Introduction to the Study of Proteins

Proteins - the most plentiful organic compounds in the body, making up more than half its dry weight.

Proteins can be categorized by function.

Catalytic proteins, or enzymes:
Catalyze the synthesis and utilization of proteins, carbohydrates, lipids, nucleic acids, and almost all other biomolecules.

Transport proteins:
Bind and carry specific molecules from place to place.

Structural proteins:
Give physical shape, and the strength to maintain it, to structures in animals.

Regulatory proteins:
Control cellular activity.

Protective proteins:
Defend against invaders and prevent or minimize damage after injury.

Contractile proteins:
Provide cells and organisms with the ability to change shape and move.

Storage proteins:
Provide a reservoir of nitrogen and other nutrients, especially when external sources are low or absent.
Proteins are Made of Amino Acids

Proteins \textit{Hydrolysis} \rightarrow \text{Amino Acids}

Mammals require all 20 amino acids for protein synthesis but can synthesize only 10 of them.

There are 10 \textbf{essential amino acids} that must be obtained from the diet.

Phenylalanine, Valine, Tryptophan, Threonine, Isoleucine, Methionine, Histidine, Arginine, Lysine, Leucine
Amino Acids

An α-amino acid

An L-amino acid
The amino acids are categorized chemically by the behavior of their R-groups:

Neutral side chains
- Aliphatic
- Polar
- Aromatic

Basic - Positively charged

Acidic - Negatively charged
Amino Acids with Nonpolar, Uncharged Side Chains

Glycine (Gly)  Alanine ( Ala)  Valine ( Val)  Leucine ( Leu)  Isoleucine ( Ile)  
Methionine ( Met)  Phenylalanine ( Phe)  Tryptophan ( Trp)  Proline ( Pro)
Amino Acids with Polar, Uncharged Side Chains

Serine (Ser)  Threonine (Thr)  Cysteine (Cys)
Tyrosine (Tyr)  Asparagine (Asn)  Glutamine (Gln)
Amino Acids with Polar, Charged Side Chains

- Aspartic acid (Asp)
- Glutamic acid (Glu)
- Arginine (Arg)
- Lysine (Lys)
- Histidine (His)
α-Amino Acids May Exist in a Zwitterionic Form

The actual structure of a neutral amino acid in neutral solution and in the solid is a charged molecule, containing a $-\text{COO}^-$ group and an $-\text{NH}_3^+$ group, called a zwitterion. The net charge on the molecule is zero.

The presence of the charged groups in the zwitterion result in very strong secondary forces between the positive and negative charges:

\[
\begin{align*}
\text{Uncharged (Nonexistent)} & & \rightarrow & & \text{Zwitterion (Exists in both solid and solution)} \\
\text{MP} = 314°C & & \text{MP} = 53°C
\end{align*}
\]
Amino Acids in Solution

1) Amino acids are also soluble in water due to the strong secondary forces between the zwitterionic charge centers of the amino acids and the dipolar water molecules.

2) Free amino acids exist exclusively in the zwitterionic form in the solid state.

3) In solution, amino acids assume a charged form based on the pH of the solution.
How do carboxylic acids and amines behave in acidic, neutral, and basic solutions?

\[ \text{R--C--OH} \quad \text{R--C--O} \quad \text{R--C--O} \]

pH = 1  \quad \text{pH} = 7  \quad \text{pH} = 12

\[ \text{R--NH}_3 \quad \text{R--NH}_3 \quad \text{R--NH}_2 \]

The behavior of the carboxylic acid and amine functional groups DOES NOT CHANGE when they are in the same molecule.
How does the amino acid alanine behave in acidic, basic, and neutral solutions?

At low pH, the functional groups have accepted all of the protons possible, and at high pH, the functional groups have donated all of the protons possible.
1) Each amino acid has a pH at which almost all of the molecules are present in a neutral form (0 net charge), called the **isolectric point**.

2) At a **pH below that of the isoelectric point**, the cation concentration increases (**net positive charge**).

3) At a **pH above that of the isoelectric point**, the anion concentration increases (**net negative charge**).

4) Most neutral amino acids have isoelectric points in the range 5 - 6.

5) “Neutral” amino acids include 15 of the 20 common amino acids.
Compounds with the ability to both release protons (acids) and absorb protons (bases) are called amphoteric compounds*. Amino acids are least soluble in their zwitterionic form, although even this form is fairly soluble.

*Is there a common chemical application for such compounds??
Amino Acids in Solution

How does the amino acid **aspartic acid** behave?

![Diagram of aspartic acid at different pH values]

- **pH = 1**: Protonated at both carboxyl and amino groups.
- **pH = 3**: Deprotonated carboxyl group.
- **pH = 7**: Protonated carboxyl group and deprotonated amino group.
- **pH 12**: Deprotonated both carboxyl and amino groups.
Amino Acids with Polar, Charged Side Chains

- Aspartic acid (Asp)
- Glutamic acid (Glu)
- Arginine (Arg)
- Lysine (Lys)
- Histidine (His)
How does the amino acid **lysine** acid behave?

