

## SCOPE AND APPLICATIONS OF PROTEIN CHEMISTRY: A REVIEW.

Shashikant R Pattan<sup>1</sup>, Nachiket S Dighe<sup>1</sup>, Manisha Kedar<sup>1</sup>, Manjusha Sanap<sup>1</sup>, Jayshari S Pattan<sup>2</sup>  
Sunil A Nirmal<sup>3</sup>, Mahesh Bhanushali<sup>4</sup>

<sup>1</sup>Department Of Medicinal Chemistry, Pravara Rural College Of Pharmacy, Pravaranagar, M S India

<sup>2</sup>Department Of Biotechnology, P V P science college, Loni,, M S, India

<sup>3</sup>Department Of Pharmacognosy, Pravara Rural College Of Pharmacy, Pravaranagar, M S India

<sup>4</sup>Department Of Medicinal Chemistry, M M College Of Pharmacy, Belgaum, Karnataka, India

### Summary

Proteins are very essential for the structural and functional growth of the body. They are the polymer of amino acid linked by covalent bonds. Proteins and peptides are widely used as pharmaceutical agents in antiviral agents, antibiotics, as prodrug and even antimicrobial agent. Proteins are of high value to increase the efficacy of immunomodulating activity. The present review is aimed to collect all the information's of proteins its scope and development.

**Keywords:** Antimicrobial Peptides, Glycoprotein, Fibrous Proteins, Plasma Membrane Proteins.

#### Address for correspondence :

Dr. Shashikant R. Pattan

Principal

Pravara Rural College of Pharmacy, Pravaranagar

A/P- Loni Bk.

Tal-Rahata, Dist-Ahmednagar -413736.

MS India.

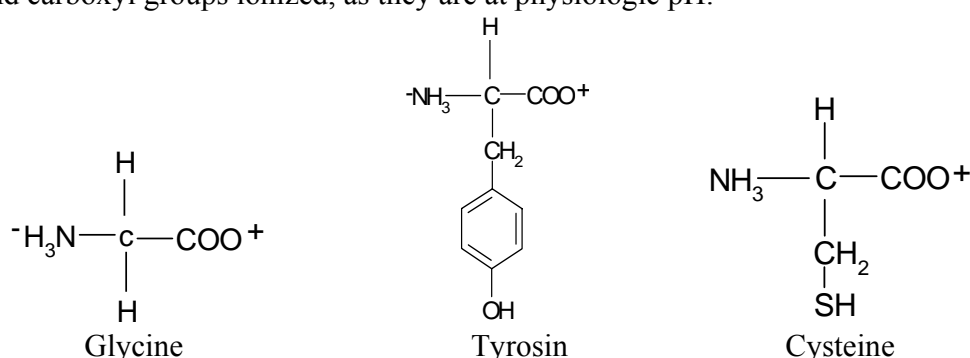
E-mail: shashipattan@yahoo.com

### Introduction

Protein chemistry is the Area of science related to Obtaining, purifying protein, Investigating protein structure & function, and Controlling and engineering proteins. Proteins are polymers of amino acids covalently linked through peptide bonds into a chain. Within and outside of cells, proteins serve a myriad of functions, including structural roles (cytoskeleton), as catalysts (enzymes), transporter to ferry ions and molecules across membranes, and hormones to name just a few.

1. Fibrous Proteins 2. Globular Proteins

As the name implies, Fibrous Proteins have fibre-like structures, they are relatively insoluble in water and unaffected by moderate changes in temperature and pH. Subgroups are-Collagens & Elastins, the proteins of connective tissues. Tendons and ligaments. Keratins, proteins that are major components of skin, hair, feathers and horn. Fibrin, a protein formed when blood clots. Globular Proteins include hormones, antibodies and enzymes and either dissolve or form colloidal suspensions in water. Such proteins are generally more sensitive to temperature and pH change than their fibrous counterparts. Proteins are polymers of amino acids joined together by peptide bonds. There are 20 different amino acids that make up essentially all proteins on earth. Each of these amino acids has a fundamental design composed of a central carbon (also called the alpha carbon) bonded to; hydrogen, a carboxyl group, an amino group, a unique side chain or R-group. Thus, the characteristic that distinguishes one amino acid from another is its unique side chain, and it is the side chain that dictates an amino acid's chemical properties. Examples of three amino acids are shown below, Note that the amino acids are shown with the amino and carboxyl groups ionized, as they are at physiologic pH.

