BIOPHYSICS 354

FALL 2000

FINAL EXAM

Date Due: Thursday Dec 14th (Take Home exam replaces scheduled exam, 7:00-10:00 pm)

Time due: Completed answers should be returned Vlad Shinkarev (at Biophysics office) no later than 9:00 am, Dec 15th

Answer all questions in Section I, three questions in Section II, and one of the questions in Section III. You may make use of material from the course WWW pages in answering these questions.

Score weighting for the three Sections will be as follows: Section I, 45% (15% for each question); Section II 24% (8% for each question); Section III, 31%.

SECTION I

Answer all questions in this section.

1. In experiments using chromatophores from *R. sphaeroides*, the redox poise of components of the photosynthetic electron transfer chain was measured following illumination by continuous light to achieve a steady state. At the same time, the membrane potential was estimated from the electrochromic change. The Table below tabulates stoichiometries and $E_{m,7}$ values, and shows the concentrations of redox components measured under these experimental conditions:

			Concentration*	
Component	Stoichiometry	$E_{m.7}$ (mV)	oxidized form (mM)	reduced form
P865 ($(BChl)_2$)	2	450	0.634	1.366
cyt c_2	1	340	0.50	0.50
cyt c_1	1	270	0.947	0.053
ubiquinone	60	90	55.26	4.74
cyt b_L	1	-90	0.396	0.604
cyt b_H	1	40	0.317	0.683

$\Delta pH = 0$ (nigericin and 100 mM KCl were present)

Δp = 223 mV (membrane potential component)

The text below is from an earlier hypothesis on the mechanism of the ubiquinol:cyt c_2 oxidoreductase (bc_1 complex) of *R. sphaeroides*:

A consequence of the suggestion that all the quinone reaction sites involve reduction or oxidation of the pool is that the mean electron potential at each site will be equal to the $E'(E_h)$ value of the pool in equilibrium with protons at the site (see Mitchell, 1976). Denoting reactants in equilibrium with the P, N, and membrane phases by subscripts P, N, and M, respectively,

$$E'\,(Q/QH_2)_P = E_{m,7} - Z(pH_P - 7) + \frac{RT}{2F}\ln(Q)_M/(QH_2)_M$$

$$E'(Q/QH_2)_N = E_{m,7} - Z(pH_N - 7) + \frac{RT}{2F}\ln(Q)_M/(QH_2)_M$$

and

$$\Delta E'(Q/QH_2)_{N\text{-}P} = Z\Delta pH_{P\text{-}N}$$

where $Z = 2.303$ RT/F. As we have already, seen at the quinol oxidase site,

$$E'(Q/QH_2)_P = (E'(b\text{-}566) + E'(FeS))/2$$

If the oxidation of cytochrome *b*-561 contributes 60% of the electrogenic process of the complex, and involves electron flow across the insulating phase, we would expect

$$E'(b\text{-}561) = E'(Q/QH_2)_N - 0\cdot 6\Delta\psi_{P-N}$$

Given that $E'(FeS) = E'(c_2) = E'(c_1)$, and that $E'(b\text{-}566) = E'(b\text{-}561) - 0\cdot 4\Delta\psi_{P-N}$, substitution shows that

$$\Delta p_{P\text{-}N} = \Delta\psi_{P\text{-}N} - Z\Delta pH_{P\text{-}N} = (E'(c_2) - E'(b\text{-}566))/2$$

Here P and N phases refer to the aqueous phases in which the proton gradient is positive and negative respectively.

Discuss the agreement or otherwise between the results above, the hypothesis outlined in the review, and the structure of the mitochondrial complex from X-ray diffraction strudies. Explain and justify your reasoning, and included calculations to support your view.

Notes: *Effective concentration either in aqueous phase of chromatophores (cyt c_2), or in membrane phase (all other components).

2. In liver cells, the degree of reduction of the NAD^+/NADH couple is much greater in the mitochondrial matrix than in the cytoplasm. You may assume that the malate/aspartate shuttle (involving the exchange of malate $\leftrightarrows$ α-KG, and of H^+-glutamate $\leftrightarrows$ aspartate through antiport carriers in the membrane) operates to transfer reducing equivalents between cytoplasm and matrix. Show diagramatically how this shuttle works, and derive an expression relating the difference in the $[NAD^+]/[NADH]$ ratio between cytoplasm and matrix to the proton motive force (Δp).

For a Δp of 190 mV, what would be the maximal difference in E' for the NAD^+/NADH couple between cytoplasm and mitochondrial matrix? (You may assume the system is at 30 ^oC)

3. The Table below shows values for P/O ratios (see note) measured in mitochondria for different spans of the respiratory chain. Use diagrams and notes to show how these values can be explained in terms of the mechanism and $H^+/2e^-$ stoichiometry of each of the different complexes of the respiratory chain, and the ATP/H^+ stoichiometry for ATP synthesis in mitochondria. You should provide a full explanation for the ATP/H^+ ratio in terms of the mechanism and stoichiometry of the ATP synthase, and of transport processes involved in import and export of substrates and products of the ATP synthase reaction.

Site	**Reactants**	**Resp. control**	**P/O ratio**
Site 2	succinate to ferricyanide	1.9	0.49 ±0.02
Site 3	ascorbate to O_2	2.6	0.98±0.09
Sites 2 + 3	succinate to O_2	7.6	1.48±0.04
Sites 1 + 2 + 3	3-hydroybutyrate to O_2	5.0	2.27±0.08

Note: P/O ratio (also called the ATP/O or ATP/2e- ratio) is the stoichiometric ratio of ATP synthesized (or phosphate incorporated into ATP) for every pair of electrons flowing through the chain ($2e^- = ½O_2 = \underline{O}$). The respiratory control ratio (Resp. control) is the ratio of rates of O_2 uptake with and without ADP plus phosphate. The lower the value, the more likely the P/O ratio will be an underestimate of the true value. However, the corrections are expected to be small.

SECTION II

Answer 3 of the following:

1) A 100 kg rock-climber scales a 2,000 m vertical rock face.
   a) He starts at sea-level, taking 3 days. He consumes 4,000 dietary Calories of food per day.
   b) He descends in 30 min by rapelling down.

   The mass of the climber is unchanged at the end of the exercise. Comment on the work available, and the work performed, considering the climber as the system.

2) What determines that the measurement of electrode potential for a redox couple is proportional to the free-energy difference with respect to the half-cell of the reference electrode? Justify your answer by use of diagrams and equations.

3) A membrane vesicle containing 100 mM KCl is suspended in a large volume of medium containing KCl at 0.1 mM. Nigericin is added, and the system is allowed to reach equilibrium. Comment on the changes in $\Delta G'$, work and heat that accompany this process.

4) When a skunk cabbage emerges in early spring, respiration in the spadix (the first shoot to break through the snow) occurs through an alternative quinol oxidase, which bypasses the coupling steps of the bc_1 complex and cytochrome oxidase without generation of Δp. What effect would you expect this to have, and why would you expect this to be beneficial to the plant?

SECTION III

Write about one of the following. Do not exceed 2000 words.

1. Mechanism of water oxidation by photosystem II
2. Role of protons in the mechanism of cytochrome oxidase.
3. What do the structures of photochemical reaction centers tell us about their evolution?
4. Mechanism of light harvesting in purple photosynthetic bacteria.
5. Structure and function of complex I (NADH:ubiquinone oxidoreductase) of the repiratory chain.