SECTION 6

Problem-Solving Challenge: LWBGase

PRE-ACTIVITY ASSIGNMENT

Following a systematic method in solving problems improves the quality of the solution. In biochemistry employing a methodology is essential because issues must be decided and assumptions must be made before a solution can be proposed. Often times several different solutions are possible depending on the assumptions made about the problem (this moves beyond always having a single answer). As a result using a method to solve biochemical problems is recommended. In order to encourage you to try a method, the problem in this activity will be graded based on your method and not on your "answer." While the method is presented in a linear fashion it can be performed in spirals in which you move ahead and then circle back to the beginning to add new items to document your understanding. It has been observed that many students do not define the problem sufficiently and as a result end up solving a problem different from the one posed by your instructor. This can be especially costly during an exam.

Problem Solving Methodology

1. Define the problem.
2. Identify key issues and determine important issues associated with the problem.
3. Collect relevant information, what is missing?
4. Identify assumptions.
5. Break the problem apart.
6. Generate solutions for the sub-problems.
7. Integrate solutions.
8. Test and validate answer.
9. Generalize the solution.
10. Communicate the solution.

The peptide shown below is acid hydrolyzed (6 M HCl @ 110 °C for 24 hours). The hydrolysed sample is then applied to ion-exchange chromatography on a column of Dowex-50 (Dowex-50 is a cation-exchange resin with strongly acidic phenyl-SO_3^- groups). The material eluting from the column is post-column derivatized with o-Phthalaldehyde, and analyzed by fluorescence. O-Phthalaldehyde reacts with amino groups to form amino acid derivatives. Draw a graph that depicts relative fluorescence versus relative elution volume. On the graph, label each peak with the name of the appropriate amino acid.

Peptide sequence: Glu-His-Leu-Val-Lys-His

IN-CLASS ACTIVITY

RIP — Investigating Structure

The company you work for, Ramachandran Integral Proteins (RIP), has just isolated a protein, lakewobegonase (LWBGase), which they have found will make all women strong, all men good looking, and all children above average. In order to understand LWBGase and take advantage of its properties, the company wants to know something about the structural nature of the protein, immediately. Most of the procedures to get information about structure, X-ray crystallography, NMR, and sequencing will take time, as the company does not have these capabilities in-house and must send out samples to get this information. While the company impatiently awaits the results, the CEO of RIP sends out the memo that follows.

MEMO

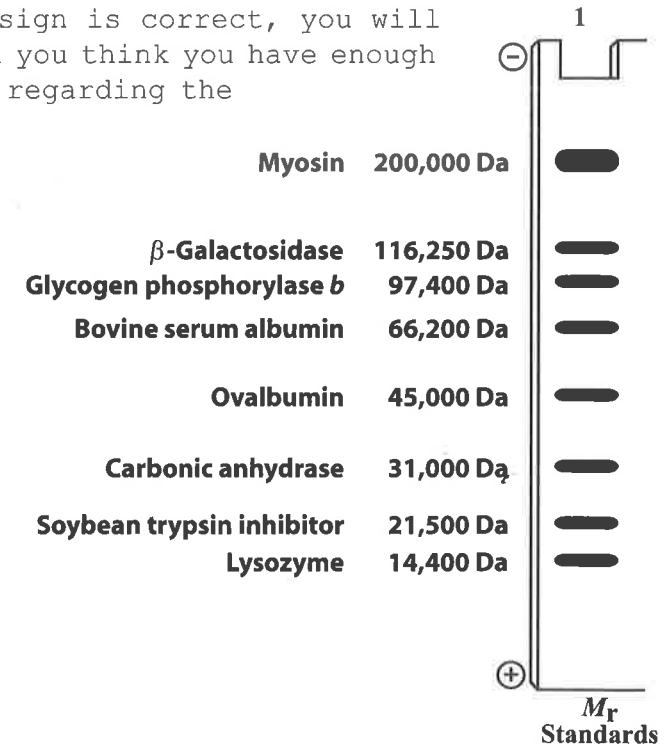
TO: all techs in the trenches (that means you)

FROM: THE BIG BOSS

RE: LWBGase Structure and $

The person who can get the most information about the structural nature of LWBGase **in the next 24 hours** will get a big bunch of stock when the company goes public next week, as well as a life-time supply of LWBGase. Send all your information and data to the head honcho of the structural group for evaluation.

Imagine you are in a lab conducting experiments. You have at your disposal some standard biochemicals, an inventory of the standard proteins given on your protein MW ladder below, and various chromatography and electrophoresis equipment. Design sequential experiments to collect relevant data. After each experiment contact your instructors. Describe your experimental design to your instructor. If your design is correct, you will receive data from your instructor. When you think you have enough information, compose a memo to the CEO regarding the structural nature of this protein.



POST-ACTIVITY SKILL EXERCISES

1. You wish to separate and isolate four proteins from a cell lysate. Below are some known physical characteristics of the proteins. Design a scheme that would facilitate the separation of all four proteins. Depict your separation scheme using a procedural flow chart.

Protein	Molecular weight (daltons)	pI	Binding Characteristics
A	80,000	6.3	No Binding
B	40,200	7.8	No binding
C	49,000	7.8	Binds to concanavalin A
D	48,000	6.2	No binding

2. A group of investigators synthesized a series of collagen-like peptides, each containing 30 amino acids residues, in order to study the important interaction among the three chains in the triple helix. Three peptides were synthesized:*

   Peptide 1, which is $(Pro\text{-}Hyp\text{-}Gly)_{10}$,

   Peptide 2, which is $(Pro\text{-}Hyp\text{-}Gly)_4\text{-}Glu\text{-}Lys\text{-}Gly\text{-}(Pro\text{-}Hyp\text{-}Gly)_5$

   Peptide 3, which is $Gly\text{-}Lys\text{-}Hyp\text{-}Gly\text{-}Glu\text{-}Hyp\text{-}Gly\text{-}Pro\text{-}Lys\text{-}Gly\text{-}Asp\text{-}Ala\text{-}(Gly\text{-}Ala\text{-}Hyp)_2\text{-}(Gly\text{-}Pro\text{-}Hyp)_4$.

   Their properties are summarized in the table.

Peptide	Forms trimers?	Imino acid content	pH	T_m(°C)
1	Yes	67%	pH = 1 pH = 7 pH = 11	61 58 60
2	Yes	60%	pH = 1 pH = 7 pH = 13	44 46 49
3	Yes	30%	pH = 1 pH = 7 pH = 13	18 26.5 19

Hyp=hydroxyproline, T_m = melting temperature

**From Kathleen Cornely, Providence College*

   a. Rank the stability of the three collagen-like peptides. What is the reason for the observed stability?

   b. Compare the T_m values of Peptide 3 at the various pH values. Why does Peptide 3 have a maximum T_m value at pH = 7? What interactions are primarily responsible?

2. You worked on developing two skills in the activity: 1) problem solving and 2) communication. For each skill, list one personal strength and why it is a strength and one personal area for improvement and how you plan to make that improvement.