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Oral protein delivery: Current status and future prospect

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ABSTRACT

Advances in biotechnology have produced therapeutically active proteins on a commercial scale, and therapeutic proteins are now extensively applied in medical practices to treat various diseases. Oral delivery of protein drugs is a highly attractive approach, and, naturally, numerous attempts have been made to develop such formulations. Despite various attempts, however, no clinically useful oral formulations have been developed, and this is mainly due to extremely low bioavailability of protein drugs. The effective oral protein delivery needs to overcome barriers related to poor absorption, poor permeation, and degradation in the gastrointestinal tract. Various strategies have been explored for enhancing the bioavailability of orally administered proteins. They include chemical modification of protein drugs, use of enzyme inhibitors, and exploration of special formulation ingredients, such as absorption enhancers and mucoadhesive polymers. This article examines the current technologies under development for oral protein delivery.

1. Introduction

Each year new therapeutic proteins are introduced into the market. Advances in biotechnology have accelerated the economical, large-scale production of proteins, vaccines, and hormones, making them readily available for therapeutic applications in medical practices and clinical studies. Therapeutic proteins have become the drugs of choice for treating numerous diseases due to their exquisite specificity and bioactivity.

Administering drugs by oral route is preferred to any other routes because of its simplicity and convenience. Oral administration of protein drugs, however, is extremely difficult due to their extremely low bioavailability. Development of oral protein formulations requires overcoming obstacles, such as low permeability of large molecules [1], lack of lipophilicity [2], and inactivation or rapid enzymatic degradation in the gastrointestinal (GI) tract [3]. These unfavorable physicochemical properties of proteins present monumental challenges to pharmaceutical formulation scientists.

The objective of this article is to review the general approaches that have been used to improve bioavailability of orally delivered proteins by overcoming various physiological barriers, and to provide the information on oral protein delivery technologies currently under investigation.

2. Main approaches used for oral protein delivery

Through the years, various strategies have been tried for improving bioavailability of therapeutic proteins. The approaches commonly used in formulating oral protein delivery systems include using specific excipients, such as absorption enhancers, enzyme inhibitors, and mucoadhesive polymers, and using formulations allowing protection of protein drugs from the harsh environment in the GI tract, as listed in Table 1.

2.1. Absorption enhancers

To improve the permeation of protein drugs through the intestinal wall, absorption enhancers have been used as formulation components, which include detergents, surfactants, bile salts, and Ca^{2+}-chelating agents [4,5]. Detergents or surfactants enhance the transcellular transport by disrupting the lipid bilayer, rendering the cell membrane more permeable [6]. Chelating agents form complexes with calcium ions and rupture tight junctions to facilitate paracellular transport of proteins. Long alkyl chain enhancers, including fatty acid sodium caprate and acyl carnitines, have shown similarly improved absorption via transient opening of tight junctions [7,8]. Zonula Occludens toxin is known to be a safe and effective enhancer, altering intestinal epithelia tight junctions transiently for passage of macromolecules, such as insulin, through mucosal barriers [9].

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Table 1
Approaches used in oral protein formulations.

Approaches	Systems	Outcomes for absorption	Drawbacks
Absorption enhancers	Bile salts, fatty acids, surfactants, salicylates, chelators, zonular occludens toxin	Increase membrane permeation	Available transport of both protein/peptide drugs and undesirable molecules present in GIT
Enzyme inhibitors	Sodium glycocholate, camostat mesilate, bacitracin, soybean trypsin inhibitor, aprotinin, CkOVM, DkOVM, polymer–inhibitor conjugates	Resist enzyme degradation present in stomach and intestine	Available inducing severe side effects in chronic therapy
Mucoadhesive polymers	P(MAA-g-EG) hydrogel microparticles, lectin-conjugated alginate microparticles, thiolated polymers Gastrointestinal mucoadhesive patch system	Site-specific delivery and improve membrane permeation	Limitation due to the natural mucus turnover in intestine
	Mucoadhesive polymer–inhibitor conjugates	Site-specific drug delivery and resist enzyme degradation	Limitation due to the extensive costs of certain enzyme inhibitors
Formulation vehicles	Emulsions –S/O/W emulsion –O/W emulsion –Enteric-coated O/W emulsion	Protect drug from acid and luminal proteases in the GIT and enhance permeation through intestinal mucosa	Physicochemical instability in long-term storage and requirement for storage at low temperatures
	Liposomes –Double liposomes –Fusogenic liposomes –Crosslinked liposomes	Improve physical stability and increase membrane permeation	Low stability of liposomes
	Microspheres –Eudragit S100 microspheres –pH-sensitive P(MAA-g-EG) microspheres	Prevent proteolytic degradation in stomach and upper portion of small intestine. Restrict release of drug to favorable area of GIT	Concerns of protein stability during processing, release and storage
	Nanoparticles –PMAA/chitosan/PEG nanoparticles –Polystyrene/chitosan/PLA–PEG nanoparticles	Prevent enzymatic degradation and increase intestinal epithelial absorption	Low loading efficiency of hydrophilic drugs, difficulty of precise size control and avoidance of particle aggregation

