Proteins are Classified by Function

Protein - a polymer of amino acids with biological activity

Enzymes - catalytic activity and functions
A -----> B

Transport Proteins - bind & carry molecules

Storage Proteins - ovalbumin, ferretin, casein

Contractile - can contract, change shape,
elements of cytoskeleton & muscles

Structural - support .... collagen of tendons,
elastin of ligaments, keratin of hair & feathers,
fibroin of silk & webs

Defensive - protect: antibodies (IgG),
fibrinogen & thrombin, snake venoms

Regulatory - regulate metabolic processes,
hormones, transcription factors & enhancers
Structure and Properties of Proteins

PROTEINS - polymer of amino acids w biological activity

STRUCTURE of Amino Acids
- alpha amino acid (20)
- aa's have a carboxyl group (-COOH) & amino group (-NH2)
- bound to an asymmetric carbon
- 20 ubiquitous aa's

ZWITTERION - 2 groups of opposite sign in same molecule
Isoelectric Point - pH where there is no net charge in molecule
pK - pH at which groups are 50% ionized & 50% non-ionized

R (side) groups... TYPES of Amino Acids
ACIDIC ... negatively charged - ASP & GLU
  R group with 2nd COOH that ionizes above pH 7.0
BASIC ... positively charged - LYS, ARG, HIS
  R group with 2nd amide that protonates below pH 7.0
POLAR UNCHARGED ... SER, THR, TYR, ASN, GLN
  are soluble in water, i.e., hydrophilic
NON-POLAR ... GLY, ALA, VAL, LEU, ILE, PRO
  all contain only hydrocarbons; R groups = hydrophobicity
AROMATIC (hydrophobic)... PHE, MET, TRP, CYS
  all contain R groups with ring structures
**peptide bond** - covalent link between carboxyl end of aa1 & amino end of aa2 forms a dipeptide shorten & stronger than C-C; longer & weaker than C=C no free rotation (attached group in same plane)

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Some examples of naturally occurring oligopeptides

**insulin** - 2 polypeptides  
alpha chain of 30 aa’s  
beta chain of 21 aa

**glucagon** - pancreatic hormone of 29 aa, opposes insulin action

**corticotropin** - 39 aa anterior pituitary hormone that stimulates adrenal cortex

**oxytocin** - 9 aa hormone pf posterior pituitary that stimulates uterine contractions

**bradykinin** - 1 aa hormone that inhibits inflammation

**thyrotropin relasing factor** - 3aa of hypothalmus that stimulates release of thyrotropin

**enkephalins** - CNS peptides that bind to brain cell receptors = analgesic reaction of pain deadening

**NutraSweet** - dipeptide of L-aspartyl-phenylalanyl (methyl ester)
Protein Fractionation Techniques

Isolation & Purification of a "new" protein....

Crude Homogenates - grind up cells in blenders & sonicators

Differential Centrifugation - subcell fractionation by centrifugal gravity... centrifuge speeds to 250,000xgravity [supernatant & pellet]

Fractionations – by Column Chromatography

gel filtration – by size in exclusion chromatography
sephadex - porous carbohydrate polymer beads

ion exchange – held by ion charge on column’s media

affinity chromatography - polymeric beads with special ligands (as substrate) to bind protein

gel electrophoresis ...porous gel by size & charge
SDS-electrophoresis (sodium-dodecyl-sulphate) bymass-mw

isoelectric focusing - migration to point of pI

2-demensional electrophoresis - isolectric focusing & SDS electrophoresis

Identification - colorimetric tests [Biuret & Bradford]
a protein is reacted with a colored dye, as Biuret or Coomassie blue; amount of color density is measured in a spectrophotometer; units are absorbance given at specific wavelengths (say 595nm) & absorbance is plotted vs. protein amount & results in a linear plot.
PROTEIN STRUCTURE

Variety of Amino Acid Sequences is infinite.....
Average prot = 300-400 aa's & MW 30,000 to 45,000

A protein of 100 amino acids made
w 20 different kinds aa's can have $20^{100}$ different
linear arrangements $[1.268 \times 10^{130}]$

Fred Sanger - 1958 Nobel prize for INSULIN sequence

to date 1,000 protein sequences are known in
computer data bases vs. e. coli makes about
3,000 proteins ; humans about 100,000 proteins

4 levels of protein structure are recognized

primary  linear sequence of amino acids
secondary regular, recurring orientation of aa’s
     in a peptide chain - $\alpha$ and $\beta$
tertiary_complete 3-D shape of a peptide
quaternary spatial relationships between
different polypeptides or subunits
PRIMARY SEQUENCE...
linear Sequence of Amino Acids... some consequences

Polymorphism...
proteins may vary in primary sequence, but
have the same biological function. ex: enzymes
\[ \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]
inter-specific: between species – diff. aa sequence
intra-specific: within a species (liver vs. kidney)

Invariants... primary sequences don't vary
ubiquitin & histones

Site Specificity...
some sequences determine intracell location
Signal Sequences, Prosthetic Binding sites, etc..

