MOLECULAR CELL PHYSIOLOGY - Protein Structure and Function

I. Different types of proteins
- **A. Enzymes (most important), transport (hemoglobin), coordinated motion (actin & myosin), nerve impulse transmission, growth factors, replication (chromosomal proteins), structure (collagen), metabolic regulation (insulin)**

II. Proteins are made of amino acids: 19 amino acids and 1 imino (proline) acid
- **A. All (except glycine are optically active) and are found as the "L" form in nature**
- **B. They exist (at neutral pH) as Zwitterion – charged at both ends**
- **C. Linked by peptide bonds to form polypeptides**
- **D. Unique shape of proteins is derived from non-covalent interactions of amino acids (AA)**
- **E. Function is determined from 3-D structure which, in turn, is determined by AA sequence**

III. Protein structure
- **A. Primary: sequence of amino acids (in peptide bond carboxyl and amide group are trans)**
- **B. Secondary: stabilized by hydrogen bonds**
  1. **Random coils vary. "Alpha" and "beta" coils studied by Mirsky & Pauling (1936)**
  2. **In the helix (Pauling & Corey, 1951), functional groups project out and H-bonds are parallel to the axis of the helix. There is a "screw" sense of the helix demonstrating clockwise (right-handed, alpha) or counter-clockwise (left-handed, beta) spin. The repeat difference for each turn of the helix (pitch) is 5.4 Angstroms (Å) and the distance between adjacent alpha-carbons is 1.5 Å, so the number of residues is 3.6 per turn of the helix (Formula: p = d X n).**
  3. **In the beta-pleated sheet, which forms a zig-zag structure, the functional groups project out and the H-bonds hold the chains together. The repeat difference (pitch) is 7.0 Å and the distance between adjacent alpha-carbons is 3.5 Å, which means there are 2.0 residues per "zig-zag" of the sheet. There are also beta turns that enable the polypeptide to loop around.**

  HELIX stabilizers include glu, met, leu; destabilizers include val, ile, ser, asp, arg
  BETA-SHEET stabilizers include val, ile, phe; BETA TURNS include pro, gly, asn, and ser
  MOTIFS: include combinations & interactions like coiled coil, helix-loop-helix, zinc finger
- **C. Tertiary: overall structure of the folded polypeptide chain, stabilized by electrostatic interactions, H-bonding, and van der Waal forces. Includes distinct domains.**
- **D. Quaternary: number and position of two or more protein subunits (dimer, trimer, etc.)**
- **E. Macromolecular assemblies (protein coat of a virus), along with lipids, carbohydrates, and nucleic acids, form organelles**

IV. Functional groups of amino acids
- **A. Polar (charged) functional groups: on protein surface, interacting with water - these include amine, carboxyl, basic, amide, and hydroxyl**
  1. **Positively charged amine (NH_2): arginine and lysine**
  2. **Negatively charged carboxyl (COOH): apartic acid and glutamic acid**
  3. **Charge dependent upon pH: histidine**
  4. **Contain amide ($CONH_2$) group: asparagine and glutamine**
  5. **Contain hydroxyl (OH) group: serine and threonine**
- **B. Nonpolar (uncharged) functional groups: form water-insoluble protein core - these include aliphatic, aromatic, and special**
  1. **Aliphatic: alanine, valine, leucine, isoleucine, and methionine**
  2. **Aromatic: phenylalanine, tyrosine, and tryptophan**
  3. **Special: cysteine, glycine, proline**

V. Amino acid mass and abundance
- **A. The average AA MW is about 113 g/mol (derived from yeast 52,738 / 466 residues = 113.15). Note that the mass of proteins is reported in daltons (same as molecular weight)**
- **B. Rare AA: cysteine, tryptophan, methionine (5% of typical protein)**
- **C. Common AA: leucine, serine, lysine, glutamic acid (32% of typical protein)**
- **D. About 60% of polypeptide chains exist as alpha helices and beta sheets, whereas the remaining include random coils and turns**

MOLECULAR CELL PHYSIOLOGY - Protein Structure and Function (continued)

VI. Post-translational folding and protein modifications
- **A. There are 8^n possible combinations for protein folding, where "n" is the number of residues (based upon 8 polypeptide bond angles)**
- **B. *Native state* is the most stable form of the protein. Denaturization (with heat, urea, and mercaptoethanol unfolds the protein. But it usually returns back to the native state through renaturization with normal temperature and dialysing away the urea and mercaptoethanol.**
- **C. Chaperones**
  - **1. Molecular chaperones: bind and stabilize unfolded / partially-folded proteins**
  - **2. Chaperonins: directly facilitate protein folding**
- **D. Note that misfolding or re-folding by prions may lead to disease such as Alzheimer's**
- **E. Protein modifications**
  - **1. Chemical modification**
    - **a) Addition of an acetyl (CH_3CO) group to the amino group of N-terminus: occurs in 80% of all proteins and increases lifespan of protein**
    - **b) Phosphorylation of serine, threonine, and tyrosine residues**
    - **c) Glycosylation of asparagine, serine, and threonine**
  - **2. Processing**
    - **a) Removal of residues from N- or C-terminus by proteases**
    - **b) Protein self-splicing to remove inteins**
- **F. Protein degradation**
  - **1. Extracelluar: digestive proteases**
    - **a) Endopeptidases (trypsin and chymotrypsin) cleave near aromatic residues**
    - **b) Exopeptidases cleave from terminus (aminopeptidases and carboxypeptidases)**
    - **c) Peptidases split larger protein fragments into di- and tri-peptides**
  - **2. Intracellular: life span of enzymes vary and vulnerable to degradation**
    - **a) Lysosomes (not in plants): acidic pH**
    - **b) Ubiquinone-mediated pathway: addition of UQ to lysine chain which is then degraded by proteasome. N-terminus residues that favor degradation include Arg, Lys, Phe, Leu, and Trp, whereas those that resist degradation include Cys, Ala, Ser, Thr, Gly, Val, and Met**

