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CONTENTS

Properties and Formulation of Oral Drug Delivery Systems of Protein and Peptides

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Semalty, *et al.*: Oral Drug Delivery of Proteins and Peptides

Although most protein pharmaceuticals are usually formulated as a solution or suspension and delivered by invasive routes such as subcutaneous injections, major efforts in both academic and industrial laboratories have been directed towards developing effective oral formulations and increasing the oral absorption of intact protein through the use of formulations that protect the macromolecule and/or enhance it's uptake into the intestinal mucosa. However, in spite of these major attempts, relatively little progress has been made. For the efficient delivery of peptides and proteins by non-parenteral route, in particular via the gastrointestinal tract, novel concepts are needed to overcome significant enzymatic and diffusion barriers. The properties of protein and peptides, which are of major interest in oral delivery, are highlighted in the article. This article reviews the various problems associated and novel approaches for formulation and development of oral protein and peptide drug delivery systems.

Key words: Oral protein delivery, protein and peptide, oral insulin

Peptide and polypeptides are low and/or high molecular weight biopolymers, which yield two or more amino acid on hydrolysis. Peptides and polypeptides are the principle component of the protoplasm of cells and are high molecular weight compounds consisting of alpha amino acid connected together by peptide linkages. These proteins serve as enzymes, structural element, hormones or immunoglobulin and are involved in metabolic process, cell growth, immunogenic defense mechanisms and other biological activities[1-4].

Peptides and polypeptides or proteins are an important class of biological substances which are not only the essential nutrients of human body, but some of the polypeptide hormones like insulin are used in treating various diseases resulting from hormonal deficiency[5]. As this use of peptides and polypeptides for systemic treatment of certain diseases is well accepted in medical practice, research activities are being directed towards the synthesis of large quantities by rDNA technology.

The most common route of administration for protein and peptide drug delivery has been parenteral, although many other routes have been tried with varying degree of success. Routes such as intranasal, transdermal, buccal, intraocular, rectal, vaginal and pulmonary route will deliver the drug to the systemic circulation while avoiding transit through the digestive system[6-12]. A major factor that limits the usefulness of these substances for their intended therapeutic application is that they are easily metabolized by plasma proteases when they reach the peripheral circulation. In addition, adverse effects associated with applying these drugs to the pulmonary or the other mucosal surfaces, may be limiting.

Delivering therapeutically active protein and peptides by the oral route has been a challenge and a goal for many decades. Currently only two biotechnology drugs (Interferon alpha and human growth hormone) that can be given orally are known to be in clinical development in the US[13]. For such drugs to be absorbed through the gastrointestinal tract, they must be protected from enzyme and must traverse through the luminal barriers into the blood stream in an unchanged form. This article reviews the problems associated with the oral delivery of proteins and peptides and presents approaches for the formulation of the delivery system for the same.

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ABSORPTION PROPERTIES OF PEPTIDES

Molecular weight and size:

Molecular weight and size influence the diffusion of drugs through the epithelial layer. As a general rule very large molecule have lower diffusivities and only small molecules (<75-100 Dalton) appear to cross the barriers rapidly[14]. However permeability falls of markedly as the molecular size increases. Several authors have investigated the effects of the molecular weight upon oral absorption of various hydrophilic compounds[15-17].

Conformation, stereospecificity and immunogenicity:

Unlike conventional drugs, peptide drugs generally have primary, secondary and tertiary structures and in solution may adopt several different conformations depending upon their size. It is the prime requisite to preserve the pharmacologically active conformation during the process of formulation and sterilization. The change in conformation can influence membrane permeability. The stereospecificity of the drug must also be preserved since the permeation systems are thought to be stereoslective[18-23]. Peptides are also recognized as often being immunogenic and the use of inert polymers like PEG, PVP and albumin for peptide delivery has been shown to increase resistance to proteolysis and simultaneously decrease peptide immunogenicity[24-25].

Electrostatic charges:

Charge distribution on the peptide change may be even more important than the value of the partition coefficient in predicting permeability of peptides through oral mucosa. Terminal charges or zwitterionic peptide have a negative effect on membrane permeability even though the effective partition coefficient is relatively high[25]. The effect of charge density can be modified to promote peptide absorption by changing the pH of the medium and thus the degree of ionization of the peptides.

PHYSICO-CHEMICAL PROPERTIES

Solubility and partition coefficient:

Peptides, being amphoteric, usually have complex solubility versus pH profile. 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. Unless the N-and C- termini are blocked, peptides are very hydrophilic with a very low octanol-water partition coefficient[26-31] (Table 1). Therefore, to improve the absorption of peptides by passive diffusion, their lipophilicity should be increased.

