Review Article

PEPTIDES AND PROTEINS IN PHARMACEUTICALS

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ABSTRACT

Peptides and proteins possesses complex architecture structure. The twenty different naturally occurring amino acids join with each other by peptide bonds and build polymers referred to peptides and proteins. Although the distinction between peptides and proteins is arbitrary, a peptide contains less than 20 amino acids, having a molecular weight less than 5000, while a protein possesses 50 or more amino acids and its molecular weight lies above this value. Different fermentation, purification processes and recombination technology produced potential protein drugs at acceptable cost which can be useful in various diseases through various routes like oral, transdermal, nasal, pulmonary, ocular, buccal, rectal. As these therapeutic proteins and peptides are made available, it will be essential to formulate these drugs into safe and effective delivery systems. Due to its wider applications in pharmaceutical industries, they will replace many existing organic based pharmaceuticals. Now days, many drugs are in the world market, while several hundreds are in clinical trials.

Keywords: Biogenerics, Complex architecture, Hexyl insulin monoconjugate 2, Smart polymer.

INTRODUCTION

In the last three decades therapeutic peptides and proteins have risen in prominence as potential drug of future. The recent advance in large scale fermentation and purification processes and analytical characterization has widened the horizons.

Ailments that might be treated with this type of therapeutics include auto-immune diseases, cancer, mental disorder, hypertension, and certain cardiovascular and metabolic diseases. Protein drug must be highly purified and concentrated and have extremely short half life and should have a shelf life of at least two years.

Recombinant technology has allowed the production of many potential protein drugs at an acceptable cost, allowing the treatment of severe, chronic and life-threatening diseases, such as diabetes, rheumatoid arthritis, hepatitis, etc. Currently, over 160 protein drugs are available on the world market, and several hundreds more are in clinical trials. The total protein drug market already exceeds 30 billion, and is expected to rise by at least 10% a year.

One of the biggest opportunity areas in the Protein Therapeutics Market will be in the field of Biogenerics, which is expected to create a multi-billion dollar market in future.
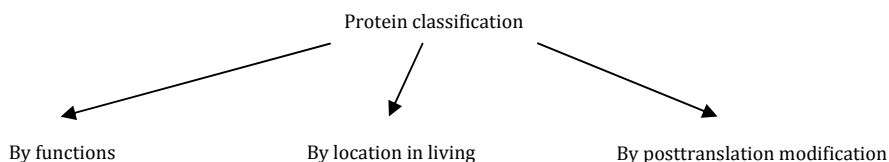
Structure of proteins:

The proteins are large molecules complex architecture. The peptide & protein are seldom linear & adapt a variety of specific folded three dimensional patterns & conformation[1].

Structure of a protein is directly related to its function, so that anything that severely disrupts the shape will also disrupt the function.

There are four types of protein structure.

1. Primary structure
2. Secondary structure
3. Tertiary structure
4. Quaternary structure

Classification of proteins:

Protein classification

By functions   By location in living   By posttranslation modification

Classification by functions of proteins:

Enzymes : DNA and RNA polymerase

Hormones : Endorphine and enkephalin.

Transport proteins : Cytochrome C, Albumin, Haemoglobin.

Antibodies : Interferon, Fibrin.

Structural proteins : Collagen, Elastin.

Motor proteins : Actin, Myosin.

Rceptors : Transmembrane proteins.

Signalling proteins : GTPase.

Storage proteins : Egg ovalbumin, milk casein.

Classification of proteins by location in the living cell:

Membrane proteins

Internal proteins

External proteins

Virus proteins

Classification of proteins by posttranslational modification:

Native protein

Glico prtein

Cleaved protein

Protein with disulfide bonds

Protein complexes

Chemically modified proteins

Prions

Physicochemical properties of peptides and proteins:

Solubility and partition coefficient:

Aqueous solubility of peptide is strongly dependent upon pH, presence of metallic ion, ionic strength and temperature. At isoelectric point the aqueous solubility of peptide is minimal where the drug is neutral or has no net charge. Peptides are very hydrophilic with a very low octanol-water partition coefficient, so to improve the absorption of peptides by passive diffusion, their lipophilicity should be increased.

Aggregation, self association and hydrogen bonding:

Self-aggregation tendency of peptides modifies their intrinsic properties. Human insulin was found to be more self-aggregating than bovine insulin. Additions of additive like non ionic surfactants (Pluronic F 68) stabilize the peptide formulation against self aggregation. Intermolecular hydrogen bonding with water decreases the permeability of protein in lipid membrane.