VII. Functional design of proteins
- **A. Molecules bound by proteins are called ligands**
- **B. Protein interactions with ligands dependent upon affinity and specificity**
- **C. Good example of protein-ligand interaction is antibody-antigen. Antibodies are Y-shaped immunoglobulins with heavy- and light-chain polypeptides**
- **D. Another example of protein-ligand interactions: enzyme-substrate complexes**

VIII. Enzymes - catalyze metabolic events
- **A. Characteristics**
  - **1. High turnover number (make lots of product per unit enzyme): rate of reaction is often enhanced 10^6 to 10^{12} times that of the uncatalyzed reaction**
  - **2. Almost all enzymes are proteins (exception is RNA - ribozymes)**
  - **3. Catalysis occurs at the active site**
  - **4. Enzymes do not change equilibrium: E + S ===> E + P**
  - **5. Enzymes exhibit specificity: typical animal cell contains 1,000 to 4,000 different types of enzymes**
- **B. Structure - complex**
  - **1. Active site formed by AA side chains**
    - **a) Recognize and bind the substrate**
    - **b) Catalyze the reaction**
  - **2. Enzyme-substrate complex**
  - **3. Induced fit model includes conformational changes**

MOLECULAR CELL PHYSIOLOGY - Protein Structure and Function (continued)

VIII. Enzymes - catalyze metabolic events (continued)

C. Mechanism of catalysis - lower activation energy

$$E + S \underset{}{\overset{K_s}{\Longleftrightarrow}} ES \overset{k_3}{\Longrightarrow} E + P \qquad \text{velocity} = k_3[ES]$$

$$E_T = [E] + [ES] \qquad K_s = \frac{[E][S]}{[ES]} \text{ or, } \frac{[E]}{[ES]} = \frac{K_s}{[S]}$$

Thus, $\frac{E_T - [ES]}{[ES]} = \frac{K_s}{[S]}$

$E_T/[ES] - 1 = K_s/[S]$ let $[ES] = \text{velocity}/k_3$

so that $k_3E_T/\text{velocity} = 1 + K_s/[S]$ and $\text{velocity} = k_3E_T/1+K_s/[S]$

and if $\text{velocity}_{max} = k_3E_T$ when [S] is high, $\text{velocity} = V_{max}/1 + K_s/[S]$

which is the Michaelis-Menten Equation

K_s (also K_m) is the Michaelis constant, a measure of enzyme affinity for its substrate, and V_{max} is the maximum enzyme velocity

D. Interactions - regulations

1. pH and temperature
2. Prosthetic groups (Fe in hemogobin or even a coenzyme)
3. Allosteric regulatory sites and feedback inhibition
4. Phosphorylation / dephosphorlyation
5. Proteolytic activation: chymotrypsinogen to chymotrypsin
6. Compartmentalization
7. Cofactors such as FAD, NAD, NADP

IX. Membrane proteins - generally integral (intrinsic) and perpheral (extrinsic)

A. Protein domains on extracellular surface: cell-cell signalling and interactions

B. Protein domains within membrane: move molecules across the membrane

C. Protein domains on the inner cell membrane: anchor proteins / intracellular signalling

X. Protein purification, detection, and characterization (NOTE: there are typically about 10,000 different proteins in a cell)

A. Solublization

1. Water-soluble proteins retain native conformation in aqueous solution
2. Transmembrane proteins, containing hydrophobic groups, may precipitate from aqueous solutions
3. Detergents (amphipathic because they have hydrophilic and hydrophobic groups) can be used to suspend hydrophobic proteins. "Charged" (ionic) detergents include sodium deoxycholate and sodium dodecylsulfate (SDS) whereas nonionic detergents include Triton X-100 [polyoxyethylene(9,5)*p*-t-octyphenol]

B. Purification: size is the best separation criterion, although charge can be just as important

1. Centrifugation (rate of sedimentation)
   a) Proteins vary in mass but density is pretty consistent - about 1.37 g/cm^3, so mass may be more closely related to the sedimentation constant than density
   b) Centrifugation is often used to separate proteins and can also be used to measure physical properties (i.e. MW, shape, size, etc.)

