

## Chapter 5

- Nitrate reductase is a dimer (2 Mo/240,000 M<sub>r</sub>).
- Phe-Asp-Tyr-Met-Leu-Met-Lys.
- Ser-Glu-Tyr-Arg-Lys-Lys-Phe-Met-Asn-Pro.
- The ionic character of proteins A, B, and C is A > C > B at the pH used in the ion exchange column. Assuming all three proteins have a similar globular shape, their sizes are C > A > B. Protein C is a flavin-binding protein; proteins A and B are not.
- The protein may consist of two polypeptide chains linked together through disulfide bridges.
- Gly-Arg-Lys-Trp-Met-Tyr-Arg-Phe.
- Actually, there are four possible sequences: NIGIRVIA, GINIRVIA, VIRNIGIA, and, of course, VIRGINIA.
- Gly-Trp-Arg-Met-Tyr-Lys-Gly-Pro.
- $$\begin{array}{c} \text{Leu-Met-Cys-Val-Tyr-Arg-Cys-Gly-Pro.} \\ | \qquad \qquad \qquad | \\ \text{S} \text{-----} \text{S} \end{array}$$
- Alanine, attached to a solid-phase matrix via its  $\alpha$ -carboxyl group, is reacted with diisopropylcarbodiimide-activated lysine. Both the  $\alpha$ -amino and  $\epsilon$ -amino groups of the lysine must be blocked with 9-fluorenyl-methoxycarbonyl (Fmoc) groups. To add leucine to Lys-Ala to form a linear tripeptide, precautions must be taken to prevent the incoming Leu  $\alpha$ -carboxyl group from reacting inappropriately with the Lys  $\epsilon$ -amino group instead of the Lys  $\alpha$ -amino group.
- Bovine ribonuclease; *Neurospora crassa* NADPH-nitrite reductase
- The mass of the myoglobin chain is calculated to be 16,947  $\pm$  1 daltons.
- Unlike any amino acid side chain, the phosphate group (or more appropriately, the phosphoryl group) bears two equivalents of negative charge at physiological pH. Furthermore, replacing an H atom on an S, T, or Y side chain with a phosphoryl group introduces a very bulky substituent into the protein structure where none existed before.
- Compound Z the ligand with the strongest affinity for the protein.
- A graph of  $\nu$  versus [L] reveals that at  $\nu = 0.5$ , [L] =  $K_D = 2.4$  mM.
- Percent Saturation of Protein with Ligands A and B

Ligand concentration (mM)	Ligand A ( $K_D = 0.3$ mM)	Ligand B ( $K_D = 1$ mM)
0.1	0.25	0.091
0.2	0.4	0.167
0.5	0.625	0.33
1	0.77	0.5
2	0.87	0.67

More B is required to achieve the same degree of saturation as any given concentration of A.  $K_D$  and affinity are inversely related.

- IRS-1 has 1242 amino acids. Its average molecular mass is 131,590.97. The amino acid sequence of the tryptic peptide of IRS-1 of mass of 1741.9629 is LNSEAAVVLQLMNIR. The sequence of the tryptic fragment containing the SHPTP-2 site is LCGAAGGLENLNYIDLVLK.
- Nucleophilic attack by the hydroxyl O of the active-site serine on the carbonyl carbon of a peptide bond.

- Amino acid changes in mutant hemoglobins that appear on the surface of the folded globin chains may affect quaternary structure.
  - Amino acid substitutions on the surface on the quaternary hemoglobin structure that create hydrophobic patches might lead to polymerization. Such amino acids would include all of the hydrophobic amino acids.
- They refer to the sedimentation coefficients of these two subunits.
  - The ribosomal subunits are quaternary complexes of protein and rRNA. The larger the S value, the larger the rRNA is and the more protein molecules the ribosomal subunit has.

Think-Pair-Share Question:

Digestion order 4:

1. Chymotrypsin: QVDGL MRTSEQMKNSRV	Edman degradation : QM
2. Trypsin: QVDGL MR TSEQMK NSR V	Edman degradation : QMTNV
3. St. Protease: QVD GL MR TSEQMK NSR V	Edman degradation : QGMTNV

Digestion order 5:

1. St. Protease: QVD GLMR-TSE QMKNSRV	Edman degradation : QG
2. Trypsin: QVD GLMR TSE QMK NSR V	Edman degradation : QGTNV
3. Chymotrypsin: QVD GL MR TSEQMK NSR V	Edman degradation : QGMTNV

The order of endopeptidase treatment did not affect the final results.

## Chapter 6

- The central rod domain of keratin is composed of distorted  $\alpha$ -helices, with 3.6 residues per turn, but a pitch of 0.51 nm, compared with 0.54 nm for a true  $\alpha$ -helix.

$$(0.51 \text{ nm/turn})(312 \text{ residues})/(3.6 \text{ residues/turn}) = 44.2 \text{ nm} = 442 \text{ \AA}.$$

For an  $\alpha$ -helix, the length would be:

$$(0.54 \text{ nm/turn})(312 \text{ residues})/(3.6 \text{ residues/turn}) = 46.8 \text{ nm} = 468 \text{ \AA}.$$

The distance between residues is 0.347 nm for antiparallel  $\beta$ -sheets and 0.325 nm for parallel  $\beta$ -sheets. So 312 residues of antiparallel  $\beta$ -sheet amount to 1083  $\text{\AA}$  and 312 residues of parallel  $\beta$ -sheet amount to 1014  $\text{\AA}$ .
- The collagen helix has 3.3 residues per turn and 0.29 nm per residue, or 0.96 nm/turn. Then:

$$(4 \text{ in/year})(2.54 \text{ cm/in})(10^7 \text{ nm/cm})/(0.96 \text{ nm/turn}) = 1.06 \times 10^8 \text{ turns/year}.$$

$$(1.06 \times 10^8 \text{ turns/year})(1 \text{ year}/365 \text{ days})(1 \text{ day}/24 \text{ hours})(1 \text{ hour}/60 \text{ minutes}) = 201 \text{ turns/minute}.$$
- Asp:** The ionizable carboxyl can participate in ionic and hydrogen bonds. Hydrophobic and van der Waals interactions are negligible.

**Leu:** The leucine side chain does not participate in hydrogen bonds or ionic bonds, but it will participate in hydrophobic and van der Waals interactions.

**Tyr:** The phenolic hydroxyl of tyrosine, with a relatively high  $pK_a$ , will participate in ionic bonds only at high pH but can both donate and accept hydrogen bonds. Uncharged tyrosine is capable of