It is generally recognized from human buccal absorption data that the absorption of drugs from whole oral cavity obeys the pH-partition hypothesis which implies a passive diffusion mechanism. Majority of the proteins are destroyed in the very low pH of the gastric region.

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 porcine or bovine insulin[32-34]. In a study it has been reported that additions of additive like non ionic surfactants (Pluronic F 68) stabilize the peptide formulation against self aggregation (fig. 1).

TABLE 1: LIPOPHILICITY OF SELECTED PEPTIDES[38]

Peptide	Partition coefficient (n-octanol/buffer, pH 7.4)
Insulin	0.0215
Thyrotropin-releasing hormone	0.0376
Luteinizing hormone-releasing hormone	0.0451
Glucagons	0.0633
Substance P	0.2750
Met-enkephalin	0.0305
Leu- enkephalin	1.1200

Peptides show very low octanol-water partition coefficient

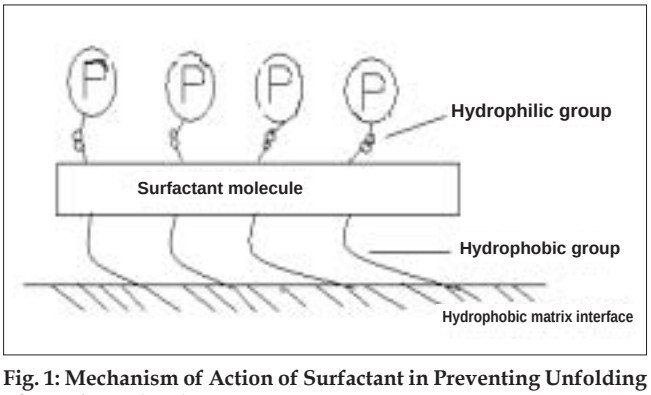

Fig. 1: Mechanism of Action of Surfactant in Preventing Unfolding of Protein Molecule
Surfactants preferentially adsorb on hydrophobic interface and thereby prevent the unfolding/denaturation of protein molecules

In aqueous solution, the three dimensional structure of a protein in its native conformation results in more hydrophobic residues being buried within the interior and more hydrophilic amino acid residues exposed to the aqueous solution. However, when the same protein comes into contact with a hydrophobic surface (like delivery matrix interface), there will be an entropic driving force for the hydrophobic residues that are normally buried within the three dimensional structure to interact with the surface and hence causing unfolding or denaturing of protein molecules. Non ionic surfactants and many other additives were found solve the problem by preferential adsorption on hydrophobic interface (Table 2). Intermolecular hydrogen bonding with water decreases the permeability of protein in lipid membrane[35-37].

BASIS OF ORAL DELIVERY OF PROTEINS

It was observed that the small amount of intact protein and peptide can enter the circulation under normal circumstances[52-54]. After these studies, some finding suggests that at higher peptide dosage the fraction absorbed may be expected to increase due to saturability of the degradation. These finding led the possibility of developing oral peptide delivery system.

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.

Approaches to circumvent metabolic barriers:

The approaches to circumvent proteolytic action should be based entirely upon the principle sight of degradation of the peptide drug; intracellular, luminal or the brush border. The approaches may include[55]; prodrug approach, co-administration of protease inhibitors, use of penetration enhancers and surfactants, use of carrier system and/or formulation approaches

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 (Table 3). Some strategies for prodrug formation include olefenic substitution, d-amino acid substitution, dehydro amino acid substitution[56], carboxyl reduction, retro inversion modification[57], polyethylene glycol (PEG) attachment to amino group[58] and thio-methylene modification. In a recent technology known as Nobex Technology, an amphiphilic protein conjugate is prepared (fig. 2). This technology reduces self-

TABLE 2: STABILIZING ADDITIVES IN PROTEIN DELIVERY

Stabilizing additive	Mechanism of action	Protein stabilized	Ref.
Sugars-trehalose, sucrose Maltose, glucose	Increase Tg thereby enhancing thermal stability of proteins	Collagan, ribonuclease, ovalbomin	39,40
Salts- potassium phosphate, sodium citrate, amm.sulphate	Increase Tg of proteins and self association of proteins, reduce the solubility	Collagan, ribonuclease, ovalbomin	39,40
Cyclodextrins-hydroxypro-pylcyclo dextrins	Not clear; probably by changing the properties of solvent	Porcine growth hormone	41
Heparin	Increase the unfolding temperature by 15-30°	Acidic fibroblast growth factor	42
Metals - zinc	Complexation	hGH against urea induced denaturation Insulin	43-45
Chelating agent- EDTA	Complexation and decrease catalytic degradation by metal	Acidic fibroblast growth factor ribonuclease A	42-46
Surfactant - Non ionic-polysorbates Cationic-cetrimide	Preferential adsorption on hydrophobic interface of delivery matrix;	Nutropin^{R} (r-hGH) with polysorbates; hGH loaded PLG polymer matrix	47-51
Anionic - SLS	Membrane perturbation		

