AMINO ACIDS

I. Function of amino acids

A. Building blocks of proteins

B. Amino acids may be functional (neurotransmitters)

- glutamate and aspartate (excitatory)
- glycine (inhibitory)

C. Precursors to other molecules

1. neurotransmitters (serotonin, dopamine, epinephrine,etc.)
2. thyroxine (thyroid hormone)
3. porphyrins
4. creatine (energy storage)
5. histamine (mediator of immune response)
6. nucleotide synthesis

II Structure of amino acids

A. 20 standard alpha-amino acids

1. **Structure.** All amino acids have alpha carbon bonded to:
   - alpha-hydrogen
   - alpha-amino group
   - alpha-carboxyl group
   - unique side chain (R group)
2. "standard" amino acids are encoded by messenger RNA
3. Amino acids are abbreviated by a 3-letter and 1-letter
4. All the 20 standard amino acids are optical active except glycine (no asymmetric carbon atom)
4. All the 20 standard amino acids are L-form. D- amino acids are never found in proteins (nomenclature from L and D-glyceraldehyde)

III Classification of amino acids:

a. according to the **chemistry** of the side chains:

- **Aliphatic** - glycine, alanine , valine, leucine , isoleucine,
- **Aromatic** - phenylalanine , tryptophan , tyrosine

  (absorb UV light with an absorbance maximum in the range of 280 nm)
- **Acidic** - aspartic acid, glutamic acid
- **Basic** – arginine, histidine ,lysine
- **Hydroxylic** - serine , threonine

- **Sulphur-containing** - cysteine , methionine
- **Amidic** - asparagine , glutamine
- **Imino** - proline

b. according to **polarity** of side chains:

- **nonpolar amino acids**
  Gly, Ala, Val, Leu, Ile, Met, Pro, Phe, Trp
  tend to orient to the inside of proteins
- **polar amino acids**
  Ser, Thr, Tyr, Asp, Glu, Asn, Gln, Cys,
  Arg, Lys, His
  tend to orient to the outside of proteins

IV. Modified amino acids

Some amino acids are not incorporated into proteins during translation

a. Hydroxyproline and hydroxylysine
   i. hydroxylated enzymatically after translation
   ii. important in collagen structure

b. phosphoamino acids
   i. Tyr, Ser and Thr
      hydroxyl groups can be
      phosphorylated
   ii. important in activation
      and inhibition of
      enzymatic activity

c. Cysteine can form disulfide bonds
   i. cysteine is the reduced form (sulfhydryl)
   ii. cystine is the oxidized form (disulfide)
   iii. disulfide bridges formed between cysteines
      areimportant in protein structure

d. gamma-Carboxyglutamate
   i. carboxylated enzymatically after translation
   ii. important in blood clotting

V. Acid –Base Properties of Amino Acids

$$\mathbf{R\text{-}COOH <\text{--------}> R\text{-}COO^- + H^+}$$

$$\mathbf{R\text{-}NH_3^+ <\text{---------}> R\text{-}NH_2 + H^+}$$

The equilibrium reactions, as written, demonstrate that amino acids contain at least two weakly acidic groups. However, the carboxyl group is a far stronger acid than the amino group. At physiological pH (around 7.4) the carboxyl group will be unprotonated (negative charge) and the amino group will be protonated (positive charge)

1. Charge
   a. Amino acids are dipolar ions (zwitterions) at neutral pH
      zwitterion is a dipolar molecule with positive and negative charges spatially separated

   b. Ionic states of amino acids depend on pH
      i. amino acids have two or three dissociable protons
      ii. pKa of the dissociable proton and the pH determine its degree of dissociation

$$pH = pKa + \log\{[A^-]/[HA]\} \quad \text{H-H equation}$$

2. Titration curve of an amino acid
   a. Titration curve of glycine
   b. Titration curve of histidine
   c. Isoelectric point (pI) - pH at which the molecule has a net charge = 0 (average of the two appropriate pKa values)

PROTEIN STRUCTURE AND PROTEIN FOLDING

I. Peptide bond

- amide bond between alpha-amino and alpha-carboxyl groups of 2 amino acids

A. Chemical properties

1. Peptide bond is
   a. electron resonance structure
   b. has partial (40%) double bond character
   c. amide group is planar, usually trans
2. Peptide bond is hydrolyzable
   a. acid hydrolysis generates free amino acids
      i. 6N Hydrochloric acid heated at 110øC for 24 hr in a vacuum
   b. cyanogen bromide cleaves at the COOH-terminal side of Met
   c. enzymatic hydrolysis of peptide bonds by proteases
      i. peptidases are specific for certain amino acids
3. Polypeptides are polyampholytes
   a. ampholyte has both acidic and basic pKa values
   b. isoelectric point - pH at which the net charge is zero

For example:

