Advanced Chemistry: Proteins & Enzymes Name

1. In an experiment a 200,000 kD (Kilodalton) molecular weight protein was isolated by gel filtration chromatography. This protein gave the following data under the conditions specified below:

a. SDS-PAGE, in the absence of β-mercaptoethanol, revealed a single band of molecular weight at 100,000 kD.
b. SDS-PAGE, with β-mercaptoethanol showed two bands of sizes 75,000 and 25,000 kD.

Based on these observations, propose the quaternary structure of this protein. A free hand diagram may suffice.

2. The hemagglutinin protein in influenza virus contains a remarkably long α-helix with 53 residues.

a. How long is this α-helix (in nm)?
b. How many turns does this helix have?
c. How many H-bonds are present in this helix?

3. A new protein of unknown structure has been purified. Gel filtration chromatography reveals that the native protein has a molecular weight of 240,000 kD. Chromatography in the presence of 6 M guanidine hydrochloride yields only a peak for a protein at 60,000 kD. Chromatography in the presence of 6 M guanidine hydrochloride and 10 mM β-Mercaptoethanol yields peaks for proteins at 34,000 and 26,000 kD. Determine the structure of this protein and how the subunits are held together.

4. Another new protein has been found. The following information has been ascertained:

a. Gel Permeation Chromatography under low salt conditions gives a single peak of 95,000 kD.
b. Gel Permeation Chromatography under high salt conditions gives 2 peaks, A & B. Peaks A and B have relative molecular weights of 15,000 and 40,000 kDs respectively
c. SDS-PAGE of the native protein, in the absence of β-mercaptoethanol reveals subunits having molecular mass of 40,000 and 15,000 kDs
d. SDS-PAGE with β-mercaptoethanol reveals subunits of 30,000, 15,000 and 10,000 kDs

Determine the structure of this protein and how the subunits are held together.

2. The initial velocity for an enzyme catalyzed reaction is measured at various initial substrate concentrations. Using the data below, calculate V_{max} and K_m for the enzyme. (You will need to use a Lineweaver-Burk double reciprocal graph)

[S]o, (μM)	Initial Velocity, μM/min
10	12.5
50	41.0
200	71.5
500	84.0
1000	89.0
2000	92.0
5000	94.0

6. The initial velocity for an enzyme catalyzed reaction is measured at various initial substrate concentrations. Using the data below, calculate V_{max} and K_m for the enzyme. (You will need to use a Lineweaver-Burk double reciprocal graph)

[S]o, (mM)	Initial Velocity, (M/sec) x 10^{-7}
0.1	0.96
0.125	1.12
0.167	1.35
0.250	1.66
0.5	2.22
1.0	2.63

7. A buffer is prepared by mixing 500 mL of 0.3 M Na_2HPO_4 and 500 mL of 0.2 M NaH_2PO_4. The pKa is 6.7 at this ionic strength

a. Calculate the pH of this buffer
b. 10 mL of 1 M HCl are added to this buffer. Calculate the new pH. (Ignore the change in volume)

8. 5. The initial velocity for an enzyme catalyzed reaction is measured at various initial substrate concentrations. Using the data below, calculate V_{max} and K_m for the enzyme. (You will need to use a Lineweaver-Burk double reciprocal graph)

[S]o, (M)	Initial Velocity, M/min
1.7 x 10^{-6}	10.0
3.8 x 10^{-6}	20.0
1.2 x 10-^{5}	45.0
2.3 x 10-^{5}	60.0
8.5 x 10-^{5}	85.0