Various additives serve to stabilize the protein delivery matrix by a variety of mechanisms which are shown in the table with the protein stabilized by them

TABLE 3: LIST OF PRODRUGS OF PROTEINS/ PEPTIDES

Parent protein/peptide	Prodrug
S-Gonadotropin Releasing Hormone	S-Gn-RH-A
	Nonopeptide with D-Arg-6
Growth Hormone	GHRP-6
Luteinizing hormone-releasing hormone	Buserelin,
	luproreline, gosereline
Vasopressin	Desmopressin
Somatostatin	Sandostatin

Some important Proteins and their respective prodrugs (Chemically modified proteins to give more stable form with increased plasma half-lives) are shown in the table

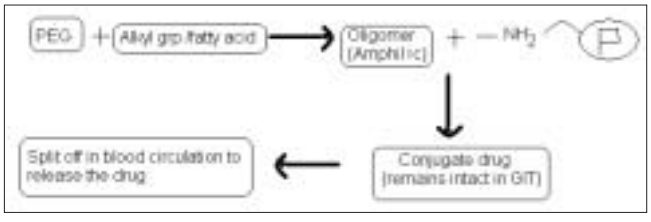

Fig. 2: Nobex Conjugated Technology for Oral Protein Drug Delivery
In this prodrug technology an amphiphilic protein conjugate is prepared by attaching short chain PEG and alkyl group to the amino groups of the protein molecule, which splits off in the blood circulation to release the parent protein.

association, increases penetration and increases compatibility with formulation ingredients than parent drug[59]. By this technology Nobex's conjugated insulin has also been prepared. In this technology short chain PEG and alkyl group are attached to Lys-29 of beta chain. Prepared conjugated insulin was found to be more absorbed and effective. Calcitonin oligomer prepared by this technology showed increased stability and absorption.

Protease inhibitors:

To alter the environment for maximum enzyme stability, protease inhibitors are co-administered with protein and peptides. Various protease inhibitors have been examined with respect to their ability to suppress proteolytic activity (Table 4). Positive results were observed in the oral absorption of tetragastrin, insulin, arginin, vasopressin, rennin inhibitors[60-63].

Use of penetration enhancers:

Penetration enhancers are compounds which, when added to a solute, increase its absorption across biological membranes. Peptides and proteins, due to their molecular size, often require penetration enhancers to achieve therapeutically significant levels of luminal absorption[67]. Surfactants are one of the classes of penetration enhancers. Addition of a surfactant can stabilize a protein against denaturation during several stages from incorporation to the release at the site of delivery. Use of surfactants decreases the self-association and absorption of protein on hydrophobic interface of delivery matrix. They increase penetration and stability of protein and peptide formulations. Besides penetration enhancement, sodium glycocholate inhibits leucine amino peptidase and protect insulin from proteolysis[68].

Use of a carrier systems:

Special types of carriers are used for the poorly absorbed proteins and peptides, which are unstable in the gastro intestinal lumen for their targeting to a specific tissue or organ. A well designed carrier system protects the drug from the intestinal proteases and localizes the drug at or near the cellular membrane to maximize their driving force for passive permeation[69]. Various novel carrier systems for protein and peptide drug delivery have been studied like lipid vesicles, particulate systems, emulsions, bioadhesive systems etc.

Formulation approaches:

A variety of approaches are adopted in formulating oral peptide delivery systems as per the nature of peptide drugs and the delivery matrix. Table 5 shows the general formulation strategies for protein and

TABLE 4: STUDIES OF PROTEIN DRUG DELIVERY WITH PROTEASE INHIBITORS

Drug	Protease inhibitor	Result(s)	Ref.
Insulin	Aprotinin, bactracin, bestatin, Camostatmesilate, chymotrypsin inhibitor FK – 448, sodium glycocholate, soyabean trypsin inhibitor	Significant reduction in insulin digestion and improvement in its intestinal absorption profile.	64 65
Insulin	Camostat mesilate Plasma glucose levels decreased in a dose dependent manner.		66
Vasopressin And its analogues	Aprotinin	Improvement in the activity profile of the drug	65
Calcitonin	Camostat mesilate	Significant improvement in calcitonin delivery	66

Co-administration of protease inhibitors with protein and peptides suppress the proteolytic activity of the enzymes and thereby improve the stability and oral bioavailability of proteins

peptide formulation. An azo polymer, which is stable in GIT but decomposes at the ileocaecal junction have been used for insulin delivery and found to be very promising for oral insulin delivery[70]. Chitosan-EDTA-protease inhibitor conjugates have been used for many peptide delivery[71]. A new class of molecules-N acylated non-α, aromatic amino acid compound was found to increase the absorption of human growth hormone (hGH) by altering the conformation of molecule reversibly and facilitate transport across intestinal mucosa[72]. Several formulations tested for the oral protein delivery include emulsions, liposomes, nanoparticles, soft gelatin coated capsules[73,74].

