—COO⁻
|
CH
H_3C CH_3
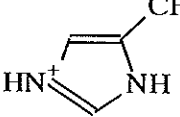
E) __Uridine__
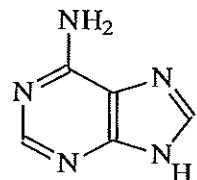
F) __Lys / K__
H_3N^+—CH—COO⁻
|
CH_2
|
H_2C
|
CH_2
|
H_2C
|
NH_3^+
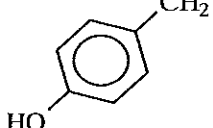
G) __Arg / R__
H_3N^+—CH—COO⁻
|
CH_2
|
H_2C
|
CH_2
|
HN
|
$CH=NH_2^+$
|
H_2N

H) __deoxycytidine mono phosphate__
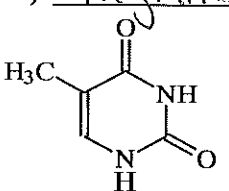
I) __Asp / D__
H_3N^+—CH—COO⁻
|
CH_2
|
⁻OOC

J) __His / H__
H_3N^+—CH—COO⁻
|
CH_2
|
HN^+ NH
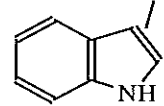
K) __Adenine__

L) __Tyr / Y__
H_3N^+—CH—COO⁻
|
CH_2
|
HO

M) __Met / M__
H_3N^+—CH—COO⁻
|
CH_2
|
H_2C
|
S
|
H_3C

N) __Thymine__

O) __Ala / A__
H_3N^+—CH—COO⁻
|
CH_3

P) __Trp / W__
H_3N^+—CH—COO⁻
|
CH_2
|
NH
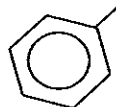
Q) __Cys / C__
H_3N^+—CH—COO⁻
|
CH_2
|
HS
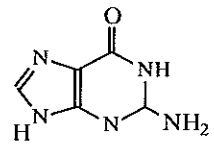
R) __Pro / P__
H_2N^+—CH—COO⁻
|
H_2C CH_2
CH_2
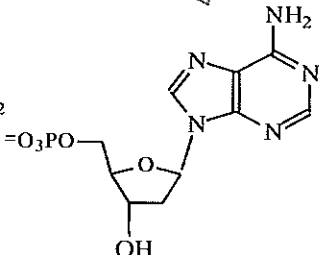
S) __Glu / E__
H_3N^+—CH—COO⁻
|
CH_2
|
H_2C
|
COO⁻

T) __Thr / T__
H_3N^+—CH—COO⁻
|
CH
H_3C OH

U) __Phe / F__
H_3N^+—CH—COO⁻
|
CH_2

V) __Asn / N__
H_3N^+—CH—COO⁻
|
CH_2
|
H_2N—C
‖
O

W) __Guanine__

X) __deoxyadenosine mono phosphate__

Y) __Gln / Q__
H_3N^+—CH—COO⁻
|
CH_2
|
H_2C
|
C=O
|
H_2N

Z) __Leu / L__
H_3N^+—CH—COO⁻
|
CH_2
|
CH
H_3C CH_3

2. You are trying to purify a mixture of the following two oligopeptides:

peptide A: **CASEMXYXD** and peptide B: **RAMACHANDRAN**

a) Estimate the charge of each oligopeptide at pH 1 and estimate the isoelectric point of each oligopeptide enter it on the lines below the oligo.

~pKa 10 8   4 10 4 2   10 12   8 6   4 12   2

H_3N^+ - C A S E M Y N D - COO^- H_3N^+ - R A M A C H A N D R A N - COO^-

charge
low pH + 0   0   0   0   0 = +1   + +   0 +   0 +   0 = +4

+1 →₂ 0 →₄ -1 →₄ -2 →₈ -3 →¹⁰ -4 →¹⁰ -5   +4 →₂ +3 →₄ +2 →₆ +1 →₈ 0 →¹⁰ -1 →¹² -2 →¹² -3