Abbreviations: CkOVM, chicken ovomucoid; DkOVM, duck ovomucoid; S/O/W, solid-in-oil-in-water; P(MAA-g-EG), poly(methacrylic acid-g-ethylene glycol); PEG, Poly(ethylene glycol); PLA, poly(lactic acid); GIT, gastrointestinal tract.

Co-administration of proteins with carrier molecules can enhance bioavailability of proteins [10–13]. For example, lipophilic carrier enhancers facilitated the absorption of proteins, such as insulin [13,14], human growth hormone [12,15], calcitonin [16], and recombinant parathyroid hormone (rPTH) [11]. Carrier molecules temporarily stabilize the partially unfolded conformations of proteins exposing their hydrophobic side chains. Thus, the carrier molecules altered lipid solubility, allowing them to gain access to pores of integral membrane transporter. This result in enhanced absorption through lipid bilayers [17]. It is noted, however, that the use of these absorption enhancers is able to enhance the transport of not only proteins but also undesirable molecules present in the GI tract when cell membranes are permeabilized or tight junctions opened [18].

2.2. Enzyme inhibitors

One of main barriers in oral protein delivery is that proteins can be rapidly degraded by various proteolytic enzymes. To minimize degradation of proteins by various proteolytic enzymes, researchers have used trypsin or α-chymotrypsin inhibitors, such as pancreatic inhibitor [19], soybean trypsin inhibitor [19], FK-448 [20], camostat mesylate [21], and aprotinin [22]. As new class of enzyme inhibitors, chicken and duck ovomucoids have been recently identified [23,24]. For example, a formulation containing insulin and duck ovomucoid offered 100% protection against trypsin- or α-chymotrypsin-mediated insulin degradation. Polymer–inhibitor conjugates such as carboxymethylcellulose–Bowman Birk inhibitor and carboxymethylcellulose–elastinal (CMC–Ela) have offered in vitro protection against trypsin, α-chymotrypsin and elastase [25]. In particular, CMC–Ela displayed higher inhibitory activity toward elastase that nearly 33% of therapeutic agent remained stable against enzymatic attack even after 4 h of incubation. However, protease inhibitors can influence the absorption of other proteins and induce severe toxic effects during chronic drug therapy.

2.3. Mucoadhesive polymeric systems

Stimuli responsive and mucoadhesive polymeric systems have been of great interest as protein delivery carriers because they exhibit dramatic changes in network structure or swelling behavior in response to changes in environmental factors, such as pH, temperature, enzymes, light, electric field or ionic strength [26].

Mucoadhesive polymeric systems could extend the residence time at the site of drug absorption. They maintain intimate contacts with the mucus to increase the drug concentration gradient and ensure immediate absorption without dilution or degradation in the luminal fluid [27,28]. The mucoadhesive controlled release systems can be designed for simultaneous release of both drug and inhibitor, allowing proteins to be efficiently protected [29]. The pH-sensitive mucoadhesive polymeric carriers have been used to protect the protein drugs from proteolytic degradation in the stomach as well as in the upper portion of the small intestine. For instance, poly(methacrylic acid-g-ethylene glycol) [P(MAA-g-EG)] exhibits pH-dependent swelling behavior resulting from the formation or dissociation of interpolymer complexes [30,31]. The polymeric microparticles loaded with insulin showed a rapid burst release with high insulin absorption in the intestine, resulting in a greater hypoglycemic effect without detectable mucosal damage [32]. P(MAA-g-EG) hydrogels showed very high (~10%) pharmacological availability of orally given insulin [33,34].

