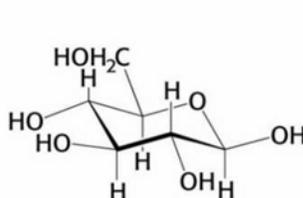
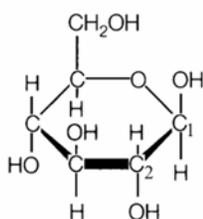


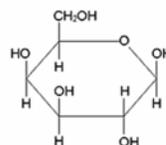
Molecular Biology Final Solutions



beta-glucose



beta-glucose



beta-galactose

1) Consider the diagrams of the chair form of beta-glucose and the Haworth projection of beta-glucose and beta galactose. e.

a) In alpha-galactose, the anomeric carbon is (equatorial) (**axial**) (both) (neither). No explanation required
Neither also acceptable, since the hydroxyl is not explicitly mentioned

b) In glucose (beta 1,4) galactose, the glycosidic bond is (no explanation required) :
(axial-axial) (axial-equatorial) (**equatorial-axial**) (equatorial-equatorial) (none of these)

c) Which of the following disaccharides contains an axial-axial glycosidic bond? Circle as many as apply.
No explanation required.

- a) glucose (alpha1,4)-glucose
- b) galactose (alpha 1,4)-glucose
- c) **glucose (alpha1,4)-galactose**
- d) **galactose (alpha1,4)-galactose**
- e) none of these

2) Which of the following types of bonds is likely to contribute to holding individual polymers of cellulose together in a strand of cotton ? Circle as many as apply. No explanation necessary.

- a) covalent
- b) ionic
- c) **hydrogen**
- d) **Van der Waals**
- e) hydrophobic forces

3) Which of the following molecules has more hydrogen atoms?

Circle one answer in each case and explain.

a) **2 glucose molecules**, lactose (a disaccharide made up of glucose and galactose), or both the same
An HOH is removed in the dehydration reaction that accompanies the formation of the glycosidic bond between the two monomer sugars.

b) **a saturated fatty acid**, a trans-unsaturated fatty acid with the same number of carbons, or both the same
Because an unsaturated fatty acid contains one or more double bonds, it will have less hydrogens, since there is only one hydrogen atom attached to such carbons compared to 2 hydrogens on carbons joined by a single carbon-carbon bond. -CH=CH- less than -CH₂-CH₂-

c) at pH7: glutamate, asparagine, or **both the same**

At pH 7, the alpha and gamma carboxyl groups of glutamic acid will be charged (de-protonated) as will the alpha carboxyl of asparagine. The alpha amino groups will carry 3 hydrogen nuclei in the -NH₃⁺ state. The amide functional group of asparagine carries no charge (neither gains nor loses a proton). Examining the structures of these two amino acids shows asparagine has 8 hydrogen nuclei and glutamic acid has 8 hydrogen nuclei.

d) **a fat containing saturated fatty acids 16 carbons in length**,
a phospholipid containing saturated fatty acids 16 carbons in length,
or both the same

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Fats are triglycerides with 3 fatty acids per molecule of fat. Phospholipids lack the third fatty acids, and the constituents forming a diester to the phosphate (e.g., serine, choline) are much smaller than the third 18-carbon fatty acid which contributes 35 hydrogen nuclei.

e) a single molecule of glycogen with a 100 carbons, a single molecule of cellulose with 100 carbon atoms, or both the same.

Glycogen has a branched structure and cellulose is a straight chain. But with the same number of glucose residues (or the same number of total carbons) each can make the same total number of glycosidic linkages and thus undergo the same number of dehydrations. Next best answer is cellulose, reasoning that the extra glycosidic bonds on each branching sugar in glycogen (3 total) should subtract more hydrogens.

4) The protein GDNF exists as a dimer in solution, with the 15000 molecular weight monomers linked by an interchain disulfide bond through the cys101 residues. Suppose a variant of GDNF exists (variant 1) in which the cys 101 has been replaced by a serine residue. Ultracentrifugation experiments under mild conditions demonstrated that the molecular weight of the variant GDNF is 30,000.

a) Fill in the table below with the expected molecular weights as measured by the indicated method [no explanation necessary] (PAGE= polyacrylamide gel electrophoresis) :

	gel filtration	gel filtration	SDS PAGE	SDS PAGE
	Normal GDNF	Variant 1 GDNF	Normal GDNF	Variant 1 GDNF
Without mercaptoethanol	30000	30000	30000	15000
With mercaptoethanol	* 30000	30000	15000	15000

You need explain only the answer in the box with the asterisk.

