February 22, 1999

ID number _________

Biochemistry Cumulative Exam

Each question is worth 10 points. 2/3 of total possible points is required for a passing grade.

The two assigned papers (Noji *et al.*, 1997; Yasuda *et al.*, 1998) describe a novel assay for F_1-ATPase. The first part of the exam will test how well informed you are about the enzyme. The second part will focus upon the papers, although general biochemical knowledge will be required for success. You may use a calculator and any journal articles that you bring with you; textbooks are not allowed.

Given constants:
$R = 1.987$ cal K^{-1} mol^{-1}
$N = 6 \times 10^{23}$ mol^{-1}
1 cal = 4.184 J
1 J = 1 N m (i.e. 1 joule = 1 newton meter)
1 N = 1 kg m s^{-2}

The topic:

1) Briefly describe what ATP synthase or H^+-ATPase does. Why are there two names? Where is it found – in which organisms, where in the cell?

2 Consider the reaction that F_1-ATPase catalyzes.

a) Write out the equation for ATP hydrolysis that is catalyzed by F_1-ATPase. Draw the complete molecular structures of all reactants (i.e. all atoms), products, and any covalent intermediates (if they exist).

b) You replace the water with $[^{18}O]$-H_2O in the reaction buffer and allow hydrolysis to proceed with F_1-ATPase as the catalyst. After the reaction is complete, you analyze the products Where would you find the ^{18}O? Indicate in your figure above where it would be (*i.e.* circle one of the oxygens).

3) Interpretation of the F_1-ATPase assay owes much to our understanding of the structural arrangement of ATP synthase. In 1994, the publication of the X-ray crystal structure of the bovine mitochondrial F_1-ATPase at 2.8 Å resolution (Abrahams *et al.*, 1994) was a crucial advance in this regard; a structure of the $\alpha_3\beta_3$ hexamer from *Bacillus* PS3 has also been reported (Shirakihara *et al.*, 1997). Yet even before these publications, we had a good idea of the general layout of the subunits and their functions from biochemical experiments.

a) What are the subunits in F_1? What is their stoichiometry in the enzyme? How are they arranged? What is thought to be the main function of each?

b) What are the subunits in F_0? What is their stoichiometry in the enzyme? What is thought to be the main function of each?

4) Explain the “binding change” mechanism of ATP synthesis. I suggest that you draw a model, and use Boyer’s terminology of “open”, “loose”, and “tight” sites (O, L, T). Is rotational catalysis required by this model? Explain why or why not.

The papers:

5) Explain in no more than 100 words and 5 sentences exactly how these two papers increase our understanding of the operation of F_1-ATPase/ATP synthase. You should plan carefully what you are to write and make it succinct. (I will stop reading your answer after the fifth sentence or 100th word.) Tell me what you think these two papers demonstrated that adds new knowledge; note that this may not be what the authors claim!

6) It is important to consider the experimental set-up and assumptions taken for the analysis of the data.

a) The authors use a mutant F_1-ATPase that has three different mutations. List the mutations and the purpose of each for this assay.

b) In the first paper (Noji *et al.*, 1997), the authors coated the flow chamber with horseradish peroxidase conjugated with Ni^{2+}-NTA. In the second paper (Yasuda *et al.*, 1998), they used Ni^{2+}-NTA polystyrene beads. What are the purpose of these? Why do you think they switched from a protein to polystyrene beads?

c) What is the purpose of streptavidin in this assay? How does it function?

d) What is the purpose of the fluorescently-labeled actin? What advantages does actin pose over other markers?

e) How do the authors know that their assay is functioning in the way that they say it is? (*i.e.* What control experiments did they perform?)

f) How is this assay fundamentally different from most assays? As a hint, make the following comparison: How many enzymes are in a 10 μL reaction containing 1 μg/mL of an enzyme (50 kDa molecular weight)? How many enzymes were examined in the first paper? Were all of the observed actin filaments included in the analysis? How many were? Do you feel that the authors were justified in selecting some and not others for their analysis?

7) The authors report that ATP is required to observe rotation of the actin filaments.

a) Calculate the maximum amount of work that can be extracted from hydrolysis of 3 molecules of ATP under the conditions of the bacterial cytosol (see below).
The $\Delta G°'$ of ATP hydrolysis (ATP –> ADP + P_i) is -7.3 kcal mol^{-1}.
[ATP] = 8 mM, [ADP] = 1 mM, [P_i] = 8 mM, pH = 7, T = 25°C

Express this in joules (J). How does this value compare with the reported estimates of mechanical work done per rotation in these papers? (Express this in joules also.) How efficient is the F_1-ATPase motor by these calculations? Does this seem reasonable? Show all of your work/reasoning.

b) Did the authors distinguish adequately between the requirement for ATP binding *vs.* ATP hydrolysis? If not, which experiments would you suggest to critically test the claim that hydrolysis of ATP in the catalytic sites of F_1-ATPase drives the unidirectional rotation of the γ subunit?

8) Explain the significance of the fact that the actin filaments rotate in only one direction in terms of the structure of F_1-ATPase and the binding change model. At what point is mechanical work done by the enzyme? Important: indicate when ATP hydrolysis occurs relative to the point at which torque is applied to the γ subunit. (I suggest that you might re-draw the “binding change” model, incorporating what we know about the arrangement of the nucleotide-binding sites from the structure.) Taking together Boyer’s “binding change” model and the crystal structure data, is anticlockwise the predicted direction of rotation?

9) In the second paper, the time resolution has improved to the point where the authors can see steps of about 120° in the rotation.

a) Is this the step size that you would expect? Under which conditions do they observe these steps? What is the mechanistic basis for this?

b) How do the authors explain the occasional backwards steps? Can this be accommodated by the "binding change" model?

c) **EXTRA CREDIT**: Using the equations given in the text, try to calculate the predicted maximal rotation rate (in revolutions per second) of a 1-μm actin filament driven by F_1-ATPase under conditions of saturating ATP. Show your work.