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From Biochemistry to Biotechnology

Answers to Chapter Review Questions

Chapter 1 - Proteins, Carbohydrates, Lipids, and Nucleic Acids

Summary

1.1. Polymers; monomers; functional groups; isomers; macromolecules; condensation; hydrolysis.

1.2. Proteins; polypeptide chains; amino acids; peptide linkages; primary structure; secondary structure; tertiary structures; quaternary structures; denatured; noncovalently; chaperone.

1.3. Carbohydrates; hexoses; pentoses; glycosidic linkages; starch; glycogen; cellulose.

1.4. Lipids; triglycerides; ester linkages; saturated; unsaturated; phospholipid; bilayer; carotenoids.

1.5. DNA; RNA; ribose; deoxyribose; pyrimidines; purines; phosphodiester linkages; complementary base pairing; sequences; transcription; translation.

Review

1. e
2. e
3. c
4. a
5. c
6. b
7. c
8. c
9. c
10. The observations support explanation "a." Glycine is small and nonpolar. Glutamic acid and arginine are larger and polar (charged). Serine and alanine are small: the protein retains its shape. But serine is polar (it has –OH as its R group), and that does not affect the structure. Valine is larger and nonpolar, and this affects shape. So the issue is size.
11. Mannose and galactose have the same atomic formula, $C_6H_{12}O_6$, but the arrangement of atoms is different: compare carbons 2 and 4. These sugars have the hydroxyl (–OH) functional group. Its polarity helps the sugars dissolve in water. The –OH group also can participate in bonding the sugar to other molecules through condensation reactions (see Figures 1.4 and 1.17).
12. High temperature disrupts weak interactions such as hydrogen bonds. Heat shock proteins might work by stabilizing the protein so that the weak interactions are not necessary to preserve its structure.
13. A change from lysine is a change in primary structure. The change could affect tertiary structure if the protein folds as a result of electrostatic attractions between charged amino acids (+ to –). In this case, the presence of a negatively charged amino acid (aspartic acid) where there should be a positively charged one (lysine) might prevent correct folding if a negatively charged amino acid elsewhere in the polypeptide chain is involved in folding (it is attracted to a + amino acid). The same forces might be at work in the interaction of separate chains for quaternary structure.

14. See Figure 1.10. Heat breaks hydrogen bonds and other weak interactions that maintain protein shape. Disulfide bonds also required for normal protein shape. Styling and perms partially denature keratin, then renature the protein in a new shape. Your investigation might involve measuring keratin protein structure of hair before and after disrupting hydrogen bonds and disulfide bonds.

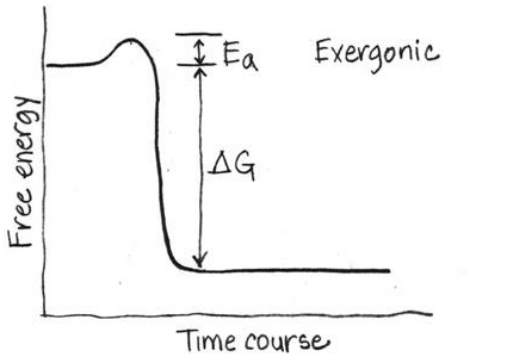
Chapter 2 - Energy, Enzymes, and Metabolism

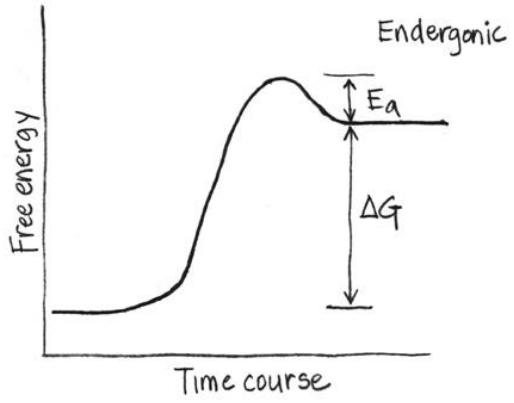
Summary

2.1. Free energy; entropy; potential; kinetic; laws of thermodynamics; chemical equilibrium; exergonic; endergonic; catabolic; anabolic.
2.2. Energy currency; hydrolysis; ATP cycle.
2.3. Energy barrier; enzymes; activation energy; substrate; complex.
2.4. Transition state; induced fit; cofactors; coenzyme; enzyme-catalyzed.
2.5. Metabolism; regulation; allosteric effector; pathway; reversible phosphorylation; pH.

Review

1. c
2. e
3. c
4. c
5. d
6. d
7. Endergonic reactions are coupled in time and space with exergonic reactions, which release the energy needed for the endergonic reactions.
8. A cytoplasmic enzyme generally has a globular structure with a hydrophilic exterior and an active site for substrate binding. An ion channel generally has a more linear structure with a hydrophobic membrane-spanning region and no active site.
9.