Weak bonds keep 2 subunits together despite the destruction of the covalent disulfide bond, as can be deduced from the fact that the protein that has serine instead of cysteine here has a MW pf 30,000. total MW at 30,000

b) Suppose there is a second variant of GDNF (variant 2) which differs form normal in that it lacks the 8 amino acid residues normally at the amino terminus (and still has cys that forms the disulfide in the normal protein). The molecular weight of the variant 2 is 28000. Suppose you carry out an Anfinsen type experiment on a mixture of equal parts of the the normal and variant 2 GDNF proteins: reduce all disulfides, subject the proteins to urea denaturation and then renaturation and then re-oxidize the disulfides.

i.)How many bands would you expect after SDS-PAGE WITH mercaptoethanol of this mixture?

(0) (1) (2) (3) (4) (5) (>5) For an explanation you need only list the molecular weight of any expected band(s).

The monomers: 14,000 and 15,000.

How many bands would you expect after SDS-PAGE WITHOUT mercaptoethanol. ?

(0) (1) (2) (3) (4) (5) (>5) You need explain only the answer in the box with the asterisk.

Three possible dimers. Of MW 28000, 29000, and 30000

The 28000 and 30000 are homodimers of the variant 2 and normal proteins respectively, while the 29000 MW species is a heterodimer consisting of one normal monomer and one variant 2 monomer.

c) Suppose GDNF normally contains 2 atoms of zinc (as the metal ion). After treatment with SDS, the protein would be expected to contain [circle all that apply and explain each possibility]:

(zinc) (peptide bonds) (beta sheet) (a net negative charge) (a net positive charge) (none of these)

Zinc is a prosthetic group; these are bound only by the native enzyme and so it is lost upon denaturation by SDS.

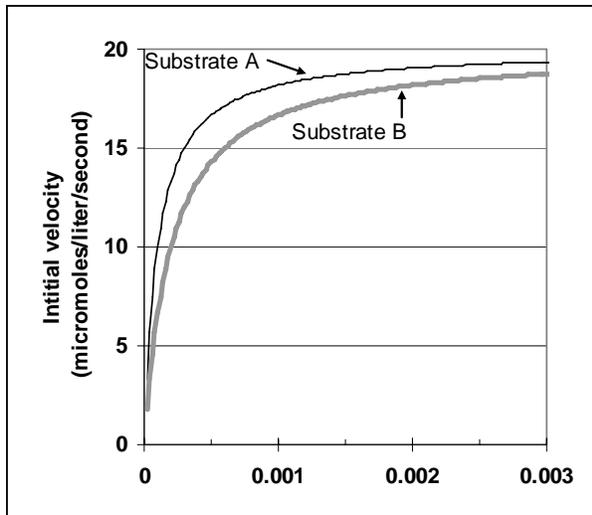
No peptide bonds are broken by SDS so these bond remain intact.

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The beta sheet is a secondary structure held together by hydrogen bonding, which would be disrupted by SDS.

All proteins treated with SDS take on a net negative charge as the SDS binds along the length of the protein. A net negative charge precludes a net positive charge.

5) The enzyme trypsin hydrolyzes peptide bonds at the carboxyl side of arginine and lysine residues. However, the amino acid residue that follows the arginine or lysine can influence either the K_m or turnover number of the reaction. Suppose the curves below were generated by comparing two substrates for trypsin:
Substrate A = val-ser-arg-phe
Substrate B = ser-val-arg-leu



Equations that may or may not help:

$$V_o = \frac{k_3 E_o [S]}{K_m + [S]} \quad (\text{Michaelis-Menten equation})$$

$$V_{\max} = k_3 E_o$$

No explanation needed for a, b, and c.

a) The K_m of the enzyme is higher for:
(A) (B) (same) (can't tell from this information)

b) The enzyme binds:
(more tightly to A) (more tightly to B) (same to both) (can't tell from this information)

