Examining the challenges underlying the delivery of therapeutic proteins

Stephen T. Buckley
Diabetes Research Unit, Novo Nordisk A/S, Denmark
spby@novonordisk.com

Introduction
Following the introduction of human insulin – the first recombinant therapeutic protein – over 25 years ago, the number of marketed biological products has grown to over 100 with many more in development; while the frequency at which they are employed is ever increasing. The inherent physicochemical properties of proteins, i.e., large molecular size, instability (both physical and chemical) and limited permeability across biological barriers, confer unique challenges with respect to their formulation and delivery. Accordingly, the vast majority of marketed protein therapeutics are currently administered via the parenteral route. A number of approaches have been successfully employed to modulate and optimise the delivery of proteins including amino acid substitution, PEGylation and acylation of the parent compound, while most recently efforts have intensified to realise the possibility of non-invasive delivery.

Modification of proteins
In their native form, proteins exhibit rapid degradation and clearance upon administration, thus giving rise to low exposure. In order to realise a protein’s therapeutic potential, engineering and chemical modification of the molecule is often necessary. In this regard, methods such as altering amino acid(s) of the backbone or attaching a chemical group can confer beneficial gains in terms of stability, half-life and efficacy.

The primary structure of the protein may be modified by means of amino acid substitution in order to achieve the desired pharmacokinetic profile in vivo. This technique has been successfully employed in the design of a number of insulin analogues. The rapid-acting insulin analogue, NovoRapid®, is one such example. By replacing the proline residue at B28 with an aspartic acid, an analogue with a much more rapid onset of action is achieved, which is capable of controlling the rapid postprandial release of glucose.

By linking the carboxylic acid group of a fatty acid to the amine group of the N-terminal residue of a protein through an amide bond, so-called “acylation”, an extended plasma half-life and stability is conferred. Insulin detemir (Levemir®), a long-acting insulin, and liraglutide (Victoza®), a long-acting GLP-1 analogue, are two such examples. Collectively, their acylated structure confers a prolongation of their pharmacokinetic profile, thus facilitating use as part of a basal bolus regimen (Levemir®) and once daily administration (Victoza®). Similarly, by means of PEGylation or conjugation to Fc fragments an analogous effect can be obtained.

Non-invasive (oral) delivery
While the aforementioned technologies confer improved pharmacokinetics and enhanced ease-of-use for patients, the invasive nature of parenteral administration remains a barrier for many. The development of non-invasive delivery systems for proteins and peptides offers the promise of improved patient compliance compared to conventional parenteral administration. In this regard, the oral route appears particularly attractive.

Moreover, in the case of certain protein therapeutics (e.g., insulin), the physiological response elicited may exhibit a pharmacodynamic profile which more closely resembles the natural physiological response. However, delivery of protein therapeutics is severely hindered by poor absorption across the intestinal barrier and extensive degradation by proteolytic enzymes (Figure 1). Thus, effective
Formulation strategies are required to overcome these impediments, in order to achieve acceptable oral bioavailability with low intra-subject variation.

![Diagram of the gastrointestinal tract with absorption and variability indicators.](image)

**Figure 1.** The challenges presented in delivering therapeutic proteins and peptides via the oral route.

On account of its intrinsic barrier properties, absorption of large molecules across the intestinal epithelium is dramatically curtailed. The native barrier structure of the gastrointestinal tract is defined by intercellular junctions (tight junctions) localised towards the apical side of differentiated enterocytes.

A multiplicity of approaches has been examined to promote efficient passage of proteins across the intestinal barrier. Amongst the most widely examined is co-administration with a permeation enhancer, which serves to modulate the barrier function and thus promote increased uptake of the concurrently delivered protein. A chemically diverse range of compounds have been shown to exhibit permeation enhancing properties (e.g., fatty acids, acylcarnitines, bile salts etc.). Via transient modulation of tight junction structures and/or membrane perturbation, such compounds can dramatically augment the extent to which an otherwise relatively impermeable protein is absorbed.

Exploitation of active transport mechanisms of the intestinal tract may also facilitate improved uptake. Receptor-mediated absorptive processes native to the intestine (e.g., transferrin, vitamin B12, FcRn receptor) can be utilised to expedite absorption into the bloodstream. However, in a number of instances, the capacity of such routes may be insufficient to ensure adequate quantities of the protein are absorbed.

Micro- and nanoparticulate-based systems composed of natural or synthetic materials such as polymers or lipids, exhibit favourable traits from the perspective of oral application of proteins. Via engineering, a delivery system which bestows permeation enhancing, mucoadhesive and/or protective properties can be achieved, while also displaying favourable formulation (e.g., rapid dissolution) and safety (e.g., biodegradable) attributes.

**Conclusion**

The fundamental properties of proteins and peptides confer significant challenges vis-à-vis their delivery via the oral route. Nevertheless, significant strides are being made to design and optimise drug delivery systems capable of achieving satisfactory and consistent bioavailability following oral administration.

**References**