Thiolated polymers (thiomers), mucoadhesive-based polymers with thiol-bearing side chains, have been considered as a promising alternative in non-invasive protein delivery. Their strong mucoadhesive properties are due to additional covalent bonds between thiol groups of thiomers and cysteine-rich subdomains of mucus glycoproteins [35]. Orally administered thiomer-based insulin tablets could significantly decrease blood glucose levels for 24 h as compared with subcutaneous injections [36]. However, the adhesion properties of thiomers might be changed because the natural mucus turnover in the human intestine is in the range of 12–24 h [37]. Thus, the limited adhesion of thiomers to the mucus

layer needs to be considered when they are applied for long-term delivery in the intestine.

Oral formulations made of mucoadhesive polymers may not have a sufficient protective effect against proteolytic enzymes. Thus, mucoadhesive polymer–protease inhibitor conjugates have been developed to achieve efficient protection. This systems could reduce presystemic metabolism of protein drugs, exclude the undesired disturbance in digestion of nutritive proteins due to the reduced dilution effects of inhibitors, exclude systemic toxic side effects of inhibitors, specifically target the sites in the GI tract, and reduce dose requirements for enzyme inhibitors [38]. Extensive costs for certain enzyme inhibitors, however, should be considered to guarantee affordable production costs in large-scale production of such conjugates [39].

2.4. Particulate carrier delivery systems

Various particulate carriers for oral protein delivery, such as emulsions, liposomes, microspheres, and nanoparticles, have been applied to protect proteins from acidic and enzymatic degradation in the harsh environment of the GI tract. These particulate systems could also provide enhanced delivery of drugs across the epithelial mucosa, control the release rate, and target drug delivery to specific sites in the intestine.

Emulsions protect proteins from chemical and enzymatic breakdown in the intestinal lumen. The solid-in-oil-in-water (S/O/W) emulsion developed by using a lipophilic surfactant-coated insulin avoided degradation and enhanced permeation through the intestinal mucosa [40]. This system showed hypoglycemic activity over several hours after oral administration to diabetic rate. But, critical drawbacks of this formulation are physical–chemical instability in long-term storage and the requirement for storage at low temperatures [41]. Their drawbacks could be overcome by developing the dry emulsion formulations prepared by spray drying [42], lyophilization [43], or evaporation [44]. A dry emulsion formulation in which the surfactant–insulin was entrapped in the oil phase of a solid formulation was further enteric-coated with a pH-sensitive polymer [41]. Such formulation showed responsiveness against the external pH change and the presence of lipase under the stimulated GI conditions.

Liposomes have been exploited to improve the absorption of proteins from the intestinal tract. For instance, liposomal system including both insulin and sodium taurocholate markedly decreased blood glucose levels after oral administration, and showed a high in vitro/in vivo correlation in the Caco-2 cell monolayer model [45]. However, orally administered liposomes are prone to degradation by the combined effects of the acidic pH of stomach, bile salts and pancreatic lipase. Langer and his colleagues developed the polymerized liposomes with double bond-containing liposomes to improve the stability of liposomes against the harsh conditions in the GI tract [46–48]. The polymerized liposomal system could also incorporate proteins into both hydrophilic and hydrophobic layers of the liposomes, either during or after polymerization. The polymerized liposomes, however, cannot increase the poor permeability of the protein drugs through the epithelial layer of the GI tract.

Microspheres manufactured from natural or synthetic polymers by spray drying, double emulsion, and phase separation-coacervation methods have been used as another oral protein delivery approach [49]. P(MAA-g-EG) microspheres containing insulin were not swollen in the acidic condition of the stomach due to the formation of intermolecular polymer complexes, and thus, insulin within the microspheres could be protected from acidic and proteolytic degradation. After the exposure to the neutral and basic pH environments in the small intestine, dissociation of the complexes causes swelling of microspheres, resulting in insulin release. Within 2 h after oral administration of insulin-containing microspheres, strong dose-dependent hypoglycemic effects were observed in both normal and diabetic rats [50]. These pH-sensitive microspheres restrict the release of proteins to favorable area of the GI tract. For this approach to be useful, the stability of microencapsulated proteins during processing, release and storage, and protein sensitivity to both polymer and solvents need to be considered, in addition to the permeability issue.