10. (a) The presence of water may prevent O_2 from reaching the enzyme. (b) Boiling denatures proteins, so polyphenol oxidase is irreversibly altered by boiling and its active site is destroyed. (c) Proteins have an optimal pH at which ionized R groups are appropriately charged to give the protein its tertiary structure. A pH 3 of may not be that optimal pH for polyphenol oxidase, so the enzyme is denatured and inactive.
11. See Figure 2.17. A competitive inhibitor binds to the active site of the enzyme and shifts the equilibrium to enzyme molecules in the active form.
12. To determine whether catalase has an allosteric or nonallosteric mechanism, perform an experiment with varying amounts of substrate and plot rate of catalase versus substrate concentration. An S-shaped curve will indicate an allosteric mechanism. A hyperbolic curve indicates a nonallosteric enzyme.
    To determine if a pollutant is a competitive or noncompetitive inhibitor, add the pollutant to the catalase to lower the rate of reaction, then add increasing amounts of substrate. A competitive inhibitor will be removed from the active site and the rate of reaction will increase. A noncompetitive inhibitor will not allow the rate to increase as more substrate is added. (There are more sophisticated kinetic experiments that you will learn in a biochemistry course).

Chapter 3 - Pathways That Harvest Chemical Energy

Summary

3.1. Oxidized; reduced; NAD^+; NADH; aerobic; anaerobic; glycolysis.
3.2. Pyruvate; glucose; acetyl CoA; citric acid cycle.
3.3. Oxidative phosphorylation; respiratory chain; proton-motive force; chemiosmosis; transfer of electrons.
3.4. Fermentation; pyruvate; ATP; glucose; synthase.
3.5. Catabolic; anabolic; gluconeogenesis; allosteric regulation.

Review

1. d
2. d
3. e
4. c
5. d
6. a
7. If cytochrome *c* remains reduced and cannot accept electrons, the electron transport (respiratory) chain stays reduced and NADH and FADH2 remain reduced. This prevents oxidation reactions in the citric acid cycle and pyruvate oxidation, so pyruvate cannot be converted to acetyl CoA. Instead, pyruvate is converted to lactic acid, regenerating some NAD that can be used so that glycolysis can continue. Because the electron transport chain is not working, there is no proton gradient set up in the mitochondria, and ATP is not made by oxidative phosphorylation.
8. See Figure 3.13. Some amino acids are converted to intermediates of glycolysis. Once they enter glycolysis these intermediates are further metabolized to a glycolytic intermediate that can be converted to glycerol, which is incorporated into triglycerides. Glycolysis and pyruvate oxidation produce acetyl CoA, which is converted to fatty acids and incorporated into lipids. Glucose is converted in glycolysis to acetyl CoA, which is then converted to fatty acids as above.
9. (a) Oxidation (removal of H from C2 and C3 of succinate)
   (b) Exergonic (because it is an oxidation)

(c) It requires the redox coenzyme NAD or FAD.
(d) The fumarate is converted to other intermediates that regenerate oxaloacetate, the acceptor for the citric acid cycle.
(e) The reduced coenzyme (NADH or FADH2) is reoxidized in the electron transport chain.

10. Anaerobes use alternate electron acceptors to generate energy, such as sulfur, sulfate, and nitrate. Also, they use substrate-level phosphorylation (direct transfer of phosphate to ADP) to make ATP.
11. The proton gradient in the experiment described in Figure 3.9 was generated artificially from the solution and did not require electron transport (a respiratory chain). The presence of antimycin A thus would have no effect on the experiment.

Chapter 4 - Photosynthesis: Energy from Sunlight

Summary

4.1. Photosynthesis; CO_2; H_2O; carbohydrates; bacteria; O_2; light reactions; light-independent reactions.
4.2. Photons; pigments; chlorophylls; electron; absorption; action; light-harvesting complexes; photosystem; noncyclic; cyclic; photophosphorylation.
4.3. Calvin cycle; RuBP; rubisco; 3PG; G3P; enzymes.
4.4. Oxygenase; photorespiration; C_4 plants; bundle sheath cells; CAM.
4.5. Cellular respiration; plant; respires; energy.