    ↑__↑   ↑__↑
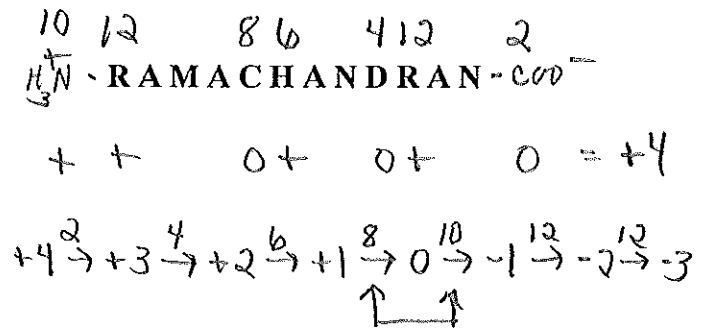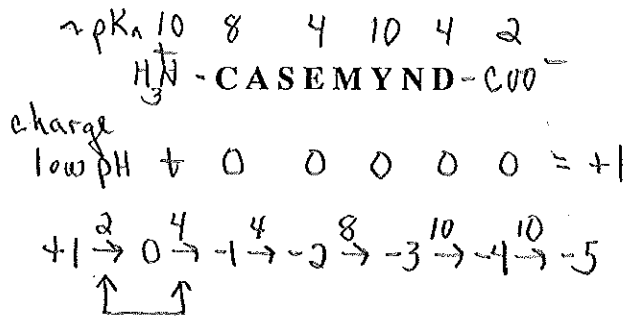
(2) charge at pH 1   +1 (1)   +4 (1)

(4) pI   3 (2)   9 (2)

b) Describe how you could take advantage of the charge properties resulting from the amino acid content of these oligopeptides to separate them. Recommend a chromatography method and conditions (pH, etc.) for their separation as well as the order of elution from the column.

(4)

(2) Very different pIs ⟹ separate by charge (IEX)

(2) At ~neutral pH (~6), CASEMYND would be (-) neg. charged and stick to an anion exchange column like (DEAE), while the other peptide passes thru.

3. a) In the blanks provided, indicate the values of φ = __180°__ (1)
and ψ = __0°__ (1) for the *second glycyl residue.* Label the phi (φ) and psi (ψ) angles on the residue to the right.
(4)
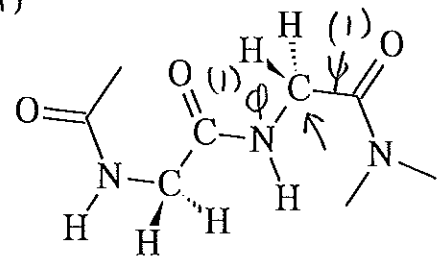
b) Identify the sugar pucker / base conformation shown to the right:
(2) __C2' endo__ / __syn__
(1)   (1)
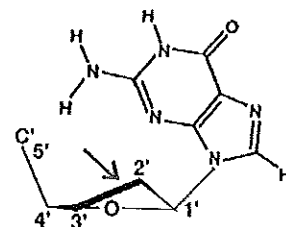
c) (2) Name one conformational difference between B-DNA and Z-DNA:

__B-DNA right handed__ (1) / __Z-DNA is left handed helix__ (1)
dG base (anti)   dG (syn)

2

4. A classmate asks for your help understanding the relationships and differences between the DNA sequencing methods Maxam-Gilbert (MG), dideoxy (DD) nucleotides, and pyrosequencing (PY).

   a) For each of the following terms, place a "DD" for dideoxy **and/or** a "MG" for Maxam-Gilbert **and/or** a "PY" depending on which sequencing method the terms apply to.

(6)
1 pt each

i) use of DNA polymerase	DD, PY	ii) "ladder" sequencing	MG, DD
iii) 5'-primer	DD, PY	iv) cleavage reactions	MG
v) luciferase	PY	vi) emulsion PCR	PY

5. Given the following dideoxy sequencing gel result, what is the sequence (5' → 3') of the original template?

   (4) 5'- A A G T C C A - 3'

   DNA polymerase I   +   4 dNTPs   + ddATP   ddTTP   ddCTP   ddGTP

6. Expression:

   a) What are two benefits of engineering a tag like His$_6$ or MBP when expressing a recombinant protein?