Proteins which are encapsulated in polymeric nanoparticulate carriers are less sensitive to enzymatic degradation through their association with polymer compared to their native counterpart. Furthermore, once orally administered, nanoparticles could be absorbed by the intestinal epithelium, especially through Peyer's patches, for systemic distribution [51]. In addition, the absorption of proteins encapsulated in nanoparticles can be influenced by particle size, surface charge, ligands, and the dynamic nature of particle interactions in the gut. Polymeric nanoparticles consisting of hydrophobic polystyrene, bioadhesive chitosan, and PLA–PEG were detected in both epithelial cells and Peyer's patches after intraduodenal administration of nanoparticles [52]. Based on a novel reverse micelle–solvent evaporation method, biodegradable polymers incorporating insulin–soybean phosphatidylcholine complex showed hypoglycemic effects up to 6 h after oral administration into diabetic rats [53]. Difficulties of using nanoparticles are the low incorporation efficiency of hydrophilic drugs, lack of precise control of drug release, tendency of particle aggregation, and the possible accumulation of non-degradable particles in tissues [54].

3. Oral protein delivery technologies developed for clinical applications

Numerous delivery systems for oral protein delivery have been actively developed, especially by pharmaceutical companies, in a hope to make them clinically useful. Although most of the development works still remain in the development stage, many of them have progressed beyond the proof-of-concept stage to the clinical trials. Those technologies for oral protein delivery under development by pharmaceutical companies are summarized in Table 2.

3.1. Emisphere: Eligen™ technology

Eligen™ technology is a macromolecule-delivering platform technology developed by Emisphere Technologies, Inc. Emisphere delivery agents, which are based on sodium N-[8-(2-hydroxybenzoyl)aminocaprylate] (SNAC), interact with the drug molecules to create a weak, non-covalent association, the drug remaining chemically unmodified [15,55,56]. As shown in Fig. 1A, the formed lipophilic drug/carrier complex is claimed to be capable of transport across the epithelial membrane [11]. Due to the weak association between carrier and drugs, the interaction is reversible, and it occurs spontaneously by simple dilution on entering the blood circulation. The released drugs are supposed to be in the therapeutically active state. Unlike the traditional penetration enhancers, Emisphere delivery agents were claimed to not cause histological damages to the intestinal epithelium and are applicable to diverse group of drug molecules ranging in size from 500 to >150,000 daltons. Eligen™ technology was used to develop various types of oral formulations including solutions, tablets, and capsules.

Emisphere delivery agents have been evaluated in various animal models as well as in humans to enhance the delivery of various macromolecules such as insulin [14], recombinant human growth hormone (rhGH) [12,15], calcitonin [16] and recombinant parathyroid hormone (rPTH) [11]. As a representative example, following oral administration of insulin in combination with SNAC in 10 fasted healthy volunteers, insulin was rapidly absorbed into the systemic circulation and peak plasma concentration occurred

Table 2
Protein oral delivery technologies under development by companies.

Company	Systems	Product name	Outcomes for absorption	Products currently available or under development
Emisphere	Carrier molecules	Eligen®	Increase membrane permeability	Calcitonin, GPL-1, PYY, Insulin, Growth hormone, parathyroid hormone, heparin
Altus	Protein crystallization	CLEC®	Stable against proteolysis and self-digestion	Calcitonin, other polypeptides, Lipases, esterases, and proteases
BioSante	Calcium phosphate nanoparticles	BioOral™	Protect proteins from acidic degradation and improve membrane permeability	Insulin and vaccines
Generex	Spray device and aerosol particles	Oral-Lyn™	Penetrate the buccal epithelium	Insulin, Macrotonin
NOBEX/ Biocon	Amphiphilic oligomers	HIM2	Resist enzyme digestion and increase membrane permeation	Insulin, enkephalin, calcitonin, parathyroid hormone
Apollo Life Science	Nanoparticles	Oradel™	Protect proteins from enzyme digestion in the stomach and facilitate the transport of proteins in the intestine	Insulin and TNF blocker
Eli-Lilly	Oral formulation	AI-401	Protect proteins from enzyme digestion	Insulin
Provalis PLC	Lipid-based microemulsion	Macrulin™	Protect proteins from proteolysis or acidic degradation, and enhance the protein absorption in GIT	Insulin, salmon calcitonin
Endorex	Polymerized-liposomes	Orasome™	Protect proteins from the stomach and upper GIT	Insulin and growth hormone, vaccines

Abbreviations: CLEC, Cross-Linked Enzyme Crystal; HIM2, hexyl-insulin monoconjugate; GLP-1, glucagon-like peptide; PYY, peptide YY; TNF, tissue necrosis factor.

within 25 min [57]. In clinical study in patients with Type II diabetes, the insulin capsule containing 10 mg of insulin and 200 mg of SNAC, when administered 30 min prior to the standardized meal, reduced post-prandial excursion, produced a marked increase in systemic insulin levels.