Review

1. e
2. b
3. d
4. d
5. d
6. d
7. In the dark, photosynthetic electron transport stops at photosystem II → reduced PQ (plastoquinone). Initially, the chlorophylls in light-harvesting complexes remain reduced, so reaction-center chlorophylls remain reduced and thus photosystem II remains reduced. In the dark, the Calvin cycle stops at the reduction phase, which requires NADH. No RuBP is regenerated, so there is no rubisco activity. The initial reactions are no oxidation of photosystem I, and no reduction of NADP to NADPH.
8. These processes can be compared using a table:

	Cyclic Electron Transport	**Noncyclic Electon Transport**
Products	ATP	ATP, NADPH, O_2
Source of electrons	Electron transport	Electron transport (photosystem I) or water (photosystem II)

9. See Figure 4.18. CO_2 carbons end up in 3PG, which is converted to pyruvate. Pyruvate goes to the citric acid cycle, where some of the intermediates are converted to amino acids, which are incorporated into protein. In the Calvin cycle, some 3PG is converted to G3P, which can enter glycolysis. Some of the intermediates of glycolysis are converted to amino acids, which are incorporated into protein.
10. (a) Here is the pathway followed by ^{14}C: $^{14}CO_2$ → cells → photosynthesis → carbohydrate → combustion → $^{14}CO_2$. Release of $^{14}CO_2$ upon combustion would be

evidence of photosynthesis (and life).
(b) In this case: $^{14}CO_2 \rightarrow$ heat denatured cells, no photosynthesis. If living things were present, $^{14}CO_2$ would be released in experiment (a), but not in experiment (b).

Chapter 5 - Gene Mutation and Molecular Medicine

Summary

5.1. Mutations; somatic; germ line; point; silent; chromosomal; spontaneous; induced; cytosine; evolution.
5.2. Proteins; amino acid; deletions; fragile-X syndrome; colon cancer; multifactorial; single gene.
5.3. Restriction enzymes; recognition sequences; gel electrophoresis; DNA fingerprinting; mutant genes; DNA barcoding.
5.4. Genetic screening; genetic diseases; expression; DNA testing.
5.5. Phenotype; cancer; inhibitors; gene therapy.
5.6. Polymerase; DNA.

Review

1. a
2. c
3. b
4. b
5. d
6. b
7. (a) In a loss of function mutation, a phenotype is not present; for example, there may be a loss of enzyme activity. In a gain of function mutation, a new phenotype is present; for example, a new signaling protein may be active.
   (b) In a missense mutation, a single base pair change results in a codon change and thus an amino acid change in a protein. In a nonsense mutation, a single base pair change results in a codon change to a stop codon and thus premature termination of a protein.
   (c) In a spontaneous mutation, DNA changes as a result of unprovoked chemical changes or replication errors. In an induced mutation, DNA changes as a result of outside physical or chemical agents.
8. (a) The mutation that leads to PKU is rare in the human population; most people do not have the harmful allele and the highest probability is that the father is homozygous normal. Because the mother has PKU (she is homozygous mutant), the developing fetus is heterozygous.
   (b) High levels of phenylalanine cause brain damage. If the mother's phenylalanine levels were too high, the baby would be born with brain problems.
   (c) The woman should be on a phenylalanine-restricted diet.
9. Testing for the cystic fibrosis (CF) allele could be done by allele-specific ligonucleotide hybridization with probes for the normal and CF alleles; see Figure 5.18. Or direct DNA sequencing of the CF gene could be done. A person who is a carrier will test positive for both the normal and the mutant alleles. To do gene therapy, the normal allele for CF could be inserted into a viral vector that can infect cells in the lung and airway tissues. Then the virus could be sprayed onto these tissues.

10. Early identification of people with multifactorial diseases, even before symptoms appear, could lead to therapeutic interventions to prevent disease development. Ethical issues might include insurability, hiring eligibility, and social stigma.
11. An enzyme test for HEXA would reveal intermediate levels in people who are carriers. This could be done on accessible cells (e.g., blood) if the gene is expressed there. A DNA test could involve sequencing the gene by allele-specific oligonucleotide hybridization (see the answer to Question 9). The advantage of DNA testing is that it can be done on any cells from the body (not just cells that express the enzyme).
    Investigation of the stop codon hypothesis would involve isolating the HEXA protein from patients with Tay-Sachs disease and showing that it is shorter in primary structure than the protein encoded by the normal allele.
12. (a) The amino acid sequence would be Leu-Ile-Ser-Ile-Ala. This is a missense mutation.
    (b) The mutation replaces proline with serine. Proline is a nonpolar amino acid that is usually part of bends or loops in a protein; serine is a polar amino acid with a smaller side chain. The mutation is likely to affect enzyme activity because it is likely to affect protein structure.
    (c) See p. 101. This region of the gene could be amplified by PCR and then digested with EcoRV. The mutant DNA will be cut, but the wild-type DNA won't be.