Degradation pathways indicating instability of proteins & peptides: [1]

Sr. No.	Degradation pathways	Mechanism
	Physical Instability:	
1.	Denaturation	Non-proteolytic modification of a unique structure of a native protein that affects definite change in physical, chemical and biological properties. Several examples of denaturing agents are urea, alcohol, acetic acid, sodium dodecyl sulphate, polyethylene glycol.
2.	Adsorption	Ampiphilic nature of protein cause adsorption at various interfaces like air-water and air-solid.
3.	Aggregation and Precipitation	The denatured, unfolded protein may rearrange in such a manner that hydrophobic amino acid residue of various molecules associate together to form the aggregates. If aggregation is on macroscopic scale, precipitation occurs.
	Chemical Instability:	
1.	Deamidation	The hydrolysis of the side chain amide linkage of an amino acid residue leading to the formation of a free carboxylic acid.
2.	Oxidation and Reduction	Oxidation occurs during isolation, synthesis and storage of proteins. Temperature, pH, trace amount of metal ion and buffers influence these reactions. Glucagon is an exception as it retains biological activity even after oxidation.
3.	Proteolysis	It may occur on exposing the proteins to harsh conditions like prolonged exposure to extreme of pH or high temperature or proteolytic enzyme.
4.	Disulfide exchange	A peptide chain with more than one disulphide can enter in to this reaction and thereby change in conformation.
5.	Racemisation	It is alteration of L-amino acids to D,L-mixtures. Racemization form peptide bonds that are sensitive to proteolytic enzymes.
6.	B- elimination	It proceed through a carbanion intermediate. Protein residues susceptible to it under alkaline conditions include Cys, Lys, Phe, Sre and Thr.

Barriers to protein drug delivery: [1]

Enzymatic barrier	Limits absorption of protein drugs from G.I. tract.
Intestinal epithelial barrier	Involved in the transport of protein drugs across the intestinal epithelium.
Capillary endothelial barrier	Involved in transport of protein drugs across the capillary endothelium.
Blood brain barrier	Involved in transport of protein drugs to brain compartment.

A protein drug has to face with a number of lipophilic and hydrophilic barriers to cross. The drug must first dissolve in the contents of the intestinal lumen if it is not already in solution; then there is a mucus layer and a water layer protecting the surface of the epithelial cells. The protein or peptide drug must have sufficient water - and lipid-solubility to pass through these layers. The epithelial tissue represents the next barrier. There are cases where a protein can be absorbed in to these cells by endocytosis, and then transported to the basement membrane on its way to the capillaries.

Various Routes of administration for protein or peptide drugs:

(1) Parenteral systemic delivery:

(2) Non-parenteral systemic delivery:

    a. Oral route
    b. Nasal route
    c. Buccal route
    d. Ocular route
    e. Rectal route
    f. Transdermal route
    g. Pulmonary route

(1) Parenteral systemic delivery: [3]

For the systemic delivery of therapeutic peptides and proteins, parenteral administration is currently believed to be the most efficient route and also the delivery method of choice to achieve therapeutic activity.

Parenteral delivery consist of three major routes: intravenous(IV), intramuscular(IM), subcutaneous(SC). Among them intravenous administration is currently the method of choice for systemic delivery of proteins and peptides. Insulin, interferons, and gammaglobulins have been reportedly metabolized and/or bound to tissue at injection sites following IM administration, and as a result, the systemic bioavailability of these protein drugs following IM administration is often less than that obtained by IV injection. The bioavailability of tissue plasminogen activator following IM administration was facilitated by the co-administration of hydroxyl amine or by electrical stimulation of muscles, which results in prompt attainment of therapeutic blood levels and achievement of coronary thrombolysis. For SC administration, insulin is best example for the treatment of diabetes. The controlled delivery of peptide or protein based pharmaceutical from subcutaneously

implanted polymeric devices was reported by Davis. He used a gel formulation of cross linked polyacrylamide-polyvinylpyrrolidone to achieve the prolonged release of immunoglobulin, luteinizing hormone, bovine serum albumin, insulin, and prostaglandin.

Biomedical application:

LHRH and Analogs*: Luteinizing hormone- releasing hormone is a naturally occuring decapeptide hormone with a molecular weight of 1182 daltons. They are poorly absorbed when taken orally, so it require parenteral delivery. There is a development of a biodegradable subdernal implant for the SC controlled delivery of goserelin(zoladex), a potent synthetic analog of LHRH for the treatment of prostate carcinoma. Another example is SC delivery of nafarelin, a potent LHRH agonist.

Insulin: It is a protein molecule, with a molecular weight of 6000 daltons. Three long acting Zn-insulin preparation, Semilente, Lente, and Ultralente insulin, have been formulated for the treatment of diabetes. For delivery of insulin by SC route require sprinkler type of needle and Novolin pen. Various types of infusion pumps like continuous, subcutaneous insulin infusion (CSII), piezoelectrically-controlled micropump (P-CRM) are use for programmed delivery of insulin.

Vasopressin*: Vasopressin, a nonapeptide with a molecular weight of 1084 daltons, is an antidiuretic hormone. Using a device prepared by covering a section of microporous polypropylene (Accurel) tubing with collodion, a long lasting and constant in-vitro release of vasopressin was achieved. By using the Alzet pump, subcutaneous delivery of vasopressin was achieved.