   (3)   Rapid purification / Works in presence of variety of agents)
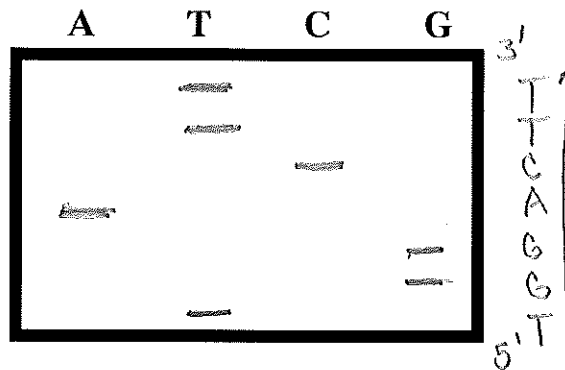   Fast tight binding (bind / wash / elute)

   b) What are two advantages and two disadvantages of using *E. coli* to express eukaryotic proteins?

   (2) Advantages   i) Cheap / easy to manipulate

   1 pt each   ii) Fast / not contaminated easily

   (2) Disadvantages i) Codon usage / Folding / Toxicity

             ii) Sugar linkage (post translational modification)

   c) Your protein contains an N-terminal proline that is crucial for activity, and you want to add a His-Tag to your expressed protein. Consider the following cloning/expression region of a vector. Assuming that none of these restriction sites are located in your gene, what restriction sites should you use to add a C-terminal His-Tag?

   T7 Promoter_____   rbs____ EcoRI ↓NdeI   His-Tag   BamHI   XhoI
   AATTAATACGACTCACTATAGGACAACGGTGAAGGAGACTTAAGTCACATATGCACCACCACCACCACCACGGATCCATTCTCGAGTAG
                                                     MetHisHisHisHisHisHisGlySerIleLeuGluEnd

   (4)   → Need to have His Tag at C-terminus, so insert
            your gene before the His-Tag.
                  EcoRI / NdeI

3

7. Complete the table by calculating the specific activities for the following steps used to purify an enzyme, and **then answer** the questions below:

Step	Volume (mL)	Protein (mg/mL)	Total Activity	Specific Activity
1. Crude extract	657.00	7.38	112455	23.2
2. 30-70% Salt cut	37.50	18.70	93874	134
3. Ion exhange Chrom	12.45	22.70	84821	300
4. Gel filtration	8.40	8.30	77650	1114
5. Affinity Chrom.	1.75	1.76	68542	22254

(2) (½ each)

(2) a) Complete the purification table by filling in the remaining blanks in the table above.

(2) b) Which step (#) exploited the differences in charge? ___3___ (2)

(2) c) Which step (#) exploited the differences in size? ___4___ (2)

(2) d) What is the overall percentage "yield" for this purification scheme? ___60.9 %___ (2)

(2) e) What is the overall fold purification for this purification scheme? ___959 X___ (2)

(2) f) Which step (#) of the purification produced the smallest % increase in specific activity? ___3___ (2)

8. Consider a "gel filtration" column that is 77 cm in length and 2.75 cm in diameter with a total volume of 455 cm^3. It is packed with spherical beads that are 0.17 mm in diameter with a V_o that is 34.0% of V_{tot}. The column is calibrated with two standards, "Std A" (~31,620 Da) and "Std B" (~125,900 Da) which gave Ve values of 395 mL and 215 mL respectively. An unknown protein is run on the same column and gave a Ve of 275 mL. (Show work / draw a Box around answers).

a) Calculate the **partition coefficients** for the two standards and the unknown.