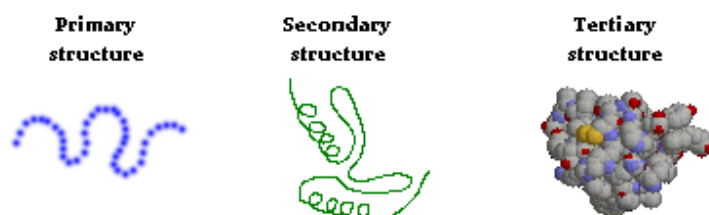


Except for glycine, which has hydrogen as its R-group, there is asymmetry about the alpha carbon in all amino acids. Because of this, all amino acids except glycine can exist in either of two mirror-image forms. The two forms - called stereoisomers - are referred to as D and L amino acids. With rare exceptions, all of the amino acids in proteins are L amino acids.<sup>1</sup>

### Structure of Proteins:

**Structural features of proteins are usually described at four levels of complexity:**

- **Primary structure:** the linear arrangement of amino acids in a protein and the location of covalent linkages such as disulfide bonds between amino acids.
- **Secondary structure:** areas of folding or coiling within a protein; examples include alpha helices and pleated sheets, which are stabilized by hydrogen bonding.
- **Tertiary structure:** the final three-dimensional structure of a protein, which results from a large number of non-covalent interactions between amino acids.
- **Quaternary structure:** non-covalent interactions that bind multiple polypeptides into a single, larger protein. Hemoglobin has quaternary structure due to association of two alpha globin and two beta globin polypeptides.



**Figure no. -1 Structure of proteins**

The primary structure of a protein can readily be deduced from the nucleotide sequence of the corresponding messenger RNA. Based on primary structure, many features of secondary structure can be predicted with the aid of computer programs. However, predicting protein tertiary structure remains a very tough problem, although some progress has been made in this important area.<sup>2,3</sup>

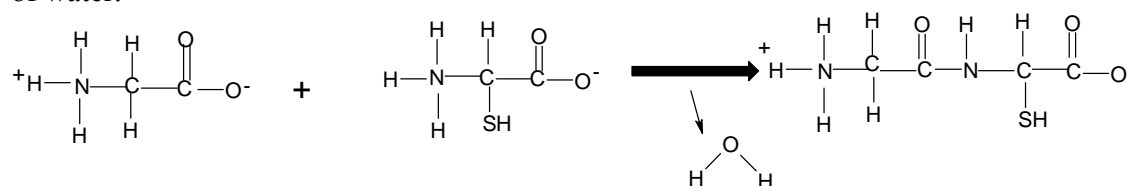
### Importance of proteins in diet:

For most of the 20th century, scientists have believed that protein needs are not altered by physical exercise. In contrast, athletes are typically convinced that additional dietary protein can significantly enhance exercise performance. Until recently, the opinion of the athletes has been largely unsubstantiated in the scientific literature. However, since the 1970s, an increasing number of studies have appeared that indicate dietary protein needs are elevated in individuals who are regularly physically active. Together, these data suggest that the RDA for those who engage in regular endurance exercise should be about 1.2-1.4 g protein/kg body mass/d (150-175% of the current RDA) and 1.7-1.8 g protein/kg body mass/d (212-225% of the current RDA) for strength exercisers. Fortunately, the typical North American diet contains protein near these quantities, so most individuals who decide to begin an exercise program will obtain sufficient protein as long as their diet is mixed and they are careful to consume adequate energy.