(2) Non-parenteral systemic delivery:

a) Oral route: [3]

The ease of administration and higher degree of patient compliance with oral dosage forms are the major reasons for preferring to deliver proteins and peptides by mouth.

Strategies for oral delivery:

Formulating for delivery through the gastrointestinal (GI) tract requires a multitude of strategies.One strategy for overcoming the body's natural processes is to alter the environment for maximum solubility and enzyme stability of the protein by using formulation excipients such as buffers, surfactants and protease inhibitors. If the enzyme attack can be defeated or delayed, the proteins can be presented for absorption. Proteins and peptides could be derivatised with polyethylene glycol (PEG) to achieve properties such as retention of activity, prevention of immunogenicity and prevention of excessive enzymatic degradation. The use of oligomers with both hydrophilic and lipophilic properties, confer the enzymatic stability necessary for proteins to survive the digestive processes in the gut.

Another strategy for oral delivery is to promote absorption through the intestinal epithelium. Absorption may be enhanced when the products formulated with acceptable safe excipients. A typical transport mechanism for proteins across the epithelial boundary is paracellular transport. There are tight junctions between each of the cells in the epithelium that prevent water and aqueous soluble compounds from moving past these cells. A number of absorption enhancers are available that will cause these tight junctions to open transiently, allowing water-soluble proteins to pass. Fatty acids, surface-active agents, EDTA, glycerides and bile salts have all been shown to be effective in opening these tight junctions. Most of the data showing the successful oral delivery of model proteins, such as insulin and calcitonin, has been generated in animal studies.

Nobex technology:

The Nobex technology involves the bonding of polyethylene glycol (PEG) and alkyl groups or fatty acid radicals to produce desired amphiphilic oligomers. These oligomers are conjugated to proteins or peptides to obtain desired amphiphilic products that can traverse the aqueous and lipid layers of the mucosa, and can resist excessive degradation of protein or peptide drugs. In this, an amphiphilic protein conjugate is prepared. This technology reduces self-association, increases penetration and increases compatibility with formulation ingredients than parent drug.

Oral delivery of insulin:

This conjugated protein is known as hexyl insulin monoconjugate 2 (HIM2), and is the first successfully delivered oral dosage form of insulin to show good oral bioefficacy in humans. The oral form of insulin goes directly to the liver and is believed to stimulate normal biochemical pathways, including glucose control. Human clinical results with conjugated insulin are a clear demonstration that a protein can be developed into a therapeutically viable product. With the conjugated form of insulin and an appropriate formulation, a product can be developed which has the attributes required for oral delivery. Oral delivery of insulin with sodium deoxycholate on ascending colon of rat causes 50% reduction in blood glucose level.

Stefanov et al. investigated the feasibility of delivering insulin systemically by oral route using a liposomes prepared from phosphatidylcholine (PC) and cholesterol (CH) as delivery system and reported no change in blood glucose level is noted in normal animals, but significant reduction is obtained with diabetic rats.

Oral delivery of arginine, lysine vasopressin, synthetic analog, 1-deamino-8-D-arginine vasopressin (DDAVP) was studied on rats and rapid anti diuretic response achieved.

Coating of peptides with polymers with azoaromatic groups and the cross linking of azopolymers to form an impervious film to protect orally administered proteins or peptides from degradation and metabolism in the stomach and small intestine.

Potential problem associated with oral protein delivery:

The oral administration of peptide and protein drugs faces two formidable problems. The first is protection against the metabolic barrier in GIT. The whole GIT and liver tend to metabolize proteins and peptides into smaller fragments of 2-10 amino acids with the help of a variety of proteolytic enzyme (proteases), which are of four major types; aspartic protease (pepsin, rennin), cystinyl proteases (papain, endopeptidase), metallo proteases (carboxypeptidase-A, ACE) and serinyl proteases (thrombin, trypsin). The second problem is the absence of a carrier system for absorption of peptides with more than three amino acids.

Prodrug approach:

Proteins are labile due to susceptibility of the peptide backbone to proteolytic cleavage, as well as their molecular size and complex secondary, tertiary and sometimes even quaternary structures. Therefore proteins can be modified chemically to give more stable prodrugs with increased plasma half-lives. Some strategies for prodrug formation include olefenic substitution, d-amino acid substitution, dehydro amino acid substitution, carboxyl reduction, retro inversion modification, polyethylene glycol (PEG) attachment to amino group and thio-methylene modification.

b) Nasal route: [1],[4]

With greater interest in delivery of protein and peptide-based drugs to the lungs for topical and systemic activity, a range of new devices and formulations are being investigated. Pulmonary protein delivery offers both local targeting for the treatment of respiratory diseases and increasingly appears to be a viable option for the delivery of proteins systemically. The lung is easy to access, has decreased proteolytic activity compared with the gut, and allows rapid absorption and avoidance of first-pass metabolism for systemically delivered drugs.

