

Chem 465
Biochemistry II

Multiple choice (4 points apiece):

1. In the reoxidation of QH_2 by purified ubiquinone-cytochrome *c* reductase (Complex III) from heart muscle, the overall stoichiometry of the reaction requires 2 mol of cytochrome *c* per mole of QH_2 because:

A) cytochrome *c* is a one-electron acceptor, whereas QH_2 is a two-electron donor.

B) cytochrome *c* is a two-electron acceptor, whereas QH_2 is a one-electron donor.

C) cytochrome *c* is water soluble and operates between the inner and outer mitochondrial membranes

D) heart muscle has a high rate of oxidative metabolism, and therefore requires twice as much cytochrome *c* as QH_2 for electron transfer to proceed normally.

E) two molecules of cytochrome *c* must first combine physically before they are catalytically active.

2. Which of the following statements about the chemiosmotic theory is correct?

A) Electron transfer in mitochondria is accompanied by an asymmetric release of protons on *one* side of the inner mitochondrial membrane.

B) It predicts that oxidative phosphorylation can occur even in the absence of an intact inner mitochondrial membrane.

C) The effect of uncoupling reagents is a consequence of their ability to carry electrons through membranes.

D) The membrane ATP synthase has no significant role in the chemiosmotic theory.

E) All of the above are correct.

3. When the ΔG° of the ATP synthesis reaction is measured on the surface of the ATP synthase enzyme, it is found to be close to zero. This is thought to be due to:

A) a very low energy of activation.

B) enzyme-induced oxygen exchange.

C) stabilization of ADP relative to ATP by enzyme binding.

D) stabilization of ATP relative to ADP by enzyme binding.

E) none of the above.

4. The most precise modern definition of a gene is a segment of genetic material that:

A) codes for one polypeptide.

B) codes for one polypeptide or RNA product.

C) determines one phenotype.

D) determines one trait.

E) that codes for one protein.

5. Which of these statements about nucleic acids is *false*?

A) Mitochondria and chloroplasts contain DNA.

B) Plasmids are genes that encode plasma proteins in mammals.

C) The chromosome of *E. coli* is a closed-circular, double-helical DNA.

D) The DNA of viruses is usually much longer than the viral particle itself.

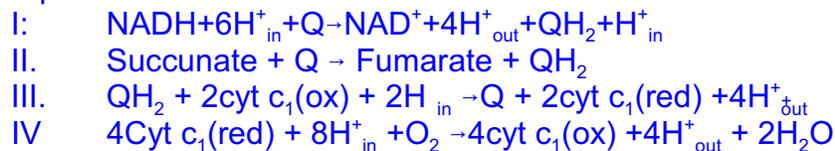
E) The genome of many plant viruses is RNA.

6. It is correct to say that DNA supercoiling cannot:
- A) be induced by strand separation.
 - B) be induced by underwinding of the double helix.
 - C) form if there is Z-DNA structure present.
 - D) occur if a closed circular double-stranded DNA molecule has a nick.
 - E) result in compaction of the DNA structure.
7. The Ames test is used to:
- A) detect bacterial viruses.
 - B) determine the rate of DNA replication.
 - C) examine the potency of antibiotics.
 - D) measure the mutagenic effects of various chemical compounds.
 - E) quantify the damaging effects of UV light on DNA molecules.

Essay Questions - Answer any 5

1. In oxidative phosphorylation we studied four different enzyme complexes that are used to transfer electrons and generate a proton gradient across the mitochondrial membrane. Describe the structure and chemical reactions that go on in each of these complexes.

A diagram like 19.17 is a good start since it shows all the complexes, their reactions and vectorial transport of protons but it lacks some details. A more complete answer would be to have diagrams like figures 19-9, 19-10, 19-12, and 19-14 with the equations



2. Describe in as much detail as you can the structure of ATP synthase and describe how it uses a proton gradient to synthesize ATP.

A diagram like figure 19-23(f) is a good place to start

Mitochondrial ATP synthase is composed of two major subunits, the membrane bound F_0 and the F_1 peripheral membrane protein.

The F_1 is composed of $\alpha_3\beta_3\gamma\delta\epsilon$ subunits, with the ATP binding site at the interface between an α and β subunit, and the γ protein making a central rod around which the alternating α and β subunits are arranged. In the crystalized structure we find one binding site that binds ATP, another binding site that holds ADP and P_i and the third site empty.

The F_0 complex consists of 3 peptides, a single a, 2 b's and 10-12 c's. The c peptide consists of 2 membrane spanning helices. The a and b make an arm that extends from the membrane up to the δ unit attached to the F_1 complex. As protons move across the membrane through the c helices, the C helices rotate. The rotation is connected to the F_1 via the ϵ and γ proteins. It appears that as the γ protein twists inside the α/β bore of F_1 it toggles the conformations of the α/β interface between the form that binds ADP and P_i , the form that changes ADP+ P_i to ATP and the third form that does not bind either ADP or ATP, so the newly formed ATP is ejected into the matrix.