Populations at greatest risk for consuming insufficient protein include any group that restricts energy intake (those on diets) or high quality protein sources (vegetarians) as well as any group that has a requirement higher than normal due to another existing condition (growing individuals). Future studies should focus on these groups. Moreover, few exercise performance measures have been made, so any negative effect of insufficient dietary protein on athletic success needs to be determined. Supplementation of several individual amino acids may be beneficial for physically active individuals, but considerable potential risk is also present. Intake of large quantities of individual amino acids is not recommended until much more information is available.<sup>4</sup>

### Peptides and Proteins:

Amino acids are covalently bonded together in chains by peptide bonds. If the chain length is short (say less than 30 amino acids) it is called a peptide; longer chains are called polypeptides or proteins. Peptide bonds are formed between the carboxyl group of one amino acid and the amino group of the next amino acid. Peptide bond formation occurs in a condensation reaction involving loss of a molecule of water.

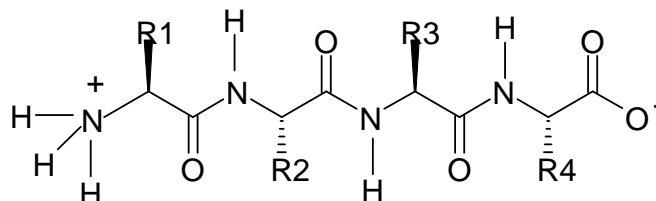


The head-to-tail arrangement of amino acids in a protein means that there is a amino group on one end (called the amino-terminus or N-terminus) and a carboxyl group on the other end (carboxyl-terminus or C-terminus). The carboxy-terminal amino acid corresponds to the last one added to the chain during translation of the messenger RNA.

### The Peptide Bond:

A peptide bond (amide bond) is a chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amine group of the other molecule, thereby releasing a molecule of water (H<sub>2</sub>O). This is a dehydration synthesis reaction (also known as a condensation reaction), and usually occurs between amino acids.

The resulting CO-NH bond is called a peptide bond, and the resulting molecule is an amide. The four-atom functional group -C(=O)NH- is called an amide group or (in the context of proteins) a peptide group. Polyamides, such as nylons and aramids, are synthetic molecules (polymers) that possess peptide bonds.<sup>5</sup>



### The Primary Structure of Peptides:

Because the N-terminus of a peptide chain is distinct from the C-terminus, a small peptide composed of different amino acids may have several constitutional isomers. For example, a dipeptide made from two different amino acids may have two different structures. Thus, aspartic acid (Asp) and phenylalanine (Phe) may be combined to make Asp-Phe or Phe-Asp, remember that the amino acid on the left is the N-terminus. The methyl ester of the first dipeptide (structure on the right) is the artificial sweetener aspartame, which is nearly 200 times sweeter than sucrose. Neither of the component amino acids is sweet (Phe is actually bitter), and derivatives of the other dipeptide (Phe-Asp) are not sweet. A tripeptide composed of three different amino acids can be made in 6 different constitutions, and the tetrapeptide composed of four different amino acids would have 24 constitutional isomers. When all twenty of the natural amino acids are possible components of a peptide, the possible combinations are enormous. Simple statistical probability indicates that the decapeptides made up from all possible combinations of these amino acids would total  $20^{10}$ . Natural peptides of varying complexity are abundant. The simple and widely distributed tripeptide glutathione (first entry in the following table), is interesting because the side-chain carboxyl function of the N-terminal glutamic acid is used for the peptide bond. An N-terminal glutamic acid may also close to a lactam ring, as in the case of TRH (second entry). The abbreviation for this transformed unit is pGlu (or pE), where p stands for "pyro" (such ring closures often occur on heating). The larger peptides in the table also demonstrate the importance of amino acid abbreviations, since a full structural formula for a nonapeptide (or larger) would prove to be complex and unwieldy. The ten peptides listed in this table make use of all twenty common amino acids. Note that the C-terminal unit has the form of an amide in some cases (e.g. TRH, angiotensin & oxytocin). When two or more cysteines are present in a peptide chain, they are often joined by disulfide bonds (e.g. oxytocin & endothelin); and in the case of insulin, two separate peptide chains (A & B) are held together by such links.<sup>5</sup>

