

MBB 694:407, 115:511
First Test Severinov/Deis
Tue. Oct. 2, 2007

Name _____
Row Letter ____ Seat Number _____

This exam consists of two parts. Part I is multiple choice. Each of these 25 questions is worth two points. Answer the Part I questions on this sheet, below. Answer the Part II questions on the question pages.

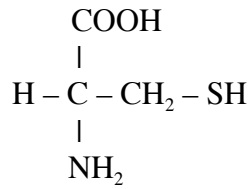
Please use BLOCK CAPITAL letters like this --- A, B, C, D, E. Not lowercase!

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|-----------------|------------------|------------------|
| 1. <u> B </u> | 10. <u> C </u> | 18. <u> D </u> |
| 2. <u> C </u> | 11. <u> C </u> | 19. <u> C </u> |
| 3. <u> F </u> | 12. <u> A </u> | 20. <u> A </u> |
| 4. <u> B </u> | 13. <u> A </u> | 21. <u> B </u> |
| 5. <u> B </u> | 14. <u> C </u> | 22. <u> B </u> |
| 6. <u> D </u> | 15. <u> B </u> | 23. <u> B </u> |
| 7. <u> C </u> | 16. <u> A </u> | 24. <u> G </u> |
| 8. <u> B </u> | 17. <u> A </u> | 25. <u>any</u> |
| 9. <u> C </u> | | |

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1. a. Draw a structural formula of amino acid C

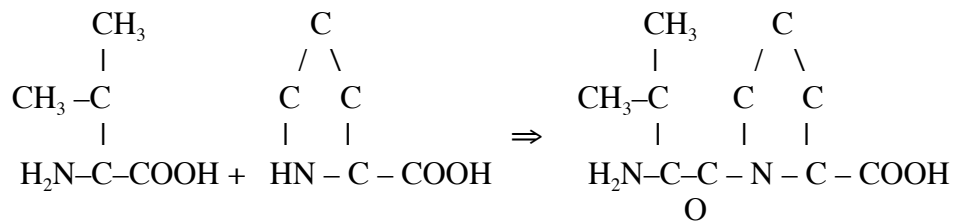
(2 points)



Of course COO(-) and NH3(+) are also OK.

b. Using structural formulas, draw a reaction of formation of a VP di-peptide from individual amino acids

(5 points) slightly tricky due to the Proline



c. Translate the following sequence into the three-letter amino acid code:

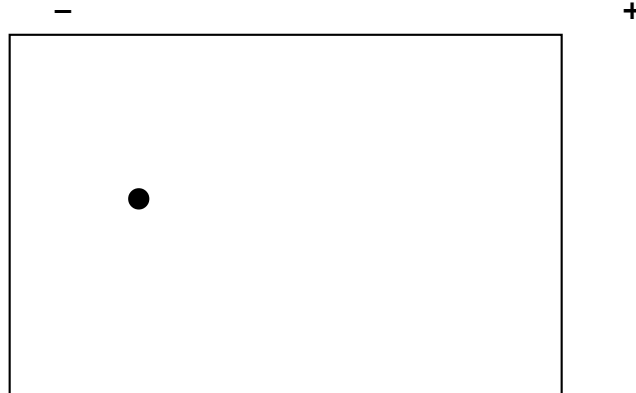
ENGYKTSFDLWKP

(3 points)

glu-asn-gly-tyr-lys-thr-ser-phe-asp-leu-trp-lys-pro

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- 2. a. Shown below are the results of a 2D gel separation of protein X. Indicate the position where **a)** the phosphorylated and **b)** the methylated form of the protein will migrate. The “-” and “+” signs indicate the polarity of electrodes during the IEF stage of the separation (5 points).



- Methylation will not change anything, since its contribution to molecular weight is negligible and it does not introduce a charge difference. Phosphorylation will introduce a negative charge, making the protein more acidic (in other words, lowering its pI). The mass change introduced by phosphorylation will have no effect on mobility in the second dimension (see above). The charge change will make the protein focus at a lower pH value during the iEF step, i.e., closer to the electrode marked by a "+" sign on the figure.

