Chemical and Enzymatic Hydrolysis of Proteins

Introduction: (By-Dr.Manju kumari)

Proteins are polymers of amino acids linked by **peptide bonds**.
Hydrolysis of proteins involves cleavage of these peptide bonds, producing smaller peptides or free amino acids. This can occur by **chemical methods** or **enzymatic methods**.

1. Chemical Hydrolysis of Proteins

Chemical hydrolysis involves breaking peptide bonds using **strong acids or bases** under high temperature and pressure. It is mainly used for **analytical purposes**.

(a) Acid Hydrolysis

- Most common method of chemical hydrolysis.
- Usually carried out using **6 N hydrochloric acid (HCl)**.
- Conditions: **110°C for 18–24 hours** in sealed tubes.
- Peptide bonds are completely cleaved, yielding free amino acids.

Important features:

- Almost all peptide bonds are hydrolyzed.
- **Tryptophan is completely destroyed**.
- **Asparagine → Aspartic acid**
- **Glutamine → Glutamic acid**
- Partial destruction of serine, threonine, and cysteine may occur.

Reaction (general):
Peptide + H_2O → Amino acids (in acidic medium)

Uses:

- Determination of **amino acid composition**
- Protein structure analysis

(b) Alkaline Hydrolysis

- Carried out using **strong bases** like NaOH or KOH.
- Requires high temperature.
- Less commonly used than acid hydrolysis.

Important features:

- **Tryptophan can be estimated** (not destroyed as in acid hydrolysis).
- Some amino acids such as **serine, cysteine, and threonine** are degraded.
- Racemization of amino acids may occur.

Advantages of Chemical Hydrolysis

- Complete breakdown of proteins
- Useful for quantitative amino acid analysis

Limitations of Chemical Hydrolysis

- Harsh conditions
- Destruction of certain amino acids
- Non-specific cleavage
- Not suitable for biological systems

2. Enzymatic Hydrolysis of Proteins

Enzymatic hydrolysis is carried out by **proteolytic enzymes (proteases)** under mild physiological conditions. It is **highly specific and controlled**.

Proteolytic Enzymes Involved

- **Pepsin**
- Works in acidic medium (stomach)
- Breaks peptide bonds near aromatic amino acids

- **Trypsin**
- Acts in alkaline medium (intestine)
- Cleaves peptide bonds at carboxyl side of lysine and arginine
- **Chymotrypsin**
- Acts on peptide bonds adjacent to aromatic amino acids
- **Papain**
- Plant protease with broad specificity

Mechanism of Enzymatic Hydrolysis

1. Formation of **enzyme–substrate complex**
2. Specific binding at the active site
3. Cleavage of peptide bond
4. Release of peptides or amino acids

Enzymes lower the **activation energy**, making the reaction fast and specific.

Factors Affecting Enzymatic Hydrolysis

- **pH** (optimum pH is enzyme-specific)
- **Temperature**
- **Enzyme concentration**
- **Substrate concentration**
- **Presence of inhibitors or activators**

Advantages of Enzymatic Hydrolysis

- Highly specific
- Mild conditions (physiological pH and temperature)
- No destruction of amino acids
- Biologically relevant

Limitations of Enzymatic Hydrolysis

- Incomplete hydrolysis
- Slower than chemical methods
- Enzymes may be expensive
- Sensitive to pH and temperature changes

3. Comparison (Summary) :

Chemical Hydrolysis	Enzymatic hydrolysis
Harsh conditions	Mild conditions
Non-specific	Highly specific
Amino acid destruction possible	Amino acids preserved
Used for analysis	Used in digestion and metabolism

Conclusion

Chemical hydrolysis is mainly used for **laboratory analysis of proteins**, while enzymatic hydrolysis is essential for **biological digestion and metabolism**. Both methods are important and complementary in protein chemistry.