The feasibility of Eligen™ technology for the development of various formulations for enhanced oral delivery of therapeutic macromolecules and small charged molecules has been explored in animals and humans. Although Eligen™ technology has shown promising results for oral delivery of various therapeutic macromolecules, the clinical application of such system appears to be difficult. The formulation is known to cause nausea in patients, and the amount of the delivery agent is orders of magnitude higher than the protein drugs, making it very inconvenient for patients to take. Most importantly, no clinical efficacy of such system has been demonstrated to date.

3.2. Altus: protein crystallization

One approach of maintaining the protein bioactivity under various conditions is to prepare protein crystals. Crystallization of therapeutic macromolecules can offer several significant advantages (Fig. 1B). First, crystallization is a powerful tool to isolate and purify proteins [58]. Second, the concentrated protein crystals are beneficial for certain drugs which require high doses at the delivery site [59]. Third, the protein stability in crystalline form is much higher than that of soluble or amorphous form [60]. Cross-Linked Enzyme Crystal (CLEC®) process mainly consists of two steps including batch crystallization of enzymes and chemical intercrosslinking of enzyme microcrystals (1–100 μm) with glutaraldehyde. These two steps require extensive optimization in order to ensure high activity and stability. Altus has produced CLEC® products of the enzymes, such as lipases, esterases and proteases. CLEC® technology, however, has some risks as follows. A protein of interest may not crystallize easily or not at all, and the protein in the crystalline state may be inactive. Thus, the protein crystallization approach still remains as the trial-and-error approach that requires numerous reiterations in finding the right crosslinking method and reagent.

3.3. BioSante: BioOral system (calcium phosphate nanoparticles)

Calcium phosphate (CAP) nanoparticles were originally developed as a vaccine delivery system. The preclinical studies demonstrated that CAP nanoparticles are non-toxic, and cause no adverse reaction at the site of administration following oral and intramuscular administration as vaccine carriers [61,62]. BioSante Pharmaceuticals discovered that CAP nanoparticles have the ability to deliver protein drugs in non-invasive and non-injectable ways [63,64]. The oral insulin formulation, called CaP–PEG–Insulin–Casein (CAPIC), was constructed by aggregating casein (the principal protein of milk) around a proprietary formulation of PEG and insulin (Fig. 1C). In CAPIC formulation, casein is one of the key components of the formulation and makes up 2–3% of the drug. Due to its non-degradability in acid and mucoadhesiveness, casein can protect the insulin as it passes through the stomach and into the small intestine, and allows the drug to remain concentrated at the site of absorption. Calcium phosphate plays an important role in reducing acid-induced degradation. These features of CAPIC lead to significantly increase in the half-life and mean resistance time of insulin in the body. In a fasted diabetic mouse model, a single dose of CAPIC administered directly into the stomach could rapidly reduce the blood glucose levels by 80% within the first hour of treatment [65]. In fed mice, CAPIC reduced 50% of blood glucose levels within 3 h, and glucose returned to previous levels after 5 h. Currently, BioSante is developing a product pipeline of hormone therapy to treat both men and women. CAP is also useful for developing novel vaccines, including bio-defense vaccines such as anthrax and ricin.

3.4. Generex: Oral-Lyn™

Generex Biotechnology Corporation has developed a platform delivery system, RapidMist™, a device similar to that used by people with asthma [66]. Human insulin is formulated with minimal amounts of generally regarded as safe (GRAS) excipients (e.g., enhancers, a nonchlorofluorocarbon propellant, and stabilizers which prevent denaturation on aerosolization and stabilize aerosol particles) resulting in micelles larger than 7 μm, which is known to be too large to enter into the lungs. The formulation, called Oral-Lyn™, is delivered by the RapidMist™ device to the oral cavity (Fig. 1D (a) and (b)) and penetrates the buccal epithelium and enters the rich network of blood vessels. The studies demonstrated that radioisotope-labeled insulin did not enter the respiratory tract (Fig. 1D (b)). Oral-Lyn™ is claimed to be a safe formulation since the administered Oral-Lyn™ formulation without insulin to 40 dogs or nearly 1000 patients did not show any abnormalities in the oral cavity. Several clinical studies showed that insulin levels

