

May 15, 2000

ID number

Biochemistry Cumulative Exam:

The Best of CH 561/562

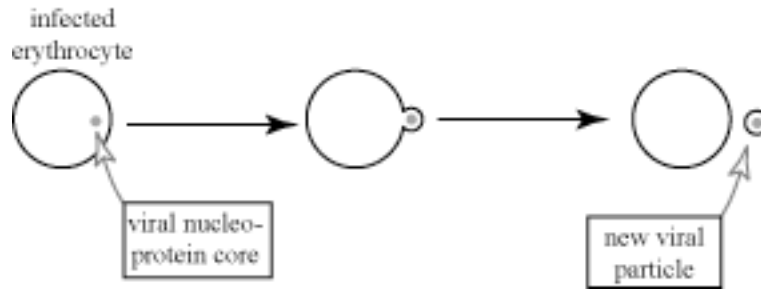
You know, I spent a lot of time carefully preparing these thought questions that integrate biochemical knowledge for the exams in the Biochem course (CH561/562). Sadly, very few of you ever took the time to work these problems. Perhaps it is because you felt that you did not have enough time during a normal exam. Well, I think it's a shame to waste good material. And now you have plenty of time to work out these problems. Plus, you have seen them before (if you took CH 561/562; if you didn't, then you must be good enough to work them anyway), so you should be ready. So I have compiled 4 of those extra credit problems that nobody bothered to try over the past 2 semesters (and lightly modified them). Work any 3 of your choice – you must get >60% total correct to pass.

Question	Points received
1	
2	
3	
4	
Total	

No book, notes, or any other material may be used during this exam.
A calculator (other than the internal one in your head) should not be necessary.

Problem 1

A recent report describes a new virus that infects erythrocytes (red blood cells). Erythrocytes are enucleated cells – they have no nuclei (or DNA), but they have plenty of ribosomes and translate mRNA that is stored in the cell. EV1 (erythrocyte virus 1) buds from red blood cells like so:



Thus, the viral particle is surrounded by membrane derived from the erythrocyte. Purified EV1 particles contain a 10,000-bp circular DNA molecule, as well as 2 major proteins of 50 kDa and 20 kDa, and a minor protein of 80 kDa (all estimated by SDS-PAGE). The 50-kDa protein is a glycoprotein.

a) If you treat the viral particles with proteases, then denature everything with SDS (sodium dodecyl sulfate) and run the products on a SDS-polyacrylamide gel, the only change is that the 50-kDa band disappears and is replaced by a 15-kDa band. Lectins (proteins that bind to carbohydrates) interact with the 50-kDa protein, but do not interact with the 15 kDa fragment. If you treat the viral particles with the detergent Triton X-100 before treating with proteases, you find that the 50-kDa protein is completely destroyed. Lastly, if you treat the viral particles with proteases and then use the treated EV1 particles to infect erythrocytes, you find that they can no longer infect them.

How do you explain all of these data? Describe as best you can the likely topology of this protein (it might be best to draw a picture). What do you think is the function (or, at least one the functions) of the 50-kDa glycoprotein, based on this information?

b) If you dissolve the viral membrane with Triton X-100, you can isolate a DNA-protein complex consisting of the viral DNA and the major 20-kDa protein. Addition of KCl at concentrations greater than 1.5 M is required to cause the 20-kDa protein to dissociate from the DNA. If you take the dissociated protein, dialyze it to dilute the salt, and then load it onto a CM(carboxymethyl)-cellulose column at pH 7, you find that it binds tightly and requires high salt concentrations to remove it. What sort of interaction do you think the 20-kDa protein uses to bind to the viral DNA?

c) You decide to analyze the carbohydrate on the 50-kDa glycoprotein. Mild acid treatment results in removal of carbohydrate from the protein and production of a 1:2:1 mixture of galactose (Gal), glucose (Glc), and mannose (Man). If you methylate the carbohydrate with dimethylsulfate before acid hydrolysis, you receive a 1:1:1:1 mixture of the following:

- 2,3,4-tri-*O*-methylgalactose
- 2,3-di-*O*-methylglucose
- 2,3,4,6-tetra-*O*-methylglucose
- 2,3,4,6-tetra-*O*-methylmannose

You treat the glycoprotein with a battery of different enzymes that catalyze the hydrolysis of specific glycosidic linkages from each of these sugars.

enzyme	releases from protein
α -galactosidase	oligosaccharide containing Gal, Glc, and Man
β -galactosidase	—
α -glucosidase	Glc
β -glucosidase	oligosaccharide containing Glc and Man
α -mannosidase	—
β -mannosidase	Man

(*e.g.* α -galactosidase catalyzes the hydrolysis of glycosidic linkages to α -galactose; this enzyme releases a carbohydrate containing the 3 sugars. The enzyme β -galactosidase has no effect.)