Hundreds of proteins and peptides are undergoing clinical investigation for a range of clinical conditions. These include growth factors, hormones, monoclonal antibodies, cytokines, and anti-infective agents. For those being investigated for delivery via inhalation, the ultimate site of action may be the airway surface (e.g. DNase), the airway cells (e.g. cyclosporin), or the systemic circulation (e.g. insulin). Careful choice of carrier and device can facilitate delivery to a specific area of the lungs. Once delivered, a carrier can further influence the distribution and rate of clearance

from the site of action.. The only protein for inhalation currently available on the market is DNase, but a growing number of proteins/peptides are in various phases of clinical trials. Systemic inhaled insulin is in late phase 3 trials. Other proteins/peptides in phase 3 trials include leuprolide and gamma-interferon. Bile salts have been used to enhance the nasal absorption of peptide based pharmaceuticals.

Nasal delivery of proteins:

Insulin*:* The nasal absorption of insulin is increased by coadministration of bile salts, surfactants. Gordon et al. reported that in case of sodium deoxycholate, a minimum concentration of 2.4 mM is required to enhance the transnasal permeation of insulin.

Interferon: Merigan et al. studied the inhibition of respiratory virus infection by intranasal administration of human leukocyte interferon.

Nasal delivery of oligopeptides:

Examples:

Dipeptides: 1-tyrosyl-1-tyrosine and its methyl esters, 1-gycyl-1-tyrosine,1-glycyl-1-tyrosinamide.

Tripeptides: Thyrotropin-releasing hormone(TRH)

Pentapeptides: Leucin-enkephalin, met-enkephamide.

Nasal delivery of polypeptides:

Examples:

Nonapeptides: Vasopressin, OXYTOCIN

Decapeptides: LHRH

Undecapeptides: Substance P

Glucagon, Calcitonin and Adrenal corticotropic hormone (ACTH) are another examples of polypeptides.

Advantages of nasal route:

Convenient, simple, practical way of drug administration

The high vascularization permits better absorption.

First pass metabolism can be avoided.

Rapid onset of action.

Disadvantages of nasal route:

Long term use may lead to toxicity to mucosa.

During disease states (e.g. common cold) some alteration in the nasal environment may occur.

c) Buccal route: [(1),(2)]

Oral mucosa, including the lining of the cheek (buccal mucosa), floor of mouth and underside of tongue (sublingual mucosa) and gingival mucosa, has received much attention in the last decade because it offers excellent accessibility and avoids degradation of proteins and peptides that occurs as a result of oral administration, gastrointestinal absorption and first-pass hepatic metabolism. Peptide absorption occurs across oral mucosa by passive diffusion and it is unlikely that there is a carrier-mediated transport mechanism. The penetration of macromolecules through oral epithelia has been studied by several investigators[(5)] .

Merkle et al.[(6)] developed a self adhesive buccal patch and reported that it is feasible to deliver peptide base pharmaceuticals such as protirelin and buserelin through buccal mucosa. Various types of polymers like sodium carboxymethyl cellulose, hydroxypropylmethyl cellulose, polyvinyl pyrrolidone, acacia, calcium carbophil, gelatin, polyethylene glycol are used for delivery of proteins or peptides via buccal route. The anionic polyacrylate type hydrogel is the most commonly used polymer.

Various strategies employed for buccal delivery:

1) Adhesive tablets: e.g. Adhesive tablet based on hydroxypropylcellulose.
2) Adhesive gels: e.g. By using polyacrylic acid and polymethacrylate as gel forming polymers.
3) Adhesive patches: e.g. Protirelin in HEC patches and buserelin.
4) Adhesive promoters: e.g. Sodium lauryl sulphate, sodium myristate, bile acids, sodium glycocholate, citric acid.

Addition of absorption promoters/permeabilizers in bioadhesive dosage forms will be essential for a successful peptide/protein delivery system.

Thyrotropin-releasing hormone, tripeptide, oxytocin, vasopressin, LHRH analogs, calcitonin, insulin have been easily applied through buccal route.

Advantages of buccal route:

It is robust, much less sensitive to irreversible irritation even on long term treatment. Absence of enzymatic barrier. Well acceptable to the patients. Easy accessibility administration as dosage forms. It is attached or removed without any pain or discomfort.

d) Ocular route : [(2)]

The ocular route holds immense potential for peptides or proteins intended for pathological ophthalmologic conditions. The ocular route is the site of choice for the localized delivery of opthalmologically active peptides and proteins for the treatment of ocular disease that affect the anterior segment tissues of eye. Christie and Hanzal [(7)] reported the observation of a dose dependent reduction in blood glucose levels following the ocular administration of insulin to the rabbit. The use of nanoparticles, liposomes, gels, ocular inserts, bioadhesives or surfactants are necessary to enhance ocular absorption of proteins or peptides. The polypeptide antibiotics like cyclosporine, tyrothricin, gramicidin, tyrocidine, bacitracin and polymyxins have often been considered potential candidates for achieving local pharmacological actions in the eyes. [(8)]

Proteins or peptides with opthalmological activities:

Affect aqueous humor dynamics: Calcitonin gene related factors, LHRH, vasopressin

Immunomodulating activities: Cyclosporine, interferons.