### Some Common Natural Peptides:

| Name (residues) | Source or Function                           | Amino Acid Sequence  |
|-----------------|--|--|
| Glutathione (3) | Most Living Cells (stimulates tissue growth) | $^{(+)}\text{H}_3\text{NCH}(\text{CO}_2^{-})\text{CH}_2\text{CH}_2\text{CONHCH}(\text{CH}_2\text{SH})\text{CONHCH}_2\text{CO}_2\text{H}$<br>$\gamma\text{-Glu-Cys-Gly}$ (or $\gamma\text{ECG}$ ) |

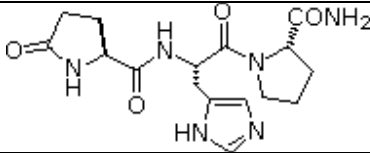
|                    |  |  |
|--------------------|--|--|
| TRH (3)            | Hypothalamic Neurohormone (governs release of thyrotropin) |  <p>pGlu-His-ProNH<sub>2</sub><br/>(or pEHPNH<sub>2</sub>)</p>                                     |
| Angiotensin II (8) | Pressor Agent (acts on the adrenal gland)                  | Asp-Arg-Val-Tyr-Ile-His-Pro-PheNH <sub>2</sub> (or DRVYIHPFNH <sub>2</sub> )   |
| Bradykinen (9)     | Hypotensive Vasodilator (acts on smooth muscle)            | Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (or RPPGFSPFR)   |
| Oxytocin (9)       | Uterus-Contracting Hormone (also stimulates lactation)     | <p>Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-GlyNH<sub>2</sub> (or CYIQNCPLGNH<sub>2</sub>)</p> <p>disulfide bonding</p>   |
| Somatostatin (14)  | Inhibits Growth Hormone Release (used to treat ulcers)     | <p>Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys (or AGCKNFFWKFTFT)</p> <p>disulfide bonding</p>   |
| Melittin (26)      | Honey Bee Venom (used to treat rheumatism)                 | <p>Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-<br/>~Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-GlnNH<sub>2</sub><br/>(or GIGAVLKVLTTGLPALISWIKRKRQQNH<sub>2</sub>)</p> |

Table no.1: Common Natural Peptides

### Applications of Peptide:

#### 1) As antiviral agents:

Peptides as a inhibitor of HSV replication.e.g. Meliacine. Naturally occurring cyclic peptide from the leaves of Melia Azedarach. Inhibits the HSV replication it is also used in Herpetic Stromal Keratitis.<sup>6</sup>

#### 2) As antibiotics:

i) Bacitracin: It is cultivated from culture broth of bacillus licheniformis. It is dodecapeptide. It inhibits cell wall formation in gram-positive bacteria by forming complex with polyisoprenyl pyrophosphate.

ii) Thiostrepton: It is cultivated from culture broth of S.azureus. It consists of highly aromatic moieties. It inhibits gram-positive bacteria by inhibiting ribosomal protein synthesis.<sup>6</sup>

**3) As prodrug:**

It shows low bioavailability. Hence prodrugs are used. Eg. THR (Thyrotropin releasing hormone). THR is used for neurological disorder.<sup>7</sup>