$$MW^{2/3} = [(6NS\pi\eta)(3/(4\pi N))^{1/3}(V + \delta/p)^{1/3}]/[1-Vp]$$

where MW=molecular weight, S=sedimentation constant, η=viscosity of water at 20 °C, N=Avogadro's number, V=partial specific volume, δ=g water / g protein, p= density of water at 20 °C

EXAMPLE (alcohol dehydrogenase) $MW^{2/3} =$

$$[6(6.023 \times 10^{23})(7.6029 \times 10^{-13})(\pi)(0.01)x(3/(4\pi(6.023 \times 10^{23})))^{1/3} x (0.769 + 0.30/1)^{1/3}] / [1-.769(1)]$$

MW = 147,395 daltons

MOLECULAR CELL PHYSIOLOGY - Protein Structure and Function (continued)

XI. Protein purification, detection, and characterization (continued)

B. Purification (continued)

1. Centrifugation (continued)

c) Differential centrifugation: pellet organelles, etc, proteins in supernatant

d) Rate-zonal centrifugation: density gradient - "banding" of proteins

e) Equilibrium density-gradient centrifugation (isopycnic centrifugation): similar to rate-zonal, although a little more sophisticated with perhaps higher speeds and longer centrifugation time

2. Precipitation: can "salt out" the protein with ammonium sulfate $(NH_4)_2SO_4$ and then dialyze out the ammonium sulfate to obtain protein

3. Electrophoresis (rate of movement in electric field according to mass:charge ratio)

a) Polyacrylamide gel electrophoresis: size or polyacrylamide cross-linking influences migration of proteins. Addition of SDS (sodium dodecylsulfate) before electrophoresis denatures protein, dissociates subunits, and thus promotes movement according to (mostly) chain length.

b) Two-dimensional gel electrophoresis

1) Charge separation: add urea to denature protein, then load sample in glass tube filled with ampholytes (contains polyanions and polycations) which produce a charge (also a pH) gradient. Proteins separate according to their "neutral" pI (isoelectric focusing - IEF).

2) Mass separation: Extrude IEF gel and run on SDS-polyacrylamainde – using the "lanes" from IEF. Proteins now separate according to mass.

4. Chromatography [rate of movement through beads based on mass (gel filtration), charge (ion exchange), and ligand-binding (affinity columns)]

a) Liquid chromatography (mass, charge, affinity): column packed with beads and then liquid containing protein extract is forced through the column. Interaction with beads determines the speed at which proteins (in solution) move.

1) Gel filtration chromatography separates by size (polyacrylamide, dextran, agarose beads). Big proteins flow around, small proteins spend time in holes.

2) Ion-exchange chromatography separates by charge (carboxymethyl, diethylamino ethyl,). Proteins of a specified charge stick to column and are then "eluted" by adding salt in increasing concentrations.

3) Antibody-affinity chromotography separates according to affinity to a particular ligand (column packed with antibody or perhaps an enzyme substrate). Can be eluted by adding excess ligand.

C. Detection, identification, and quantification

1. Light-producing reaction or reactivity (may need enhancement)

a) Enzyme assay example: Use $\Delta A = \varepsilon b \Delta c$

Given that the extinction coefficient for NADH is 6223 $M^{-1}cm^{-1}$, calculate the MDH activity in units of μmol/mg protein/hour when 1.0 mL of 0.150 mg protein/mL extract is mixed with 2.0 mL of saturated substrate causes an increase in absorbance of 0.075 units in 10 minutes:

$0.075 = (6223)(1)(\Delta C)$, therefore $\Delta C = 1.2052 \times 10^{-5}$ M;

$(1.2052 \times 10^{-5}$ mol/L)[(1+2) x 10^{-3} L per assay](1 mL extract/0.150 mg protein) =

2.4×10^{-7} mol/mg protein;

2.4×10^{-7} mol/mg protein)(1/10 minutes)(60 minutes/hour)$(1 \times 10^{6}$ μmol/mol) =

1.44 μmol/mg protein/hour

MOLECULAR CELL PHYSIOLOGY - Protein Structure and Function (continued)

XI. Protein purification, detection, and characterization (continued)

D. Detection, identification, and quantification (continued)

2. Antibody interactions (particularly in Western blotting)

a) Run SDS gel and then transfer proteins onto a membrane

3. Autoradiography

a) Radioactive precursor is incubated with cells/crude extract

b) Extract is purified; fractions identified

c) Radioactivity of factions is measured - qualitative / quantitative

4. Edman degradation

a) Initiate with phenylisothiocyanate to cleave N-terminus

b) Hydrolyze to get PTH-reside derivative and identifiy

c) Repeat steps again as the polypeptide shortens

5. To cut up big proteins and then sequence, use:

a) CNBr: Met

b) Trypsin: Lys, Arg

c) Chymotrypsin: Phe, Tyr, Trp, Met

d) Pepsin: Phe, Tyr, Trp

6. X-ray crystallography, cryoelectron microscopy; NMR spectroscopy

a) X-ray crystallography: use diffraction pattern to obtain structure

b) Cryoelectron microscopy: low dose of electrons used to scan image

b) NMR: Spacial relation of certain atoms