From amino acid analysis, you know that the glycoprotein is rich in threonine, and you guess that the oligosaccharide is linked to a Thr residue. Assuming this to be the case, draw the structure of the oligosaccharide that is linked to the 50-kDa protein (including Thr).

d) The 80-kDa protein is purified from EV1 particles and is found to have an enzymatic activity: if supplied with ribonucleoside triphosphates and viral DNA, it will synthesize a polynucleotide chain. What type of enzyme is this? Why do you think it would be packaged into viral particles?

Besides the proteins mentioned, what other enzyme(s) will be required for production of new viruses? In each case, explain why you include it.

Problem 2

Scientists in your marine biology group have sequenced the genome of a blue-backed dolphin. Among the many genes, they find one resembling the gene for hemoglobin. However, the gene predicts a protein that would have 2 copies of globin (each having the 8-helix globin fold) connected by a stretch of 24 amino acid residues rich in glycine and alanine. They find that messenger RNA corresponding to this gene is only present in young dolphins but not in adults. Thus, it is named Hb-jd (for hemoglobin of juvenile dolphins), to distinguish it from the adult form (Hb-ad), which is known to be a conventional tetrameric ($\alpha_2\beta_2$) hemoglobin, similar to the one used by all mammals

When you analyze the red blood cells from dolphins, you find that adult dolphins have only the Hb-ad protein present. Newborn dolphins have a mixture of Hb-ad and a novel hemoglobin. Isoelectric focussing allows you to estimate a pI of 6.7 for Hb-ad and a pI of 5.6 for the novel hemoglobin. When you examine the genes for Hb-ad and Hb-jd, you find that both proteins should have the same amount of arginines, histidines, and lysines, but that Hb-jd should have a few more aspartates and glutamates due to the linker connecting the globins.

Consider this hypothesis:

The novel hemoglobin that you have purified corresponds to Hb-jd.

Are the data consistent with this hypothesis? Explain your answer.

Briefly detail an experiment you would use to test this hypothesis. Describe the experiment and how you would do it. (*i.e.* this should be a **reasonable** experiment – one that you yourself could do in a few weeks time.)

When run on a SDS-polyacrylamide gel, the novel hemoglobin has a band corresponding to a polypeptide of about 35 kDa. What method would you use to estimate the molecular weight of the native protein?

Using your method (assuming you picked a good one), you find that the native protein has a molecular weight of about 70 kDa. Make a hypothesis about the tertiary and quaternary structure of the protein that accounts for all of the information above. Also try to explain how such a protein could have evolved.

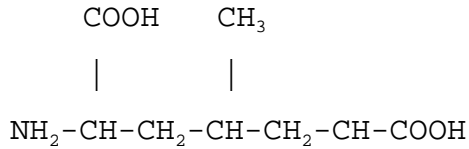
In young dolphins, as in many young animals that are growing, a lot of carbohydrates from the mother's milk are being metabolized to make ATP as well as intermediates from which to make amino acids.

You find that the novel hemoglobin also binds glycerate-2,3-bisphosphate (GBP), which serves as an indicator of glycolytic activity in dolphins too. The novel hemoglobin has a K_d for GBP that is much lower than that of Hb-ad.

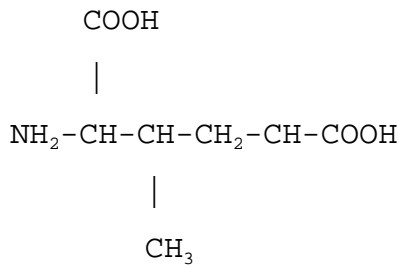
What effect would this have on this hemoglobin's ability to bind and deliver oxygen? Does this make physiological sense, given the considerations above? Explain your answer.

