

Name

Student number

University of Guelph
Department of Molecular and Cellular Biology

BIOC*2580 Introductory Biochemistry
Fall 2010

Midterm
October 23, 2010 1:30 pm RozH 101 & 104

Instructors: Drs. John Dawson, Enoka Wijekoon

Total Marks: 50
Total Time: 75 minutes

Total pages in this booklet: 10
This exam determines 25% of the final course grade

**CONTROL
CODE**

A

Enter this code in
question 21 on the
scantron card

Instructions:

1. Print your full name and student number in the spaces provided at the top of the page.
2. This exam consists of 2 sections. Please attempt all questions.
3. Please answer the multiple choice questions in section 1 on the scantron card provided. Please answer section 2 in the space provided in the booklet.
4. Use only pen to answer the questions in section 2. Do NOT use red pen.
5. Hand in all materials including this exam booklet.

Scantron Instructions:

1. Use a soft black lead pencil only on the Scantron card and erase cleanly if needed. Do NOT use white out or ink on the Scantron card.
2. Fill out your ID number on the score sheet.
3. In the event of a discrepancy between the Scantron card and what has been noted on the exam booklet, the results on the Scantron are taken as final.

Notice:

1. Short answer questions answered in pencil will not be regraded, in accordance with the Rules on Academic Misconduct.
2. No marks will be given for illegible and otherwise unreadable, incomprehensible, or unclear answers or writing.
3. You are not allowed to have / use programmable calculators, wallets / purses, cell phones, palm pilots, blackberries, etc. or any such aid under any circumstance unless previously authorized.
4. You are not allowed to leave before 40 minutes or during the last 15 minutes. If you need to use the bathroom, notify an invigilator and you will be escorted to the bathroom.

2.1	2.2	2.3	2.4	2.5	Total
3	3	4	25	0	12.5
5 marks	4 marks	4 marks	4 marks	3 marks	20 marks

Section 1: Multiple Choice Questions (30 marks)

Answer questions 1-20 on the Scantron Card provided with pencil. Each question is worth 1.5 marks.

- 1) The nonstandard amino acid γ -carboxy-glutamic acid has a side chain with the structure -CH₂-CH₂-CH₂-COOH (pK_a = 5.6). What net charge does γ -carboxy-glutamic acid have at pH 6.3?

A ✓

A. -0.83
 B. -0.17
 C. +0.17
 D. +0.83

$pH = 6.3$ $pK_a = 5.6$ $= 0.83$ $glu = C=O - dep - 1$
 $6.3 = 5.6 + \log$ $pro = 0$
 $5.01 = \alpha$ $6.3 > 5.6 \therefore de$

- 2) Which one of the following amino acids will bind to anion exchange resin at pH 7.0?

- D ✓
- A. Arg
 B. Thr
 C. Leu
 D. Asp

contains SO^- will bind

- 3) Which of the following statements is **true** about a peptide containing four amino acids at its isoelectric point, or pI?

- A ✓
- A. The total net charge is zero.
 B. The amino and carboxyl termini are not charged. ✗
 C. Two internal amino acids cannot have ionizable groups. ✗
 D. Only the amino and carboxyl termini contribute charge. ✗

- 4) Aminocellulose paper is made from cellulose fibres with bound (-NH₃⁺) groups at neutral pH. In a thin layer chromatography experiment, a mixture of amino acids is applied to a sheet of aminocellulose paper and dipped in an aqueous buffer at pH 7.0. Which one of the following amino acids would possess the **lowest** relative mobility (R_f)?

- * B
- A. Ala ✗
 B. Arg-polar
 C. Trp ✗
 D. Glu-polar

b polar
HL

- 5) When determining protein sequences using tandem mass spectrometry, why is it important for the peptide fragments generated with trypsin to be at very low pH?

- # D
- A. To make the N-terminus a better nucleophile. ✗
 B. So the fragments have net positive charges. ✗ \rightarrow
 C. For better ionization of the peptide in the ionspray chamber.
 D. To increase the rate of peptide bond hydrolysis.

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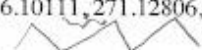
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- 6) A polypeptide is cleaved into peptides by treatment with trypsin and cyanogen bromide, and then the peptides are purified and sequenced. The sequences of the peptides are shown below.