General method for production of protein formulations are emulsification, coacervation, extrusion, spray drying and polymerization. In all these processes it is highly emphasized that high stress, high temperature, heat and crosslinking agent must be avoided (or minimized), to ensure the stability during the formulation[75,76]. Special procedures like double emulsion method and Prolease microsphere technology may also be adopted[77]. The Prolease process is a spray method of producing microparticles containing proteins using a cryogenic process. In this method, the protein drug is incorporated as a lyophilized powder, and all manipulations involving the matrix polymer (PEG) and the proteins are performed at low temperature (≤- 80°).

As proteins are more stable in solid state than in liquid, its incorporation in solid form in delivery matrix is advantageous. Spray drying and lyophillization are widely used for formulation of protein and peptide delivery system[78]. In a study, hGH was lyophilized to get stable form with reduction in aqueous solubility. It decreased the potential for degradation during release due to decrease in protein molecule mobility and thereby ensured the stability and improved the bioavailability of orally administered peptide hormone.

RECENT ADVANCES IN ORAL PROTEIN DRUG DELIVERY

Biosante Pharmaceuticals has developed a delivery system based on calcium phosphate to administer an oral form of insulin called CAPIC. Calcium phosphate particles containing insulin was synthesized in the presence of PEG-3350 and modified by aggregating the particles with caseins (the principle protein of milk) to obtain the calcium phosphate-PEG-insulin-casein (CAPIC) oral insulin delivery system. The formulation CAPIC was created through a nano-particulate technology, using microscopic particles of calcium phosphate. Studies in diabetic mice showed that oral insulin administration through the new system was effective in reduction and maintenance of normal blood glucose levels[79,80].

A group of research scientists developed mucoadhesive oral Insulin delivery systems using lectin functionalized complexation hydrogels[82,83]. They developed a class of environmentally responsive complexation hydrogels composed of methacrylic acid grafted with ethylene glycol chains (P(MAA-g-EG)) functionalized with wheat germ agglutinin (WGA) to overcome the challenges of oral administration. The drug carriers were designed to firstly minimize the effects of the harsh environment of the gastrointestinal tract and secondly to target delivery of insulin to the upper small intestine by exploiting the pH shift between the stomach and the upper small intestine. Insulin entrapment in the polymer network was unaffected by the WGA functionalization and loading efficiency was determined to be 75% in both functionalized

TABLE 5: APPROACHES OF FORMULATION OF ORAL PROTEIN DRUG DELIVERY SYSTEM[81]

Protein's nature	Formulation approach
Unstable in solution	Lyophilization using cryoprotectants and incorporating drug into delivery matrix as a solid powder.
Adsorb to delivery matrix (PLG)	1. Incorporation of hydrophilic surfactants (Polysorbate 20/80, Pluronic F.68) 2. Addition of another protein as a competitor for adsorption surface
High protein concentration required in delivery system-prone to aggregation	1. Addition of surfactant to reduce self-association 2. Use of less soluble prodrug e.g.- complexation with metal (zinc-insulin)
Poor stability at low pH	1. Lyophilization 2. Formulation in high pH buffer 3. Addition of soluble basic salt in delivery matrix to neutralize acid degradation products of delivery matrix 4. Formulation of microporous delivery matrix rather than monolithic device
Heat sensitivity	Using low temperature homogenization encapsulation process.

On the basis of the nature of protein, various approaches can be explored to formulate the oral protein drug delivery system.

and unfunctionalized microparticles. Recently, on 27 January 2006, Pfizer Inc. obtained the US FDA approval for launching human insulin (rDNA origin) inhalation powder (Exubera) spray. The product had been introduced in the US market in December 2006[84].

CONCLUSIONS

The scientific community has reached a new stage in the understanding of the properties of peptides and proteins and in the manufacturing of these therapeutic agents. In the past, administration of peptides and proteins was believed to be impossible, while nowadays it is expected that the obstacles for effective delivery of therapeutic peptides and proteins will be overcome and delivery systems with better compliance would be made available to the patients.

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