H3N+-Ala-Lys-Ala-Ala-COO-

pKa of the Alpha-carboxyl group = 3.6
pKa of the Alpha-amino group = 8.0
pKa of the delta-amino of the Lysine = 10.6

at pH = 1 the net charge is +2
at pH = 6 the net charge is +1
at pH = 14 the net charge is -1

the isoelectric point pI = (pKa2 + pKa3)/2 =(8 +10.6)/2 = 9.3

B. Nomenclature

1. Size
   a. dipeptide, tripeptide, etc.
   b. oligopeptide - several amino acids (up to 20)
   c. polypeptides (more than 20 amino acids)
      - all proteins are polypeptides

II. Levels of Protein Structure

A. Primary Structure - amino acid sequence of a polypeptide

1. primary structure determines 3-dimensional structure (Anfinsen)
2. always represented NH2-terminus to COOH-terminus

B. Secondary structure - regular local conformation of linear segments of the polypeptide chain

1. Secondary structure are stabilized by hydrogen bonds between amide and carbonyl groups
2. Several types of secondary structure
   a. alpha-helix
      1. right handed helix
      2. 3.6 amino acids per turn
      3. carbonyl oxygen hydrogen bonded to 4th amide hydrogen
      4. amino acid R-groups orient out
      5. proline breaks the helix
   b. beta-pleated sheet
      i. polypeptide chains side by side
      ii. polypeptide chains can be parallel or antiparallel
      iii. carbonyl oxygen hydrogen bonded to amide hydrogen
      iv. beta-strand is a single pass of the polypeptide
   c. reverse turn, beta-bend
      i. allows a sharp turn in polypeptide chain
      ii. carbonyl oxygen hydrogen bonded to 4th amide hydrogen
      iii. Glycine is required

C. Tertiary structure - overall folded conformation of the polypeptide

1. Physical forces affect tertiary structure
   a. Hydrophobic forces
      i. hydrophobic residues orient to inside
      ii. hydrophilic orient out
   b. salt bridges, electrostatic forces
   c. Van der Waals radii
   d. Hydrogen bonds
   e. Disulfide bridges

D. Quaternary structure - subunit structure

1. aggregation of 2 or more subunits
   a. hetero- or homo- polymers
2. same forces drive tertiary and quaternary structure

III. Protein folding

A. Folding occurs step-wise with several intermediates

B. Folding is driven by hydrophobic forces

C. Proteins can self assemble but in vivo folding is facilitated by proteins (Chaperones)

D. Denaturation is unfolding

1. Requires some input to overcome hydrophobic forces
   a. heat
   b. denaturant (urea or guanidinium)
2. Requires reductant to reduce disulfide bridges to sulfhydryls

IV. Analytical Techniques in Protein Biochemistry

A. Determination of Amino Acid Composition

1. Amino acid analysis provides % of each amino acid in protein
   a. Hydrolysis of polypeptide with 6N HCl
   b. Derivitization of amino acids with dansyl chloride PITC, or 0-phthalaldehyde (OPA)
   c. Liquid chromatographic separation of the tagged amino acids
   d. Quantitation
2. Composition of a protein can be used to identify a protein

B. Determination of primary sequence of a polypeptide

1. Preparation of peptides for sequencing
   a. Removal of disulfide bridges
      i. reducing agent (-mercaptoethanol or dithiothreitol)
      ii. derivatize sulfhydryls to block disulfides from reoxidizing
   b. Digestion with cyanogen bromide
      i. CNBr cleaves at the carboxyl side of methionine residues
   c. Digestion with proteolytic enzymes
      i. use at least two different enzymes
      ii. overlapping enzymes allows determination of peptide sequence
   d. Separation of peptides
      i. peptides separated by chromatography
      ii. based on differences in ionic, polar, and/or hydrophobic characteristics
2. **Edman degradation** is used to sequentially determine a.a. sequence
   a. PITC reacts with the N-terminal amino acid

b. Strong acid cleaves the peptide bond between the 1st and 2nd amino acids
c. Product is a PTH derivative of amino acid #1
d. Determine identity of amino acid-PTH using HPLC chromatography
e. Repeat steps a-d

Note: Edman degradation has limited success with very long polypeptides

C. Determination of Molecular Mass

1. Gel Filtration (Molecular exclusion chromatography
   a. protein is loaded on a column of porous beads
   b. small molecules can enter the beads, large ones cannot
   c. an aqueous buffer moves the protein through the beads
2. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)
   a. protein is unfolded and coated with sodium dodecyl sulfate(SDS) detergent
   b. proteins are loaded on an acrylamide gel matrix
   c. electricity moves the proteins through the matrix
   d. low molecular weight proteins move faster (farther)
   e. large molecules migrate faster because they bypass beads

D. X-ray Crystallography and NMR

1. Physical techniques to identify 3-D structure of a pure protein
2. Requires tremendous time, effort, and analytical resources