Problem 3

Researchers discover a new bacterium, *Bacillus annoyingus*, that has a unique cell wall, the core of which is a polymer of the unusual amino acid, 2-amino-4-methyl-6-carboxy-hexanoic acid (AMCH):



Many mutants of *B. annoyingus* have been isolated that cannot make a cell wall. Of these, a subset of mutants in 5 genes are defective in the synthesis of AMCH. These genes have now been cloned and sequenced and are found to strongly resemble genes that encode enzymes involved in the synthesis of Ile/Val and Leu. Interestingly, another mutant that makes an altered cell wall has been found. The gene that is inactivated in this mutant resembles the gene for α -isopropylmalate synthase, which catalyzes the first committed step in leucine synthesis. In this mutant, the polymer is made of 2-amino-3-methyl-5-carboxy-pentanoic acid (AMCP):



Based on this information, delineate the pathway of AMCH synthesis. Make sure to indicate the starting point for this sequence of reactions and the point of origin for other carbons and nitrogen. Explain why AMCP is made in the mutant described above.

Problem 4

In yeast cells, as in any other eukaryotes, it is important to coordinate expression of nuclear and mitochondrial genes. The cytochrome bc_1 complex is composed of three main subunits: cytochrome b, cytochrome c_1 , and the Rieske iron-sulfur protein. While cyt c_1 and the Rieske protein are encoded by nuclear genes, the cytochrome b gene is on the mitochondrial genome. Since every cyt bc_1 complex contains one copy of cytochrome b and one copy of the Rieske protein, it is important that they be expressed at the same rate. Available evidence suggests that the major regulation of the cyt bc_1 complex is by the growth rate of the cells. This makes sense, as every time a cell divides, it must double the number of mitochondria, and thus, the complement of mitochondrial proteins.

When cells are starved or growth is stopped by any of a number of means, production of the Rieske protein is reduced significantly. However, under these conditions, it is observed that the level of Rieske mRNA is not reduced, although the mRNA is found associated with a novel protein. When the cells resume growth, the Rieske mRNA is no longer found associated with this protein. Geneticists have isolated a mutant, *rce1*, that expresses the Rieske protein even when growth is inhibited. The *RCE1* gene is cloned and the sequence reveals that the putative protein has three zinc-finger domains followed by a region rich in Ser and Thr. An antibody is raised against this protein (after expressing the gene in bacteria and injecting the recombinant protein into rabbits), and the antibody recognizes a cytosolic protein with the same molecular weight as the novel protein. Moreover when cells are not actively growing, the Rce1 protein is found associated with the Rieske mRNA. When growth resumes, Rce1p becomes phosphorylated and is found unassociated with RNA. Molecular biologists begin to "dissect" the Rce1 protein by removing parts of the gene encoding various domains: loss of any of the Zn-finger domains results in a high constitutive expression of Rieske, while loss of the Ser/Thr-rich region results in a very low constitutive expression of Rieske. Another mutant with a phenotype like *rce1* (*i.e.* constitutive high expression of Rieske) has been isolated, and the mutation is found to be in the Rieske gene itself – a 10 bp deletion in the 3' untranslated region.

Regulation of cyt b in the mitochondrion appears to be different. When cells are growing, levels of cyt b mRNA are high; when cells are not growing, cyt b mRNA levels are very low. A sequence element has been identified upstream of the cyt b open reading frame that is involved in its regulation; when it is deleted, levels of cyt b mRNA remain high even when cells are not growing. A nuclear mutant, *cbe2*, is isolated that has the same phenotype (constitutive high cyt b expression). The *CBE2* gene is cloned and found to have an N-terminus that resembles a mitochondrial import signal and a C-terminal region with strong similarity to helix-turn-helix motifs. An antibody raised against Cbe2p recognizes a mitochondrial protein with the expected molecular weight. Interestingly, it is found that cyt b expression is not strictly regulated by growth rate: in growth-inhibited *rce1* mutants that maintain high expression of Rieske, cyt b mRNA levels remain high. Someone performs a "risky" experiment and finds that the Cbe2 protein binds to free Rieske protein, but cannot bind to Rieske when it is incorporated into the cyt bc_1 complex. When bound to Rieske, Cbe2p is unable to bind to DNA.

Putting all of these facts together, construct a model to explain how the expression of the Rieske and cyt b subunits is regulated. Your model should explain:

- a) how expression of cyt b is coordinated with that of Rieske
- b) how expression of the whole complex is regulated by growth