Act on inflammation : Substance P, enkephalins.

Affect wound healing: Epidermal growth factor, fibronectin.

The systemic bioavailability achieved by this route is very low. Ocular tissues are sensitive to the presence of foreign substances and patient acceptance could be rather low in ocular route.

e) Rectal route: [(3)]

The use of the rectum for the systemic delivery of organic and peptide based pharmaceuticals is a relatively recent ideas. The coadministration of an absorption promoting adjuvants such as sodium glycocholate, has been reported to enhance the rectal absorption of insulin. Touitou et al. [(9)] achieved hyooglycemia in rats by administering insulin via rectal route, in a dosage form that contained polyethylene glycols and a surfactant. It was recently reported that a solid dispersion of insulin with sodium salicylate can produce a rapid release of insulin from the suppositories achieve a significant reduction in plasma glucose levels in normal dogs, even at doses as low as 0.5IU/kg.[(10)] Bile salts, such as sodium salts of cholic, deoxycholic and glycocholic acids, have also been shown to enhance the rectal absorption of insulin in rats[(11),(12)] and human volunteers.[(13)] Vasopressin and its analogs[(14)], pentagastrin and gastrin[(15)], calcitonin analogs[(16),(17)] and human albumin[(18)] have been investigated for rectal delivery of protein or peptide based pharmaceuticals.

Advantages of rectal delivery:

It is highly vascularized. It avoids first pass or presystemic metabolism. Drug can be targeted to the lymphic system. It is

suitable for drugs that cause nausea/vomiting and irritate GI mucosa on oral administration. A large dose of drugs can be administered.

f) Transdermal route:

Transdermal delivery has attracted considerable interest as a route for administering peptides and proteins. As early as 1996, Tregear investigated the feasibility of administering proteins and polymers through skin excised from human and animals[19]. More recently, Menasche et al.[20] studied the percutaneous absorption of elastin peptides through rat skin and its subsequent distribution in the body. The small peptides such as thyrotropin releasing hormone (TRH) [21] vasopressin[22],[23] have great difficulty in permeating the skin barrier.

Approaches for transdermal delivery:

Iontophoresis : It is use of electric current to drive charged drug molecules into skin by placing them under an electrode of like charged.

DC Iontophoresis: Siddiqui et al. [24] used the phoresor system or recently marketed DC iontophoretic device, as the power source for direct current and were able to deliver insulin transdermally to diabetic hairless rats, with attainment of a reduction in hyperglycemia. Recently a body wearable DC iontophoresis delivery device, called power patch applicator, was developed [25] and applied to diabetic rabbits[26],[27].

Pulse DC iontophoresis: By delivering a pulse current with a 20% duty cycle (4μsec), followed by an 80% depolarizing period(16μsec), a β-blockers was successfully delivered systemically to five human subjects without polarization induce skin irritation[28]. A transdermal periodic iontotherapeutic system (TPIS) was designed [29] and it is capable of delivering the pulsed direct current with variable combinations of waveform, frequency, on/off ratio and current intensity for a specific duration treatment. Proteins or peptides drugs like insulin, TRH, salmon calcitonin, delta sleep inducing peptide, LHRH, vasopressin, leuprolide can be applied through iontophoresis.

Phonophoresis: In this method ultrasound is applied via a cupling contact agent to the skin. Insulin, IFN γ, erythropoietin can be delivered by this method.

Penetration enhancers: Penetration enhancers like oleic acid, dimethylsulphoxide. Surfactants and azone have been used for topical delivery of peptide or proteins.

Prodrugs; Prodrug with modeled physicochemical characteristic permeated well across the skin. LHRH, TRH, neurotensin can be delivered by this method.

Advantages of transdermal route:

Avoids the hepatic first-pass effect and gastrointestinal breakdown.

Provides controlled and sustained administration, particularly suitable for the treatment of chronic disease.

Reduces side-effects, often related to the peak concentrations of the circulating agent;

Enables self-administration and improves patient compliance, due to its convenience and ease of use.

Permits abrupt termination of drug effect by simply removing the delivery system from the skinsurface.

Limitations of transdermal route:

A low rate of permeation for most of protein drugs due to their large molecular weight.

High intra- and inter-patient variability.

Because the skin has a relatively low proteolytic activity, the peptide drugs have poor skin permeability.

g) Pulmonary route:

The respiratory tract offers an alternative site for systemic non-invasive delivery of peptides/proteins . Most of these drugs are readily absorbed through the lung, once they entered the deep lung tissues via transcytosis.