Homologous Proteins....
evolved in a related fashion
perform same cellular function in diff species
Hb & cyto-C ex: in duck & chickens = 2 variants
in yeast & horses = 48 variants

Example - LYSOZYME:
Functions as a bactericidal agent (breaks cell walls);
an enzyme found in egg whites & human tears;
MW = 14,600 & 129 aa's with 8 CYS residues [4 S-S]
hydrolyses polysaccharides in bacterial cell walls.
Secondary Level
alpha Helix - peptide backbone wound around a long axis; core forms a rigid helix cylinder
R-groups radiate out
3.6 aa per turn
right handed helix - (counterclockwise)
formed by H-bonds
[Hof N (of any aa) & -O=C (of 4th aa)]
¼ of aa's in globular proteins
Beta sheet - a linear extended ZIG-ZAG pleated sheet formed by H-bonds intra- & inter-chain

Tertiary Level
3D shape of a protein ...
often the most stable conformation involves weak forces:
H-bonds, hydrophilic & hydrophobic interactions & stronger bonds as: ionic bonds & disulfide bonds
Denaturation vs. Renaturation (loss biological activity)

Quarternary Level
shape between more than one polypeptide or subunit of a protein
hemoglobin, RNA polymerase, ASP-transcarbamylase
Nomenclature of Proteins

Two classes - Simple & Complex
Based on solubility of Proteins in Solvents... esp. Water

SIMPLE
1. Albumins - soluble in water; globular; many enzymes
2. Globulins - soluble in dilute aqueous solutions;
3. Prolamines - insoluble in water; soluble in 50% to 90% simple alcohols
4. Glutelins - insoluble in most solvents; soluble in dilute acids/bases
5. Protamines - not based upon solubility;
   low MW proteins w 80% Arg & no Cys
6. Histones - unique - complexed w DNA
   high [ basic aa's ] - 90% Arg, Lys, or His
7. Scleroproteins - insoluble in most solvents
   fibrous structure - cartilage & connective tissue
   Collagen = high Gly, Pro & no Cys
   when boiled makes gelatin
   Keratins - proteins of skin & hair -
   high basic aa's w Cys

COMPLEX
   lipoproteins... blood, membrane, & transport proteins
   glycoproteins... antibodies, cell surface proteins
   nucleoproteins... ribosomes & organelles
ENZYMEs - “in yeast”

1878 1st described on Pasteur's lab
1926 Sumner crystalizes 1sy enzyme enzymesUrease

regulate metabolic reaction rates: controls metabolism molecules (mostly protein) that accelerate or catalyze chemical reactions (A ---> B) in cells by breaking old covalent bonds and forming new covalent bonds. biological catalyst... but, differs from metal catalysts:

1. have complex, specific structure (sequence of aa's)
2. act only upon a specific substance (substrate)
3. do not change direction (energetics) of a reaction
4. function by lowering Energy of Activation $E_a$
catalyzes reactions by:
gains/loses e-; transfers group; breaks bond.
many require cofactor or coenzyme
cofactor - small inorganic ion that catalyzes reaction 
Cu+2; Mg+2; Mn+2, etc...
coenzyme - smaller, non-protein ligand which

Reaction path: $E + S$ <--- $ES$ <--- $E + P$

Active site - portion of enzyme protein that holds the substrate by means of weak chemical bonds (H-bonds, ionic bonds, hydrophobic forces, etc...) lock & key vs. induced fit.
ENZYME KINETICS

mathematical and/or graphical expression of the reaction rates of enzymes

Catalase \[ 2 \text{H}_2\text{O}_2 \longrightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]

Characteristic Enzyme Curves:

or how to determine if a reaction \( \text{A} \longrightarrow \text{B} \) is enzymatic

1. Rate \((0.8 \text{ ml O}_2/\text{min})\) Vs. \([\text{E}]\)
2. Rate (optimum) Vs. pH
3. Rate (optimum) Vs. Temperature
4. Rate (saturates) Vs. \([\text{S}]\)
A plot of rate (amount of product per unit time) vs [S] i.e., rate vs. substrate concentration saturates..... at [S] = Vmax (maximum velocity)

\( \text{Km} = \) substrate concentration at which rate is \( \frac{1}{2} \) of the maximal velocity (in above Km = 2 mg) is a measure of affinity of enzyme for its substrate i.e., amount of [S] needed to reach 1/2 Vmax

**Inhibition** - where action of inhibitor is ...reversible competitive... inhibitor binds to active site lower Km same Vmax noncompetitive... binds to allosteric site same Km, but lower Vmax
ENZYME NOMENCLATURE...

4 digit Number System [1.2.3.4.] Enzyme Commission #

1st Major Class of Activity
2nd Subclass (type of bond acted upon)
3rd Subclass (group acted upon, cofactor required, etc...)
4th Serial number ... sequence order

MAJOR CLASSES

1. **Oxidoreductases** [dehydrogenases] ..... catalyze oxidation-reduction rxns, often w coe NAD+/FAD
   Alcohol dehydrogenase  [EC 1.1.1.1]
   ethanol + NAD+ -------> acetaldehyde + NADH

2. **Transferases**... catalyze transfer of functional groups
   Hexokinase  [EC 2.7.1.2]
   D-glu + ATP -------> D-glu-6-P + ADP

3. **Hydrolyases**... catalyze hydrolysis –
   adds water across C-C bonds
   Carboxypeptidase A  [EC 3.4.17.1]
   [aa-aa]n + H2O -----> [aa-aa] n-1 + aa

4. **Lyases**.... add or remove groups to C= bonds
   Pyruvate decarboxylase  [EC 4.1.1.1]
   PYR -------> acetaldehyde + CO2

5. **Isomerases** [mutases] .... catalyze isomerizations
   Maleate isomerase  [EC5.2.1.1.] (cis-trans isomerization)
   maleate -------> fumarate

6. **Ligases**... condensation 2 substrates w splitting ATP
   Pyruvate carboxylase [EC 6.4.1.1.]
   PYR + CO2 + ATP -------> OAA + ADP + P