Trypsin peptides	Cyanogen bromide peptides
T-1 GSMDPVALMTLR	C-1 TLRNFEEGSKFENYA
T-2 ITGLAIHQK	C-2 ITGLAIHQKELIM
T-3 NFEEGSK	C-3 VPKGSM
T-4 ELIMVPK	C-4 DPVALM
T-5 FENYA	

Based on sequences of the overlapping peptides generated by treatment with trypsin and cyanogen bromide (shown above), which of the peptides represents the N-terminus of the polypeptide? *Norm*

- C
- A. T-3
 B. C-1
 C. C-2
 D. T-4
- ITGLAI*
ITGLA

- 7) Steve the grad student completed mass spectroscopy-based sequencing of a peptide from his protein and then accidentally shredded his peptide sequencing results! He was able to find the mass values for the peaks of the fragments, listed here in Daltons: 156.10111, 271.12806, 372.17574, 486.21867, 649.282, 748.35042, 849.3981, 978.44069. 

Using the list of masses of amino acids given below, what is the identity of the *third* amino acid in the peptide when the sequence is written N- to C-terminus?

Amino Acid Masses (Da)

G: 57.02147

R: 156.10111

K: 128.09497

V: 99.06842

S: 87.03203

H: 137.05891

D: 115.02695

E: 129.04259

T: 101.04768

Y: 163.06333

N: 114.04293

F: 147.06842

A. Thr

B. Asp

C. Val

D. Glu

- 8) Which level of protein structure describes the sequence of amino acids in a polypeptide?
- A. Quaternary structure
 B. Secondary structure
 C. Primary structure
 D. Tertiary structure

* 9) In the alpha helix the hydrogen bonds are:

- C ✓
 A. perpendicular to the axis of the helix.
 B. mainly between electronegative atoms of the R groups.
 C. mainly between electronegative atoms of the backbone.
 D. only near the amino and carboxyl termini of the helix.

10) Which one of the following amino acids is a secondary structure breaker?

- C ✓
 A. Arg
 B. Trp
 C. Pro
 D. Ala

11) Which one of these amino acids would most likely be located at the surface of a protein?

- A ✓
 A. Arg
 B. Trp ×
 C. Pro ×
 D. Ala ×

12) To which family of tertiary structures does the following sequence most likely belong?

Val-Thr-Tyr-Thr-Ile-Asp-Ile-Thr-Trp
 β β β β β α β β β

- B ✓
 A. A four-helix bundle
 B. A β-barrel
 C. An α/β barrel
 D. A globin fold

13) Which of the following experiments provided the first evidence that the amino acid sequence of a polypeptide chain contains all the information required to fold the chain into its native, three-dimensional structure?

- B ✓
 A. When ribonuclease was treated with urea, it lost its catalytic activity.
 B. When denatured ribonuclease was allowed to renature, it regained its catalytic activity.
 C. When renatured ribonuclease was allowed to denature, it regained its catalytic activity.
 D. Addition of mercaptoethanol caused ribonuclease to regain catalytic activity.

14) What name is given to the three amino acids involved in the catalytic mechanism of chymotrypsin, trypsin and elastase?

- B ✓
 A. The catalytic triumvirate.
 B. The catalytic triad.
 C. The catalytic triangle.
 D. The catalytic triune.

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15) Which of the following catalytic mechanisms is not provided by any amino acid?

- ✓
B
- A. Nucleophilic catalysis ✓
 - B. Electrophilic catalysis
 - C. General Acid catalysis ✓
 - D. Transition state stabilization ✓

16) When an enzymatic activity is purified from a complex mixture of proteins, what usually happens to the total amount of protein and the specific activity during the purification process?

- *
✓
- A. The total amount of protein and the specific activity both increase.
 - B. The total amount of protein and the specific activity both decrease.
 - C. The total amount of protein increases and the specific activity decreases.
 - D. The total amount protein decreases and the specific activity increases.

$$100 = \frac{1}{5} \uparrow \frac{SP}{E}$$

17) A sample of pure actin protein was placed in a thin cuvette with a pathlength of 0.75 cm and an absorbance of 0.45 at 290 nm was measured. Given that the extinction coefficient of actin at 290 nm is $42680 \text{ M}^{-1}\text{cm}^{-1}$, what is the concentration of actin in the sample? (Be careful with unit conversions.)