They provide larger surface area (70 m^2) as compared to the other mucosal sites including nasal, buccal, rectal and vagina. A simple diffusion and carrier mediated transport mechanism operate in lungs. Three devices are currently available for the pulmonary delivery of the protein/peptide drugs : metered dose inhaler, nebulizer and powder inhaler (insufflator).

Insulin can be delivered by this route by using devices such as aerosol, dry powder or as a administered with penetration enhancers like 1% azone, 1% fusidic acid or 1% glycerol. Calcitonin can be delivered as dry powder by this route.

Advantages of pulmonary route:

Provide a direct route to the circulation.

Reduction in dose requirement upto 50 fold and thus cost effective option for pharmaceutical industries

Fast absorption

Safe route for drug entry even in patient with lung diseases

No triggering of immune function.

Increase patient compliance with a minimum of discomfort and pain

Disadvantages of pulmonary route:

Most of the drug is delivered to the upper lung, an area with low systemic absorption.

Only a small amount of drug can deliver.

Paracellular delivery of peptides—a rational approach:

Currently, a new route—the paracellular pathway—is being explored for delivery of peptides. As opposed to the transcellular pathway, the paracellular pathway is a water-filled pathway, which is amicable to the delivery of polar molecules like peptides and proteins.Another advantage is that by traversing through the area between the two cells the peptide also circumvents the intracellular lysosomal enzymes.A major problem is the low pore size of the paracellular pathway. The pore size can be regulated by various cellular signaling pathways. Paracellular delivery of proteins and peptides may become a clinical reality as it would be possible to design simple and easy to use dosage forms for non-parenteral delivery of proteins and peptides.

Polysaccharide hydrogels useful in the development of controlled release formulations for protein drugs.

Polysaccharide microspheres;

Polysaccharide-conjugated protein drugs;

Polysaccharide matrix in protein drug delivery; and

Microencapsulation of protein drugs.

Smart polymer based delivery system for proteins and peptides: [30]

Biodegradable polymeric systems represent promising means for delivering many bioactive agents, including peptide and protein drugs. The development of smart polymer-based injectable drug delivery systems has gained attention over the past few years. The advantages of this delivery system are ease of application, localized delivery for a site-specific action, prolonged delivery periods, decreased body drug dosage with concurrent reduction in possible undesirable effects common to most forms of systemic delivery, the non toxic degradability, and improved patient compliance and comfort.

Smart polymers are macromolecules that display a dramatic physicochemical change in response to small changes in their environment. Smart polymer-based injectable formulations are easy to prepare and form implants at the site of injection upon administration. Smart polymers can be classified according to the

external stimulus they respond to (i.e. temperature, pH, solvent, magnetic field, ions, and pressure).

Various types of polymers are used e.g.

1) Temperature sensitive polymers:
   e.g. Poly (ethylene oxide)-poly (propylene oxide)-poly(ethylene oxide) triblock copolymers (PEO-PPO-PEO), Poly nisopropylacrylamide (PNIPAAM)
2) Phase sensitive polymers:
   e.g. Poly (D,L-lactide), Poly (D,L-lactide-co glycolide)
3) pH sensitive polymers:
   e.g. Poly(methacrylic acid g-ethylene glycol) P(MAA-gEG)
4) Photo sensitive polymers:
   e.g. PEG, Poly(vinyl alcohol), PEO-PPO.

Hybrid protein delivery systems: [31]

Heterologous hybrid proteins can be designed bearing the combined or reordered features of one or more proteins that display effector functions, protection abilities and recognition properties. Site specific hybrid proteins may be produced by employing ligated gene fusion processes or by chemical linkages of protein fragments.

Ligated gene fusion hybrid delivery systems:

Gene fusioin techniques can be applied to blend the diverse properties of parent proteins and there by bioactive protein with improve pharmacological effects can be produced. Hybrid protein between interferon γ and TNF β have been developed. This hybrid preserved the cell specificity of the growth factor, antiviral activity and increase antiproliferative activity.

Synthetically linked hybrid conjugates:

Biological disposition and fate of the proteins can be modified by linking them to other proteins. The toxin gelonin has a circulation half life of 3.5 min(in mice) but on conjugate it to immunoglobulin, the terminal phase blood half life was increased to days.

Development of delivery system for peptide based pharmaceuticals: [32]

1) Formulation consideration:

a) Preformulation studies of therapeutic peptides and proteins:

Preformulation data must be generated to serve as the basis for the formulation development of dosage forms or for the design of delivery system to achieve optimum physicochemical stability and maximum systemic bioavailability.

b) Surface adsorption behaviour of peptide and protein molecules:

Protein and peptide molecules have a tendency to be adsorbed to a variety of surfaces including glass and plastic.

c) Aggregation behavior of protein and peptide molecule:

The potential problem is the self aggregation of peptide and protein molecules such as insulin [33],[34]. This has been minimize by the incorporation of additives like urea, dicarboxylic amino acid such as aspartic acid and glutamic acid or other reagents such as glycerols, EDTA, Lysine.