**4) Antimicrobial Peptide:**

These are an evolutionarily conserved component of the innate immune response and are found among all classes of life. Fundamental differences exist between microbial and mammalian cells that may represent targets for antimicrobial peptides. These peptides are potent, broad spectrum antibiotics which demonstrate potential as novel therapeutic agents. Antimicrobial peptides have been demonstrated to kill Gram negative and Gram positive bacteria (including strains that are resistant to conventional antibiotics), mycobacteria (including *Mycobacterium tuberculosis*), enveloped viruses, fungi and even transformed or cancerous cells. Unlike the majority of conventional antibiotics it appears as though antimicrobial peptides may also have the ability to enhance immunity by functioning as immunomodulators.<sup>8</sup>

| Sr. no. | Class               | Source   | Biological activity  |
|---------|---------------------|--|--|
| 1       | Cathelicidin        | Human myloid cell  | Antimicrobial  |
| 2       | Granulysin          | Human cytolytic T-lymphocyte                                   | Broad spectrum Antimicrobial   |
| 3       | $\beta$ -defensin   | Human  | Wound healing and Otopic eczema  |
| 4       | $\alpha$ - defensin | Human Neutrophil elastase                                      | Physiological function in a host defence against bacterial infections and matrix remolding following tissue injury. <sup>5</sup> |
| 5       | Interleukins        | Released from an intracellular preformed pool in keratinocytes | Wound healing  |
| 6       | Thrombocidin        | Human blood platelet   | Antibacterial peptide  |
| 7       | Other               | Human  | Cosmetics  |

**Table no.2: Antimicrobial Peptides.**

**Functions of Proteins:**

1. Proteins necessary for structure and strength of Body.a) Collagen and elastin: found in bone matrix, vascular system b)  $\alpha$ -keratin in epidermal tissue.
2. Proteins act as Enzymes, Hormones, Blood clotting Factor, Immunoglobulins, Membrane receptors, Muscle contraction & respiration etc.
3. Other functions of Proteins we have antibodies, connective tissue (collagen), fluid media, transportation vehicles (Haemoglobin, serum albumin), buffers (serum albumin), signal transducers (rhodopsin), etc.
- 4.

**Examples:**

1. **Cytoskeleton** – Actin (muscle), Tubulin (cell motility), Intermediate filaments Spectrin (cytoskeletal protein, particularly found in erythrocytes)
2. **Human Plasma** – Albumin (osmotic regulation, buffering, transport), a-Globulins (transport), b-Globulins (iron transport {transferring}, histocompatibility antigen {b2-Microglobulin}), a-Globulins Antibodies, Fibrinogen (proteolysed by thrombin to form fibrin clot), Complement A (11 different protein types working to complement the immune system)

3. **Extracellular Matrix** – Glycosaminoglycans (hydrated gels), Proteoglycans, Collagen (extracellular matrix; Type I-III tissue supporting fibrils, Type IV laminar network), Elastin (random coil protein gives elasticity to tissues), Fibronectin (cell adhesion), Integrin (integral membrane proteins, also adhesion of cells to extracellular Matrix)
4. **Digestive Enzymes of Digestive Tract** – Amylase (starch to disaccharides), Pepsin, Trypsin, Chymotrypsin (proteins to large peptides), Peptidases (large peptides to small peptides; small peptides to amino acids), Lipases (lipids to fatty acids and glycerol), Ribonuclease (RNA into oligonucleotides), Disaccharidases (disaccharides to monosaccharides)
5. **Cytosol Proteins** (300-1000 types) – Synthesis of most small molecules, proteins, carbohydrates & lipids of cell
6. **Nuclear Proteins** – Histones (5, complex to DNA to make chromosomes), Nucleic Acid polymerising enzymes (5-10, used in DNA and RNA synthesis)
7. **Mitochondrial & Chloroplast Proteins** (300-1000) – Energy production from metabolites or light
8. **Endoplasmic Reticulum & Golgi Apparatus Proteins** (50-200) – Protein modification, oligosaccharide and lipid synthesis
9. **Lysosome & Peroxisome Proteins** (300-1000) – Degradation processes of undesirable compounds
10. **Plasma Membrane Proteins** (100-500) – Transport across membranes, Transmission of important metabolic signals across plasma membrane.<sup>10</sup>