[Deduced from the numerical definition of K_m as the concentration of substrate that yields a half-maximal V_o and an examination of the graph, seeing that the substrate B curve is further to the right]

c) From the graph, the approximate K_m for Substrate A is:
(0) (0.0001) (0.0005) (0.001) (0.002) (>0.002)

c) The enzyme binds more tightly with the product:
(val-ser-arg) (ser-val-arg) (same) (can't tell from this information)

One would have to vary the products and measure the reverse reaction to measure the K_m for a product. Since only the initial velocity for the forward reaction is being measured, the product concentration is negligible, and the ability of the enzyme to bind the tripeptide as a substrate for the reaction in reverse cannot be assessed. Next best answer is val-ser-arg, since Substrate A bound more tightly to the enzyme than Substrate B, and there is some similarity between substrate and product here. .

d) Suppose a similar experiment using the same enzyme and Substrate A was also carried out by another laboratory, but the V_{\max} was only one-tenth of that found in the experiment shown above. This difference could be explained by the other laboratory's use of (circle as many as apply):
(a lower amount of substrate) (a lower amount of time) (a lower amount of enzyme)
(a lower turnover number) (none of these)

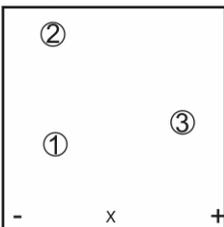
Lower enzyme would yield a lower V_{\max} since V_{\max} is proportional to the amount of enzyme used. ($V_{\max} = k_3 E_o$). Too low an amount of substrate would not allow an estimation of V_{\max} , too low time makes no sense since all rates are obtained by dividing by time, a lower turnover number or K_m make no sense as these are properties of the enzyme and not experimental variables.

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e) Suppose the enzyme concentration in the experiment graphed above was 0.1 micromoles/liter. was 0.1 micromoles/liter. Which of the following is the best guess for the turnover number of this enzyme for Substrate A? (<10) (10) (20) (100) (**200**) (500)(>500) per sec.

The plateau seems to be approaching 20 micromoles/liter/sec for both reactions. From the $V_{max}=k_3E_0$ equation, $k_3 = V_{max}/E_0 = 20/0.1 = 200$. The plateau is clearly > 10 and < 50, ruling out 100 and 500 and all other choices.

6) An oligopeptide (a decapeptide) is subjected to fingerprint analysis using the usual separation methods. The enzyme used was trypsin, which cleaves after arginine and lysine residues. The hydrolysate was spotted onto the center of one edge of a square blotting paper (at the X), the electrophoretic step was carried out at pH7, and the second separation direction was from bottom to top. Suppose the pattern of spots seen after staining for peptides and amino acids looked like this:a) Match the peptides below to the lettered spots in the diagram.



Spot 1 _____ Spot 2 _____ Spot 3 _____

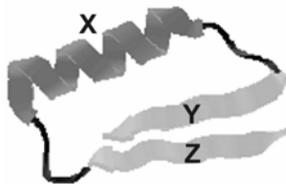
- A) ser-ser-arg
- B) ser-ala-glu
- C) val-val-lys

1=A; 2=C; 3=B. 1= A = positively charged and hydrophilic so migrates toward the cathode and does not migrate far in paper chromatography. 2= C= positively charged and hydrophobic, so migrates to the cathode and moves far in paper chromatography. 3= B= negatively charged so migrates toward the anode

b) The N-terminal (amino terminal) amino acid in the original decapeptide must be:
(A) (B) (C) (A or B) (**A or C**) (B or C) (A or B or C) (cannot tell from the information given)

Since the carboxy-terminal sub-peptide must be B, as it contains no arg or lys.

7) Consider the protein domain pictured below.



a) The bonds holding Y and Z together include [circle as many as apply]:
(ionic) (**hydrogen**) (hydrophobic forces) (**backbone atoms**) (side chains)

The picture shows the 3-dimensionsal structure of a polypeptide or polypeptide region (domain) in a ribbon format. The regions Y and Z denote two strands of a beta sheet (secondary structure) in this type of diagram. They are held together by hydrogen bonding between backbone atoms (secondary structure) .

b) The bonds holding X and Y together could include [circle as many as apply]:
(ionic) (**hydrogen**) (**hydrophobic forces**) (backbone atoms) (**side chains**)

A is an alpha helix and B is part of a beta sheet. These secondary structures are formed themselves using H-bonding between backbone atoms but associate with each other via their side chains, which could include all the weak bonds (4) used in forming tertiary structure.