2) Pharmacokinetic considerations:

Due to the very short half life of protein and peptides, it is more critical to get information about pharmacokinetic of therapeutic peptide and protein. Metabolic degradation by peptidases and proteinases can occur in the vascular endothelium, liver, kidney and non target tissues and even at a site of administration.

3) Analytical consideration:

Bioassay method has been available for detection and potency determination of peptides and proteins, but it is very time consuming, labor intensive. Now a days spectroscopy, chromatography, electrophoretic methods and immunoassays have been available for an analytical determination of protein or peptide. The most commonly used analytical methods include HPLC and RIA. Fast atom bombardment mass spectroscopy is also very useful in peptide and protein analysis.

4) Regulatory consideration:

Biotechnology products regulated under the authority of four federal agencies:

The food and drug administration (FDA),

The environmental protection agency (EPA),

The occupational safety and health administration (OSHA).

U.S.Department of agriculture (USDA).

• PolyXen® : [35]

PolyXen® is an enabling technology for protein drug delivery. It uses the natural polymer polysialic acid (PSA) to prolong the active life and improve the stability of therapeutic peptides and proteins. It can also be used for small molecule drugs.

PSA is a polymer of sialic acid (a sugar). When used for protein and therapeutic peptide drug delivery, polysialic acid provides a protective microenvironment on conjugation. This increases the active life of the therapeutic protein in the circulation and prevents it from being recognized by the immune system.

The use of PSA makes PolyXen® a particularly effective form of protein drug delivery. PSA is a naturally occurring polymer, and it is biodegradable, non-immunogenic and non-toxic. This is particularly important where a polymer is to be used to deliver therapeutics chronically or in large dosages

• ImuXen® : [36]

ImuXen® is a group of liposomal technologies designed to improve the delivery and effectiveness of DNA, protein and polysaccharide vaccines.

ImuXen® technology can help to generate strong protective immune responses, in some cases with a single injected dose.

The potential advantages of ImuXen® for DNA, protein and polysaccharide vaccines include:

Vaccine protection against degradation.

Efficient delivery of vaccines to the immune system.

Increased immune responses.

Protective immunity with a single injection.

Rapid, simple and scaleable manufacture.

• The benefits of ImuXen® for DNA, protein and polysaccharide vaccines include:

Multiple vaccines delivered with a single injection.

Reduction in the number of doses required.

Reduction in side effects.

Potential for oral administration of vaccines.

Humira is the best-selling new monoclonal antibody in the market.

Pegasys is the best-selling newcomer to the therapeutic protein, and global pharma market.

Epogen is the second best-selling therapeutic protein.

Aranesp, Neulasta and Enbrel are the most successful therapeutic proteins in terms of growth. Aranesp is the fastest-growing therapeutic protein.

Table 1: Example of proteins and peptides for inhalation:

Disease State (Local and Systemic)	Peptide/Protein
Adult Respiratory Distress Syndrome	Surfactant Proteins (approved)
Cystic fibrosis (CF)	DNase (approved)
Emphysema/CF	Secretory leukoprotease inhibitor, Alpha-1-antitrypsin
Cancer	Interferon-γ, Interleukin-2
Alpha-1-antitrypsin deficiency	Alpha$_1$ proteinase inhibitor
Asthma	IL-1R, Anti-IgE Mab
Anti-TB vaccine	Muramyl dipeptide
Osteoporosis	Calcitonin, Parathyroid hormone
Growth deficiency	Human growth hormone
Multiple sclerosis	Interferon-β
Diabetes	Insulin
Viral infections	Ribavirin, Interferon-α
Neutropenia	rhG-CSF
Anemia	Erythropoetin
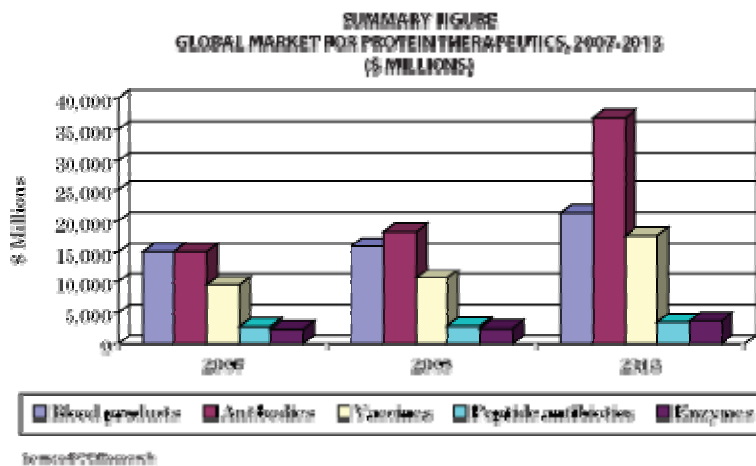
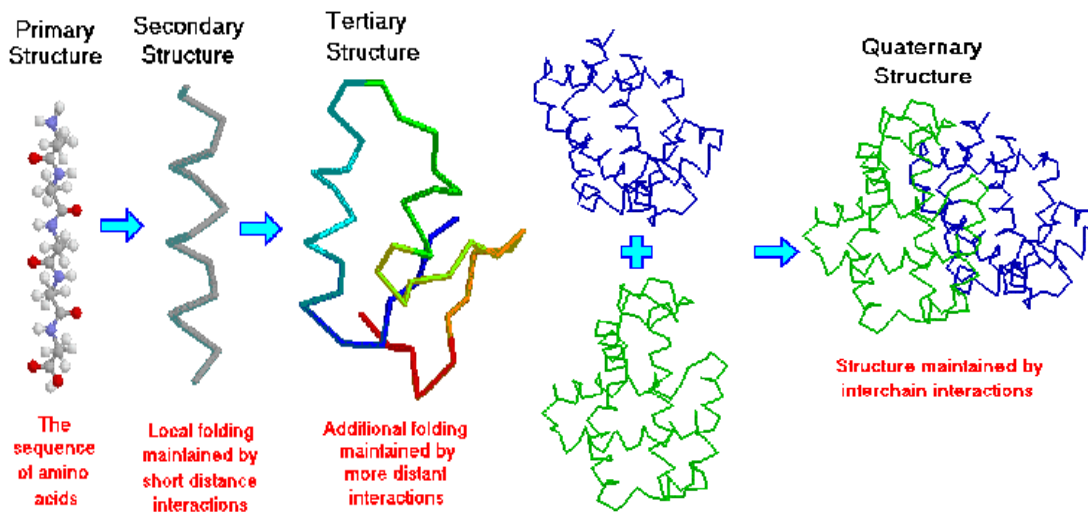Fig. 1: Global market for protein therapeutics, 2007-2013

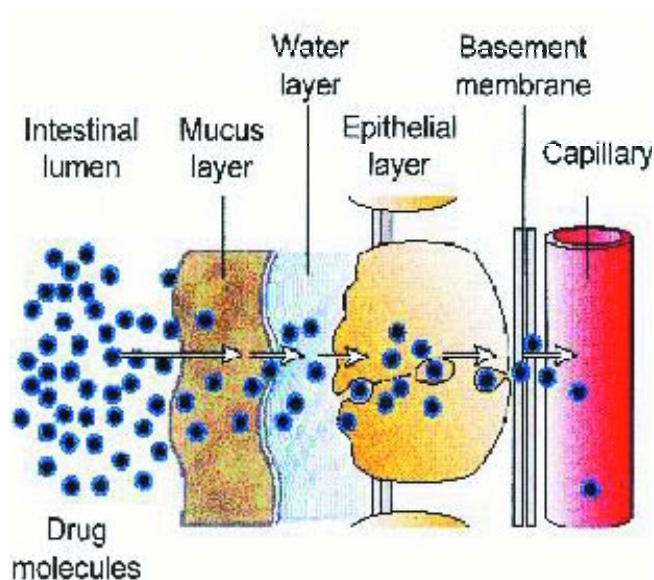Fig. 2: Structure of proteins

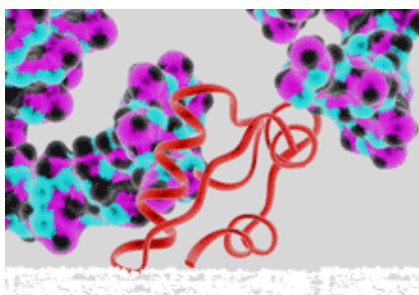**Fig. 3: Barriers to the absorption of the drug in the intestine**

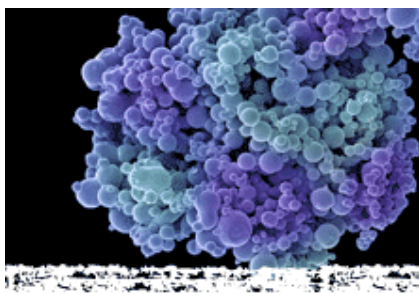**Fig. 4:   A model of the molecular structure of a polysiaylated peptide**

Fig. 5: Scanning electro micrograph of liposomes

CONCLUSION

The peptide and protein based pharmaceutical are rapidly becoming very important class of therapeutic agents and are likely to replace many existing organic based pharmaceuticals in the very near future. The peptide and protein based pharmaceuticals will be produced on a large scale by biotechnology processes and will become available commercially for therapeutic use. With the key benefits including favorable time to market and high rate of success in clinical trial compared with traditional pharmaceuticals, therapeutic proteins will play a pivotal role in the treatment of diseases.

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