**Methods of analysis of Protein chemistry:**

- Paper chromatography of amino acids and peptides
- High-V paper electrophoresis of amino acids and peptides
- Ion-exchange chromatography of amino acids and peptides
- Disulphide bonds
- Selective cleavage of peptide chains
- N-terminal sequence determination
- C-terminal sequence determination
- Dialysis and gel filtration
- Column Chromatography of proteins
- Zone electrophoresis of proteins
- Plasma desorption mass spectrometry
- Mass spectrometry
- Micro flow based automated chemistries.<sup>11,12</sup>

**Uses of Protiens:**

1. Creatinine Ratio Measurements on Random Urine Samples for Prediction of Significant Proteinuria
2. An altered peptide ligand mediates immune deviation and prevents autoimmune encephalomyelitis.
3. Induction of circulating myelin basic protein and proteolipid protein -specific transforming growth factor-beta1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients.
4. Immunogenic and encephalitogenic epitope clusters of myelin proteolipid protein.
5. Therapeutic Vaccines and Autoimmunity
6. Synthetic Vaccines

7. Immunogenicity and Antigenic Specificity
8. Therapeutic proteins used to treat cancer, heart attacks, Strokes, cystic fibrosis, diabetes, anemia and hemophilia. These proteins are produced by using microbial fermentation on cell culture, in transgenic plant and transgenic animal.
9. Glycoprotein - a threat to effective drug therapy its role-
  - Antioxidant
  - Increased bile acid secretion
  - Prostate cancer
  - Breast cancer
  - Anti-Atherosclerotic activity.<sup>13</sup>

### Future prospects of Protein Chemistry:

#### 1) Protein engineering of redox-active enzymes:

Redox-active enzymes perform many key biological reactions. The electron transfer process is complex, not only because of its versatility, but also because of the intricate and delicate modulation exerted by the protein scaffold on the redox properties of the catalytic sites. Nowadays, there is a wealth of information available about the catalytic mechanisms of redox-active enzymes and the time is propitious for the development of projects based on the protein engineering of redox-active enzymes.<sup>14</sup>

#### 2) Enzyme design by chemical modification of protein scaffolds:

Covalent modification methods allow an almost unlimited range of functionality to be introduced into proteins. In concert with genetic techniques, chemical strategies have had significant impact in the field of enzyme design. Major recent developments include introducing catalytic activity into inactive proteins, modifying the selectivity and/or reactivity of existing enzymes and designing novel enzyme-based biosensors.<sup>15</sup>

#### 3) Immobilized Derivatives of Leucine Aminopeptidase and Aminopeptidase M:

Leucine aminopeptidase and aminopeptidase M have been covalently bound to an arylamine derivative of porous glass. Both bound enzymes will catalyze the hydrolysis of the aminoethylated A and B chains of insulin nearly to completion ( $\approx 87\%$  recovery of free amino acids in all cases). These digests are carried out at pH values near neutrality in a volatile buffer with no activating metal. Immobilized pronase (Royer, G. P., and Green, G. M. (1971) *Biochem. Biophys. Res. Commun.* 44, 426) was used in concert with bound leucine aminopeptidase and bound aminopeptidase M for the hydrolysis of  $\beta$ -lactoglobulin.<sup>16</sup>