3. Explain the differences and similarities between type I topoisomerases, type II topoisomerases and gyrases. Which of these enzymes is not found in eukaryotic cells? What role do these enzymes play in chromosome structure, DNA replication, and how might they be involved in DNA transcription (DNA→RNA)?

Type I topoisomerases add or remove DNA twists by breaking a single strand of the DNA, and allowing the other strand to twist. This changes the linking number by 1.

Type II topoisomerases add or remove DNA twists by breaking both strands of a DNA helix and passing it around a second strand of DNA, changing the linking number of DNA by 2.

A gyrase is the name given specifically to a type II topoisomerase found in bacteria that uses ATP energy to put negative supercoils into DNA. Even though the DNA in eukaryotic cells are negatively supercoiled, eukaryotic cells do not have a gyrase enzyme.

In replication one starts with a large piece of negatively supercoiled DNA, this must be unwound so the individual strands can be replicated, and then the two new pieces must be converted back to negatively supercoiled DNA. So both winding and unwinding will require topoisomerases. Similarly in transcription the DNA must be unwound so RNA can be transcribed from it, and then the DNA has to be rewound back into its original form, so topoisomerases are again needed. Topoisomerase activity is also needed to resolve DNA that is twisted around itself in recombination events or in separating DNA molecules at the very end of replication.

Even in eukaryotic cells when DNA is looped around a histone, topoisomerase is needed to relieve the positive supercoils that remain in the unbound part of the DNA after histone binding.

4. Describe the structural hierarchy of DNA going from a piece of B-form DNA up to a eukaryotic chromosomal rosette. What proteins are involved at each level of structure?

B-form DNA is first looped twice around a histone to make a nucleosome. The nucleosome core is made of 2 each of H2A, H2B, H3, and H4, and H1 is involved in the linking region between one histone and the next.

The nucleosomes are then coiled around each other to make a 30 nm fiber. This 30 nm fiber is then looped to make rosettes. Each rosette consists of an inner core of nuclear scaffold protein with 6 loops of the 30 nm fiber going out from the core.

5. DNA ligase is an important enzyme in both DNA replication and repair. What does DNA ligase do? What is its mechanism? How do viruses and eukaryotic cells differ from bacteria in this mechanism?

Ligase is the enzyme that seals a nick in the ribose phosphate backbone of DNA. The mechanism is best shown in a diagram like figure 25-16, where either ATP or NAD are used as a source of AMP that is covalently attached to an amine in the ligase protein. The AMP is then transferred from the protein to the phosphate on the 5' end of a nick to make a diphospho-linkage, which can then be attached by the 3' OH of the nick to seal the nick and release AMP. In this mechanism viruses and eukaryotic cells use ATP while bacteria use NAD.

6. Describe how the following potential mutation events are repaired in the cell:
- A. An A on one strand of DNA is accidentally misread and replicated as a C on the newly made DNA strand.
 - B. An A on one strand of DNA is deaminated and becomes Hypoxanthine.
 - C. A G on one strand of DNA is methylated to O⁶-Methylguanine.

A. This is a mismatch. In this repair mechanism the mismatch is first recognized by the MutS and Mut L proteins which bind tightly to the mismatch site with the hydrolysis of ATP. Mut H protein then binds to the complex, and, with the use of more ATP energy the DNA is threaded through the complex and looped out until the complex finds the hemi-methylated GATC site that is closest to the mismatch. The mut H protein then nicks the non-methylated strand of the DNA. If the nick is on the 5' end of the mismatch the DNA is unwound, the unmethylated strand degraded in the 3'-5' direction with exonuclease I or X, then the missing DNA is replaced with DNA pol III. If the nick is on the 3' end of the mismatch, similar steps are taken, but exonuclease VII or RecJ nuclease are used to remove DNA in the 5'-3' direction.

Note: if you said that the proofreading ability of pol III could catch and repair this error I also gave full credit!

B. Deamination repair is accomplished by base-excision repair. A glycosylase specific for the base Hypoxanthine removed it from the ribose sugar leaving the DNA backbone intact. AP endonuclease then locates the DNA missing a base and nicks the DNA at the 5' end of the ribose sugar. DNA pol I then replaces the damaged DNA, and ligase seals the nick where Pol I stops

C. The O⁶-Methylguanine is repaired by a direct repair system. In this system a cystine on the protein O⁶-Methylguanine-DNA methyl transferase picks up the methyl group from the base to become a methylated cystine. The protein cannot regenerate itself so is now inactive. The presence of this inactive protein serves as a signal to turn on the synthesis of a host of other DNA repair proteins.

1. Ans: B
2. Ans: A
3. Ans: D
4. Ans: B
5. Ans: B
6. Ans: D
7. Ans: D