- D ✓
- A. $1.40 \mu\text{M}$
 - B. $7.91 \mu\text{M}$
 - C. $10.5 \mu\text{M}$
 - D. $14.0 \mu\text{M}$

$$C = \frac{A}{\epsilon l} = \frac{0.45}{(0.75 \text{ cm})(42680 \text{ M}^{-1}\text{cm}^{-1})}$$

$$= 1.4050 \times 10^{-5} \times \frac{1000000}{1 \text{ M}}$$

18) What is the benefit of measuring the **initial** rate of a reaction v_0 at the beginning of an enzyme-catalyzed reaction?

- * ✓
- A. $[P] = 0$, so the reverse reaction can be ignored
 - B. $[ES]$ can be measured accurately
 - C. $v_0 = V_{\text{max}}$ ✗
 - D. Changes in K_M are negligible, so K_M can be treated as a constant ✗

19) At what percentage of its V_{max} is an enzyme functioning when the substrate concentration is 20% of the K_M ?

- *
C
- A. 10%
 - B. 16.6% ✓
 - C. 20%
 - D. 60%

$$v_0(K_M) + v_0(K_M) =$$

$$\frac{v_0}{v_{\text{max}}} = \frac{20K_M}{K_M + 20K_M}$$

$$\left(\frac{v_0}{v_{\text{max}}}\right)(K_M + 20K_M) = 20K_M$$

$$\left(\frac{v_0}{v_{\text{max}}}\right)K_M =$$

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20) On a Lineweaver-Burk plot, what is the label of the y-axis?

- B
- A. $1/[S]$
 - B. $1/V_{max}$
 - C. $[S]/v_o$
 - D. $1/v_o$ ←

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Enter this code in **question 21** on the Scantron card,
if you haven't already

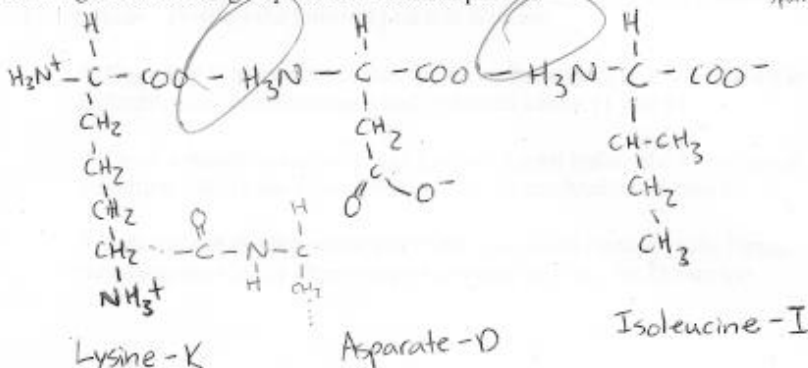
Nothing on page 6 will be marked. Use for rough work.

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Short Answer Questions

2.1. Structure (5 marks)

The tripeptide KDI is a biologically active fragment of the neurite outgrowth-promoting protein called laminin-1 that is being studied for its potential to regenerate neuronal tissues and be used in the treatment of spinal cord injuries. Draw the complete structure of the KDI peptide, showing the ionizable groups in their forms at pH 7.0.



3

2.2 Protein Structure (4 marks total)

a) What two structural properties make certain amino acids prefer beta sheet structure? (2 marks)

i) They are big

ii) They are branched on the beta carbon.

b) Briefly explain why it is important to have a few strategically placed breaker amino acids in a protein sequence to attain a compact, overall globular three-dimensional structure? (1 mark)

A few strategically placed breaker amino acids allow for turns in the protein sequence. More than a few will allow for loops

c) To which family of protein tertiary structures does the myoglobin protein belong? (1 mark)