Chapter 6 - Genomes

Summary

6.1. DNA sequencing; miniaturization; genomes; noncoding.
6.2. Prokaryotes; metagenomics; transposon mutagenesis; composite; transposon mutagenesis.
6.3. Model organisms; development; gene families; pseudogenes; eukaryotic; moderately repetitive sequences.
6.4. Haploid; noncoding; introns; SNP genotyping; personalized medicine; pharmacogenomics.
6.5. Proteome; protein-coding; mass spectrometry; metabolome; hormones.

Review

1. c
2. b
3. e
4. e
5. b
6. b
7. c
8. c
9. a
10. One gene can produce several proteins by alternative splicing, which makes the proteome highly complex. In addition, many proteins are modified after translation, and this contributes to even more protein diversity. The metabolome is highly variable from cell to cell and from one time to another. It is determined not only genetically but also by responses to environmental conditions.
11. While all of these plants have the same basic genes for "life" as well as "plant" functions (e.g., photosynthesis, cell-wall formation, flowering), there are some

genes (and proteins) that are specialized for each plant (e.g., rice genes for growing under water, genes for timing flowering, genes for seed-storage proteins).

12. (a) Extract genomic DNA from the patient's cells and analyze it for SNP polymorphisms. If the SNP that correlates with kidney cancer is present, he has an increased susceptibility.
    (b) Isolate both normal and cancerous kidney cells. Do a metabolomic profile on the kidney cancer cells and the normal kidney cells using chemical analyses for small molecules. By comparing the profiles, generate a metabolomic "signature" for the kidney cancer cells. Next, examine the metabolomic profile of kidney tissue from the patient and compare it with the metabolomic signature for kidney cancer cells.
    (c) For possible drugs involved in kidney cancer treatment, isolate many cancers (or examine stored tissues) and do a SNP analysis, correlating tumor response to the drug with the SNP polymorphism. Then isolate some of the patient's tumor cells and examine the DNA for SNPs that relate to drug response. Use the drug that the patient's genome indicates will be effective.

Chapter 7 - Recombinant DNA and Biotechnology

Summary

7.1. Recombinant DNA; sticky ends; restriction enzyme; DNA ligase.
7.2. Clone; transformation; transfection; *Agrobacterium tumefaciens*; reporter genes; selectable markers; replicon; vectors.
7.3. Genomic library; mRNAs; reverse transcription; PCR.
7.4. Knock out; antisense RNA; DNA microarray; cDNA.
7.5. Biotechnology; expression vectors; transgene.
7.6. Proteins; pharming; biotechnology; transgenic; food production.
7.7. Genomic equivalence; stem cells; intercellular signals; embryonic; induced pluripotent.

Review

1. b
2. c
3. e
4. a
5. e
6. d
7. b
8. c
9. Both PCR and cloning begin with a gene sequence. In PCR, the sequence is amplified in the test tube. In cloning, the sequence is amplified by an organism (typically bacteria). In PCR, amplification is achieved by synthesizing primers that bind to either end of a target DNA sequence and adding nucleotides and DNA polymerase. The doubled DNA is them denatured, and the process is repeated 20 to 40 times.
   In cloning, the target DNA is inserted by restriction and ligation into a vector, which has an origin of replication that will function in the organism where amplification will occur. The vector is added to the host cells, which are cultured and divide many times, amplifying the target DNA along with the host chromosome. The vector is then removed from the host cells and cut with a restriction enzyme, releasing amplified, cloned target DNA.

PCR is much simpler and faster but has artifacts where inappropriate fragments of DNA are amplified or sequence errors are introduced by DNA polymerase. Cloning yields the correct DNA without mutations but involves host cell culture and time-consuming DNA purification steps. See Figure 7.12.

10. A simple table can answer this question.

	Conventional	**Recombinant DNA**
(a) Sources of new genes	Other plants of same species	Any organism or synthetic DNA
(b) Number of genes transferred	Often many	One
(c) How long it takes	At least one growing season, usually many	Weeks

11. (a) The target gene would be inserted into an expression vector with a promoter such that the gene would be expressed in the developing seed. The vector could be added to cultured wheat cells, and those cells carrying the vector selected (the vector could carry a reporter gene for resistance to an antibiotic). The cells could be induced to form a wheat plantlet, which would be transferred to the field and the seeds examined for the new protein.
    (b) The target gene could be inserted into a sheep expression vector containing the lactoglobulin promoter so that the gene would be expressed in milk glands. The recombinant vector would be inserted into sheep egg cells. After the female offspring grew up, their milk could be tested for the presence of the human enzyme.
12. Public concerns include the artificiality of unnatural interference with nature, the safety of these foods for human consumption, and environmental dangers if non-host plants receive recombinant genes.
13. One could analyze mRNA in egg cells, in the parent differentiated cells, and in the reprogrammed cells. This could be done by reverse transcriptase PCR or by gene expression arrays.