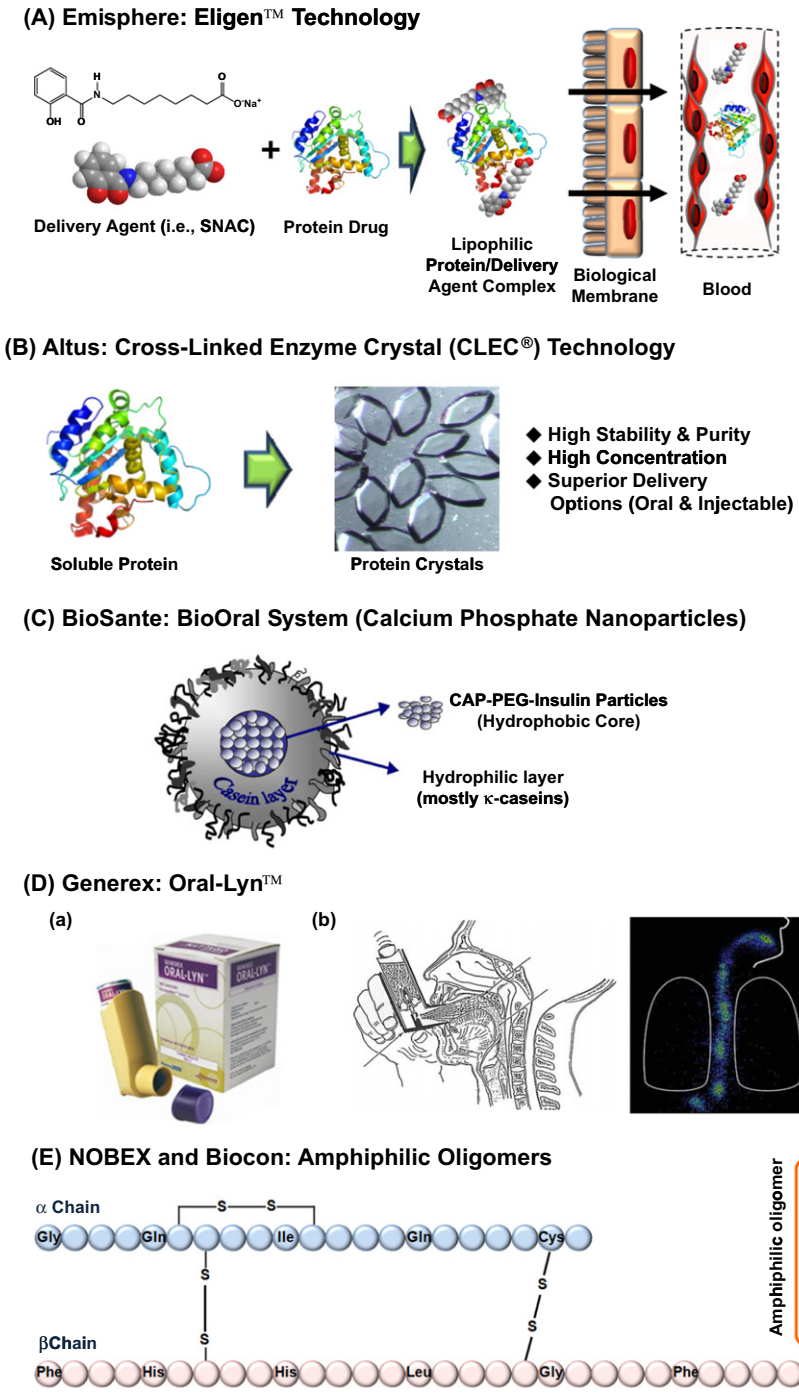

Fig. 1. Representative examples of oral protein delivery technologies developed for clinical applications. A. Emisphere: Eligen™ technology. Delivery agent associates with protein drug to create a lipophilic and transportable complex. Due to the weak association, complex dissociates by simple dilution on entering the blood stream [11]. B. Altus: Cross-Linked Enzyme Crystal (CLEC®) Technology. C. BioSante: Calcium Phosphae–PEG–Insulin–Casein (CAPIC) oral insulin delivery system [65]. D. Generex: Oral-Lyn™: (a) the RapidMist™ device, (b) the formulation is delivered to the oral cavity for absorption via the oral mucosa. Radionucleide studies demonstrated that no labeled insulin entered the respiratory tract [66]. E. NOBEX and Biocon: Amphiphilic Oligomers: Chemical structure of hexyl-insulin monoconjugate 2 (HIM2), in which a single amphiphilic oligomer is linked to the primary amine group of the Lys-29 residue in beta chain of human insulin.

rose in a dose-dependent manner. Oral-Lyn™ formulation also showed the promising results in Type II patients who did not respond to conventional treatments, such as diet and exercise, piogliazone, metformin and sulphonylureas. Oral-Lyn™ is in various stages of clinical development around the world, and now, this product is under development for treatment of Types I and II diabetes [67,68]. Recently, Oral-Lyn™ was approved in India for import, commercial marketing and sale for the treatment of Types I and II diabetes and Generex Biotechnology has been marketed under the brand name Oral Recosulin™ since January 2009 [67].