- b. If $S = 4 \text{ mM}$ and $V_{\text{max}} = 100 \text{ mM/sec}$, with $K_m = 6 \text{ mM}$, calculate the initial rate for this enzyme in the absence of inhibitors. State equation, show work, circle answer.

- (5) $V = V_{\text{max}} \left(\frac{[S]}{[S] + K_m} \right) = 100 \times 4 / 10 = 40 \text{ mM/sec}$

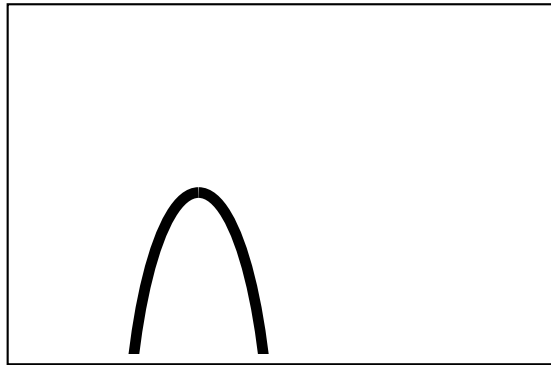
- 3. a. Enzyme X activity shows a sigmoidal rather than hyperbolic behavior from the substrate concentration S. Offer an explanation for such a behavior (5 points).

Allosterically modulated. Affinity for substrate increasing when substrate conc is increased.
Enzyme is multimeric

- b. Enzyme Y has a k_{cat} value of 10,000. What does this mean? Describe specifically what k_{cat} is. Can we assume K_m indicates the value of K_{diss} for this enzyme? (5 points).

- High k_{cat} means more catalytic conversions per second. High turnover ($\uparrow k_{\text{cat}}$) does not correspond to binding. So high k_{cat} invalidates the approximation $K_m = K_{\text{diss}}$.

- 4.
- Gel-filtration sorbent Sephadex G-25 has pore sizes into which proteins with molecular weights over 25 kDa do not enter. A mixture of three proteins with molecular weights of 10, 20, and 50 kDa was loaded on a Sephadex G-25 column and fractions were collected from time 0 to 60 (minutes). The peak where the 50 kDa protein eluted is indicated below. Schematically indicate expected positions of elution peaks for the remaining two proteins. How will the elution profile change if the 10 and 20 kDa proteins formed a complex? Draw and label all peaks.
- (10 points).



Time
of chromatography

0

60

- The smaller proteins will elute from the column later than the 50 kDa protein (20 kDa first, then 10 kDa). If the 10 kDa and 20 kDa proteins formed a complex (with a total molecular weight of 30 kDa), then peaks corresponding to individual 10 kDa and 20 kDa proteins would disappear. Instead, an earlier eluting peak corresponding to a molecular weight of 30 kDa will appear. This peak will elute after the 50 kDa protein but sooner than the peak corresponding to 20 kDa protein alone.
5. a. The standard free energy change for the reaction $[A] + [B] \rightarrow [C]$ is 12 kJ/mol

If the concentrations observed are $[A] = 2 \text{ M}$, $[B] = 2 \text{ M}$, and $[C] = 8 \text{ M}$, calculate 1) the equilibrium constant for this reaction and 2) the actual free energy change for the conditions described here. State equations, circle both answers. $R = 8.3 \text{ J/mol deg}$ and $T = 300 \text{ K}$.

(5 points) $\Delta G^{\circ} = -RT \ln K_{eq}$ therefore $K_{eq} = e^{-\Delta G^{\circ}/RT} = e^{-12000/2490}$

$$K_{eq} = e^{-4.82} = 0.0081$$

$$\Delta G = 12000 + (2490) \ln(2) = 12000 + 1720 = 13.7 \text{ kJ or } 13,700 \text{ Joules}$$

- b. Luca had ribosomes, ATP, cofactors (NAD), enzymes that were proteins, genes that were nucleic acids (probably RNA) fats, tRNA, amino acids, everything that is universal in living cells