The α - β -barrel

alpha-helical family

Name

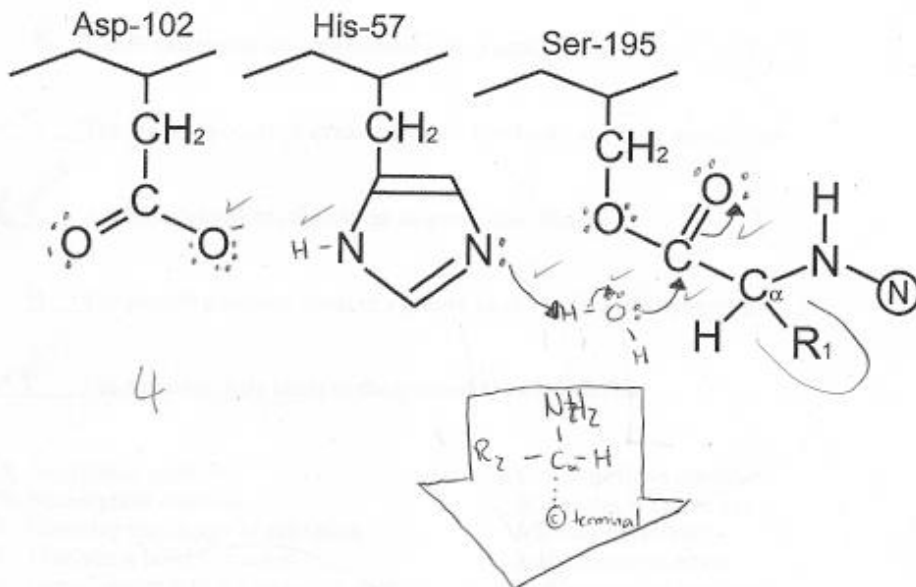
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2.3. Enzyme mechanisms: Chymotrypsin (4 marks total)

A. Chymotrypsin increases the rate of hydrolysis of peptide bonds by breaking down the natural reaction mechanism into two lower energy steps. At the end of the first step, the peptide bond is broken, releasing the C-terminal peptide, while the N-terminal peptide remains covalently bound to the chymotrypsin enzyme in the acyl-enzyme intermediate.

Shown below is a representation of the active site in the acyl-enzyme intermediate of chymotrypsin. Perform the following on this diagram:

1. Complete the structures of the amino acids participating in the catalytic mechanism, indicating any formal charges and hydrogen atoms. (1 mark)
2. Draw a **water** molecule where it would appear before the formation of the second transition state in the chymotrypsin catalytic mechanism. (1 mark)
3. Indicate **the movement of electrons** that occurs leading to the formation of the second transition state in the chymotrypsin catalytic mechanism. (2 marks)



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2.4. Enzyme mechanisms: Chymotrypsin (4 marks total)

The chymotrypsin enzyme vastly increases the rate of peptide bond breakage by water. Match the general examples of enzyme modes of action and binding forces to the specific examples from the chymotrypsin mechanism. Write the letter associated with the general examples in the space beside the specific example. Not all of the general examples are used.

Example:

L The binding of a large amino acid in the binding pocket of chymotrypsin positions the bond to be broken.

B Water is deprotonated, leaving it with three lone pairs of electrons in its valence shell. \

H X E The binding pocket of chymotrypsin is the right size to fit a large amino acid.

C Chymotrypsin breaks the natural reaction into two easier steps. \

A His-57 donates or accepts protons during catalysis. \

2.5

S The binding pocket of chymotrypsin is lined with nonpolar amino acids. \

I X K Asp-102 delocalizes the charge on protonated His-57.

F X H The peptide substrate binds in a groove on the surface of chymotrypsin.

F The oxyanion hole binds to the tetrahedral carboxyanion.

- A. Acid / base catalysis
- B. Nucleophilic catalysis
- C. Lowering the energy of activation
- D. Disulphide bond formation
- E. Complementary to the transition state
- F. H-bonds

- G. Competitive inhibitor
- H. van der Waals forces
- I. Ionic interactions
- J. Hydrophobic effect
- K. Electrophilic catalysis
- L. Proximity and orientation

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2.5 Enzyme Inhibition (3 marks)

The rate data for two enzyme experiments were collected: (a) in the presence of $1.8 \mu\text{M}$ inhibitor, and (b) in the absence of inhibitor. These data were then plotted on a Lineweaver-Burk plot. The linear regression lines from both data sets crossed at the y-axis. The **x-intercept** value for the experiment containing inhibitor was only **10%** of that observed in the absence of inhibitor. What is the K_i of the inhibitor? (Show all your work for full marks. Hint: try sketching the Lineweaver-Burk plot)