3.5. NOBEX and Biocon: amphiphilic oligomers

Introducing hydrophobicity to proteins by chemical modification with lipophilic moieties may be of particular benefit to transcellular absorption or to the increased protein stability. NOBEX

Corporation and Biocon have jointly developed a covalently modified insulin product, hexyl-insulin monoconjugate 2 (HIM2), in which a single amphiphilic oligomer is chemically linked to the primary amine group of the Lys-29 residue in beta chain of human insulin [69,70] (Fig. 1E). The altered physicochemical properties of HIM2 resist enzymatic degradation and facilitate absorption when administered as an oral semisolid formulation in hard gelatin capsules. Single oral dosage of HIM2 is effective in controlling postprandial glycemia in Type II diabetes [69]. HIM2 also prevented the expected rise in plasma glucose concentrations in fasting, insulin-deprived adult patients with Type I diabetes [70,71]. HIM2 may better reduce the risk of hypoglycemic events than subcutaneous insulin therapy and may be useful in the management of Types I and II diabetes with inadequate post-prandial glycemic control. Recently, Biocon completed Phase IV trials for insulin and marketed it as Insugen in India.

3.6. Apollo Life Science: Oraldel™

Apollo Life Sciences have developed Oraldel™ drug delivery technology with the features to protect and transport insulin molecules by encapsulating them in the matrix of nanoparticles made up of a sugar-based (carbohydrate) protective polymer coated with vitamin B12 molecules [72–74]. Nanoparticles containing insulin were claimed to be pulled through the wall of the intestine using an active transport mechanism in the GI tract for the absorption and cellular uptake of the relative large vitamin B12 molecules [75]. Vitamin B12 molecules are suggested to protect insulin from enzymatic digestion in the stomach as well as facilitate the transport of insulin across the small intestine into the blood stream. Recently, Apollo Life Sciences has indicated that it has a unique production method whereby up to 100% of insulin molecules are entrapped in the matrix of the Oraldel™ nanoparticles. When received orally with Apollo's Oradel™ formulation, blood glucose levels could be lowered to within normal range for up to 12 h in diabetic rats. Apollo has also manufactured nanoparticles of different sizes to deliver a range of other drugs such as anti-inflammatory proteins (TNF blockers) for the treatment of rheumatoid arthritis.

3.7. Eli-Lilly: oral formulation (AI-401)

AI-401 is an oral formulation of recombinant human insulin. Unlike Oral-Lyn™ and HIM2, AI-401 technology is not in trials solely for the treatment of Type 1 diabetes but also for prevention of progression. The theory behind this technique is called "oral tolerance therapy". Small drug dosages (too small to affect blood glucose levels) are claimed to result in suppression of autoreactive T cells that have been attacking the pancreas. Autoimmune Inc. has agreements with Eli-Lilly to develop and market the product. The interim results of US Phase II trial of AI-401 for the treatment of diabetes showed the feasibility of oral insulin therapy, delaying beta-cell destruction in the pancreas, and thus preserving endogenous insulin secretion in newly diagnosed Type I diabetic patients. The Eli-Lilly company is AutoImmune's worldwide partner in autoimmune (Type I) diabetes [76]. The final data from the oral insulin arm of the NIH sponsored Diabetes Prevention Trial-Type I showed a statistically significant benefit for patients with Type I diabetes [77].

3.8. Provalis PLC: Macrulin™

The lipid-based microemulsions can load protein drugs, and have been proposed to enhance the bioavailability of orally administered protein drugs. This system may protect proteins from proteolysis or acidic degradation to enhance the protein absorption in the GI tract. Provalis PLC has developed an insulin-based oral pill (Macrulin™) [78]. The technology uses a water-in-oil (W/O) microemulsion in which aqueous phase contains insulin and the oil phase contains cholesterol, lecithin (a biological surfactant and a major component of membrane lipids) and non-esterified fatty acids. The lipid phase acts as a vehicle to enhance the uptake of insulin from the small intestine following oral administration. Macrulin™ is in Phase II clinical trials for the management of Type II diabetes.