At different pH levels:

- **pH = 1**
- **pH = 7**
- **pH = 10**
- **pH = 12**
Amino Acids with Polar, Charged Side Chains

Aspartic acid (Asp)

Glutamic acid (Glu)

Lysine (Lys)

Arginine (Arg)

Histidine (His)
Electrophoresis of Amino Acids

Electrophoresis can be used to analyze mixtures of α-amino acids.

In this procedure, a strong electric field causes anions (acidic amino acids) to move towards the anode (positive electrode) and cations (basic amino acids) to move towards the cathode (negative electrode).

Amino acids whose isoelectric pH is close to the pH of the solution remain stationary in the electric field.

A form of electrophoresis called paper electrophoresis is often used for this analysis:
Electrophoresis of Amino Acids

Beginning

- cathode
- anode
- wet with pH 6 buffer solution

End

- cathode
- anode
- Asp$^-$ moves toward the positive charge
- Ala does not move
- Lys$^+$ moves toward the negative charge

streak containing Ala, Lys, Asp
Peptides
Peptides

How are amino acids joined together in proteins?

A peptide bond may join two amino acids \[ \rightarrow \text{dlpeptide} \]

The two amino acids are joined through a dehydration reaction.

- Alanine
- Valine

Alanylvaline (Ala-Val)

Peptide bond
Peptides

The two amino acids are joined through a dehydration reaction.

OH and H combine to form water.

Amide bond (peptide linkage)

Glycine  Alanine  Gly–Ala dipeptide + H₂O
More than two amino acids may be joined.

Phe-Ser-Asp-Ala-Gln

Polypeptides - greater than 10 amino acids

Proteins - more than 50 amino acids; >10,000 molecular weight
The peptide bond is usually drawn as a single bond, but actually has considerable double bond character which prevents free rotation about the bond. The atoms of the double bond, and those directly attached to it, all lie in the same plane.
The trans-planar nature of the peptide bond accounts for the very high melting and boiling points and a lack of basicity in the simple amides, and plays an important role in determining three-dimensional structure and function in polypeptides.
1) Different **constitutional isomers** are possible whenever amino acids react to form peptides. (\(\text{Ala-Val} \neq \text{Val-Ala}\))

2) All peptides have one free \(\alpha\)-amino group (**N-terminal** or **amino-terminal residue**), and one free \(\alpha\)-carboxyl group (**C-terminal** or **carboxyl-terminal residue**).

3) When drawing (or naming) peptides the standard convention is to place the **N-terminal residue** at the left and the **C-terminal residue** to the right.
Drawing a Peptide

Dipeptide formation

\[ \text{condensation reaction} \quad \text{removal of water} \]

\[ \text{bond forms here} \]

\[ \text{water} \]

\[ \text{peptide bond} \]
Ionization of Peptides

1) As in the case of amino acids, each peptide has an **isoelectric pH (or “pI”)** at which it does not migrate in an electric field.

2) The pI value of a peptide containing only neutral amino acid residues or equal numbers of acidic and basic residues or both is in the range of pI values for neutral amino acids (pH 5.05–6.30).

3) The pI of a peptide containing acidic and basic amino acid residues is
   a) on the acidic side (lower than 5.05–6.30) if there is an excess of acidic residues, and
   b) on the basic side (higher than 5.05–6.30) if there is an excess of basic residues.

4) All amino and carboxyl groups, including those on side groups of acidic and basic amino acid residues, are charged at physiological pH.
Physical Properties of Peptides

1) Peptides have **solubility and electrophoresis properties** that are pH dependent.

2) **Peptide solubility** is lowest at its isoelectric point.

3) At a given pH, each peptide has a particular electrical charge depending upon its isoelectric point and the number of ionizable groups it contains.

4) **Electrophoresis can be used** to separate peptides of differing charges.

5) Peptides are often distinguished from proteins by the **number of amino acid residues**. Molecules having fewer than 50 amino acid residues are generally called peptides, regardless of physiological activity.
Chemical Properties of Peptides

1) Hydrolysis to amino acids

2) The amino acid cysteine contains a sulfhydryl group, -SH.
   a) Pairs of cysteine residues often link two peptide chains or two parts of one peptide chain through disulfide bridges:
   b) Disulfide bridges in peptides may be represented using the 3 letter amino acid abbreviations.
   c) Disulfide bridges generally survive hydrolysis reactions.
Examples of Physiologically Active Peptides
Aspartame - 2 amino acids
An Artificial Sweetener

L-aspartyl-L-phenylalanine methyl ester
Aspartate  Phenylalanine  Methanol
Met enkephalin - 5 amino acids
Reduces Pain Sensation
Bradykinin - 9 amino acids
Powerful Vasodilator-Released by mast cells after injuries and during allergic responses. Similar to histamine in actions
Oxytocin

9 amino acids; 1 disulfide linkage

Causes contraction of uterine muscles during labor.
**Insulin**

51 amino acids ; 3 disulfide linkages

Regulator of carbohydrate metabolism and absorption
**Insulin**

51 amino acids; 3 disulfide linkages

Regulator of carbohydrate metabolism and absorption

*Structure of insulin*

![Insulin Structure Diagram](image-url)
Insulin Maturation

Preproinsulin

Proinsulin

Insulin
Insulin Gene Mutations

SIGNAL PEPTIDE

B CHAIN

A CHAIN

C-PEPTIDE