(3)
$$V_T = 455 \text{ cm}^3 \quad ; \quad V_0 = .34 \times V_T = 155 \text{ cm}^3 \quad ; \quad V_T - V_0 = 300 \text{ cm}^3$$

$$K = \frac{V_e - V_o}{V_T - V_o}$$

$$K_A = \frac{395-155}{300} = 0.80 \quad ; \quad K_B = \frac{215-155}{300} = 0.20 \quad ; \quad K_{unk} = \frac{275-155}{300} = 0.40$$
(1)   (1)   (1)

b) Estimate the molecular weight for the unknown protein. ___79,400___ (5)

(5)

	K	log M
A	0.80	4.50
unk,	0.40	
B	0.20	5.10
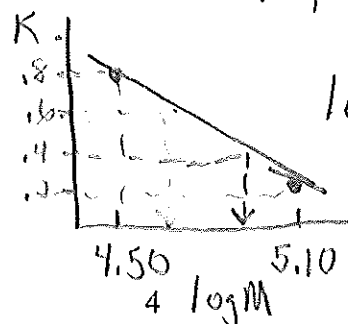
$$\log M_{unk} = 4.50 + \frac{.4}{.6}(.6) = 4.9$$

$$M_{unk} = 79,400$$

4 log M

9. Given the sizes and pI's of the following proteins, predict the order in which these proteins would be eluted off a DEAE (diethylaminoethyl) column run at pH 7.0 and eluted with a salt gradient from 0.10 M NaCl to 2.0 M NaCl. (+)

	A) Hemoglobin	B) Serum albumin	C) Chymotrypsin
Size (kD):	64.5	68.5	23
pI:	6.8	4.9	9.5
	~ O change	(-)	(+)

(3)   Order off column: (1st off) _C_ _A_ _B_ (last off)
                                    (1) (1) (1)

10. You have the following amino acid sequence from a protein that you believe is part of a "smart hormone" that recognizes DNA. P-Y-V-N-V-K-L-P-G-R-S-D-E-Q-L-K-N-L-V-S-E-V-T-D-A-V-E. You want to search for homologues to find out more about the properties of this protein's relatives. You use your web browser to go to the NCBI web site that is a well known bioinformatics site providing access to many sequence databases. You search for homologues using the BLASTP routine and obtain the following results.

		Score	E
1.	gi[1042879] HUMAN PROTEIN	482	2e-85
2.	gi[2346879] HYPOTHETICAL PROTEIN Mus	123	4e-18
3.	gi[5310428] UNKNOWN PROTEIN FROM 2D-PAGE	79	7e-13
4.	gi[2385419] Y531 protein [Methanobacterium]	55	1.45

a) What is the meaning of the E?

(2)   Changes of getting a better score for a random alignment of this sequence vs. the given alignment

b) What can you conclude from the search results? Are there any significant homologues?

(2)   Yes, first three have low E values.

c) Aligning the amino acid sequences gives the following results:

```
            1       5        10       15       20       25
                                      *        *
1. gi[1042879]  P-Y-V-K-V-Q-L-P-G-P-S-D-E-Q-L-K-N-L-V-R-E-V-T-D-A-V-E
2. gi[2346879]  P-F-L-S-L-R-L-P-G-P-S-N-E-Q-L-K-N-L-V-R-E-L-S-E-A-V-E
3. gi[5310428]  P-F-I-N-V-K-L-P-G-P-S-S-E-Q-L-K-D-I-V-R-E-I-T-Q-A-V-E
4. gi[2385419]  P-I-V-N-V-I-T-G-G-D-V-A-H-E-S-P-L-V-S-M-V-I-K-A-D-V-A
```

The protein is believed to bind non-specifically to the phosphate backbone of nucleic acids, place an (*) above those residues most likely to be involved in this binding. **Why?**

(3)   Only the first 3 sequences need to be considered. There are 2 conserved (+) residues (K 16 & R20) that probably fall on same side of a helix.

11. Identify the purification technique that you would associate with the conditions indicated in the blanks provided.

(5)

	Start Conditions	Technique	End Conditions
1)	Low ionic strength	Ion exchange	High ionic strength or pH change
2)	Small sample volume	Gel filtration	Diluted sample
3)	30% ammonium sulfate	Salt cuts	70% ammonium sulfate
4)	High ionic strength	HIC	Low ionic strength
5)	Low imidazole conc.	Ni - NTA column	High imidazole conc.

1 pt each

(Please sign your name on the back of your exam in a manner that you can recognize it when it is returned.)