#### 4) The redox chemistry of the Alzheimer's disease amyloid beta peptide:

In AD there is an over accumulation of the Amyloid beta peptide (A $\beta$ ), this is the result of either an elevated generation from amyloid precursor protein (APP) or inefficient clearance of A $\beta$  from the brain. A $\beta$  can efficiently generate reactive oxygen species in the presence of the transition metals copper and iron in vitro. Under oxidative conditions A $\beta$  will form stable dityrosine cross-linked dimers which are generated from free radical attack on the tyrosine residue at position 10. There are elevated levels of urea and SDS resistant stable linked A $\beta$  oligomers as well as dityrosine cross-linked peptides and proteins in AD brain. Since soluble A $\beta$  levels correlate best with the degree of degeneration. We suggest that the toxic A $\beta$  species corresponds to a soluble dityrosine cross-linked oligomer. Current therapeutic strategies using metal chelators such as clioquinol and desferrioxamine have had some success in altering the progression of AD symptoms. Similarly, natural antioxidants curcumin and ginkgo extract have modest but positive effects in slowing AD development. Therefore, drugs that target the oxidative pathways in AD could have genuine therapeutic efficacy



### Conclusion

Protein chemistry has attracted the attention of physician, pharmacists for their wide variety of applications. Proteins are employed for the treatment of chronic viral diseases, Alzheimer's disease, extracellular matrix, human plasma, digestive enzymes for digestive tract. Most of the common natural peptides have been used in the antibiotic preparations. The mechanism of peptide bond and the structural features of proteins are discussed in the review. Proteins and peptides will be the promising agent for treatment of all chronic diseases. Most of the drugs, probiotics, nutrients and supplements are supported by proteins.

### References

1. Brown, W.E., Howard, G.C., Practical Methods in Advanced Protein Chemistry, *CRC Press*, 2000, 0-8493-9453-8.
2. Sumner, JB. "The isolation and crystallization of the enzyme urease. Preliminary paper", *Journal of Biological Chemistry*, 1926, 69: 435–41.
3. Text Book of Biochemistry, by U.Satyanarayana, U. chakrapani, 3<sup>rd</sup> edition, 46-66.
4. Maton A, Hopkins J, McLaughlin CW, Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall,1993,12:123-126.
5. Wong, S.S., Chemistry of protein conjugation and cross linking, *CRC Press*, 1991, 0-8493-5886-8
6. Burger's Medicinal chemistry and drug discovery, Volume 2, 5<sup>th</sup> edition, by Manfred E. Wolff. *A wiley interscience publication*,498-500.
7. "Research advances in the development of peptide antibiotics", *Journal of pharmaceutical sciences*, vol.-97, Number-3, March-2008,1060
8. "Therapeutic protein production & delivery", *Indian journal of pharmaceutical education & Research*, vol.42 (2), Apr-Jun 2008, 104-112.
9. "Skin peptides: Biological activity and therapeutic opportunities", *Journal of Pharmaceutical sciences*, vol.-97, Number-7, July-2008,2524
10. Nagradova, N.K., Lavrik, O.I., Kurganov, B.I., Chemical Modification of Enzymes, Nova Science, Inc., 1995, 1-5607-2238
11. Michael Powell<sup>†</sup> and Paul Tempst, Microflow-Based Automated Chemistries: Application to Protein Sequencing Protein Center and Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, *Anal. Chem.*, 2001, 73 (4), 776–78
12. Peter Roepstorff, Per F. Nielsen, Klaus Klarskov, Applications of plasma desorption mass spectrometry in peptide and protein chemistry, Department of Molecular Biology, Odense University, DK-5230 Odense M, Denmark
13. Magdassi, S., Surface Activity of Proteins: Chemical and Physicochemical Modifications, Marcel Dekker, Inc., 1996, 0-8247-9532-6.
14. Saab-Rincón G, Valderrama B, Protein engineering of redox-active enzymes. *Antioxid Redox Signal*. 2009 Feb; 11(2): 167-92
15. Tann CM, Qi D, Distefano MD., Enzyme design by chemical modification of protein scaffolds. *Curr Opin Chem Biol*. 2001 Dec;5(6):696-704
16. Garfield P. Royer and John P. Andrews , applications in protein chemistry, From the Department of Biochemistry, The Ohio State University, Columbus.1999,12:321-332.