3.9. Endorex: Orasome™

Orasome™ delivery system, a proprietary stable liposome technology, was claimed to promote the oral bioavailability of several hormone polypeptide therapeutics, insulin and human growth hormone. Orasome technology developed by Endorex Corporation essentially encapsulated drugs in a protective coating using liposomes through the stomach and upper GI tract, allowing them to be released in the lower intestine and eventually into the blood stream. As previously described, the Orasome™ system is based on the polymerized liposomes containing drugs [48]. This system can withstand the harsh conditions of the GI tract such as exposure to acidic pH, bile salts, and detergents by polymerization [46,47]. Currently, Orasome™ system is under development for oral delivery of growth hormone and insulin.

4. Conclusions and future prospects

The technologies that have been used to improve bioavailability of orally delivered proteins are based on specific approaches of either preventing degradation by acid and enzymes in the GI tract or increasing the permeability of proteins through the epithelial layer of the GI tract. While the approaches taken to date have shown some effects in protecting protein drugs from degradation and in increasing absorption from the GI tract, none of the approaches proposed or under development have been proven to be good enough for clinical applications. The review on the literature clearly indicates that the current approaches on oral protein delivery are based on a simple assumption that may not reflect the reality. The current assumption is that the bioavailability of orally delivered proteins will increase by protecting the proteins from degradation and by increasing the permeability. Protecting proteins from acidic and enzymatic degradation is relatively easy to achieve. Increase in protein permeability through the epithelial cell layer of the GI tract, however, is extremely difficult, if not impossible. A variety of manipulations, such as chemical modification of proteins and adding absorption enhancers, have shown some increase in oral bioavailability. But, no approaches have ever demonstrated that the oral protein delivery can be achieved in a reproducible manner after repeated dosing.

Of the many protein drugs, insulin is at the top of the list for oral delivery. Since the diabetic patients require multiple daily administration of insulin throughout their lifetime, the potential market is so enormous that almost all oral protein delivery technologies are focused on insulin delivery. Numerous references have shown the efficacy of orally administered insulin in small animals, usually mice, but almost all references fail to clarify three important criteria necessary for insulin delivery. For oral insulin delivery to be clinically useful, it should deliver an accurate amount of insulin fast enough to control the glucose concentration in blood, and the function has to be reproducible each time oral insulin is delivered. Simply showing some bioavailability after oral administration of insulin is rather meaningless in treating the diabetic patients.

To bring oral protein formulations into the market, i.e., clinically useful, there are many conditions to be satisfied. For instance, the low bioavailability implies a large variation in absorption and a high manufacturing cost, which is unacceptable for development of most proteins. Furthermore, recently developed technologies must be assessed in terms of easy scale-up production, protein quality, bioavailability, and toxicity. Since most therapeutic proteins require chronic administration, the effects on intestinal absorption of age, genomic factors, physiological conditions, individual variations, and long-term oral administration of absorption carriers must also be carefully evaluated.

In developing oral protein formulations in the future is that one needs to consider developing new biomaterials that can be more effectively protect and deliver protein drugs. The integrated and personalized 'smart' delivery systems capable of fully autonomous delivery and site-specific receptor targeting will have a considerable impact on drug administration. Additionally, integration of advanced biotechnology, biopharmaceuticals, and a wide variety of molecular imaging techniques has reached a new stage in the understanding of the properties of proteins and in the manufacturing of these therapeutic agents. In protein oral delivery, increase of oral bioavailability requires more than protecting protein drugs in the GI tract, but it is the first step in achieving successful oral bioavailability of protein drugs. The real challenge in oral protein delivery is to somehow enhance the absorption from the GI tract. Showing that proteins can be absorbed from the GI tract is far different from using oral administration as a means to deliver protein drugs on a regular basis. Protein drugs have to be delivered with the predetermined doses at predictable absorption kinetics with reproducible manner. The formulation scientists need to clearly understand the challenges facing oral protein delivery, and furthermore, oral delivery of therapeutic proteins is lot more than simply showing the feasibility of oral protein delivery in mice. Quite often, the development of formulations for oral protein delivery has been based on commercial reasons, rather than the scientific potential, and incremental improvements in formulation are not expected to solve the huge challenges facing the oral protein delivery field. It is time for all the formulation scientists to examine the combination of problems as a whole to come up with breakthrough solutions in oral protein delivery.

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