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Review

Glycopeptide drugs: A pharmacological dimension between “Small Molecules” and “Biologics”

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Highlights

- Glycosylation can dramatically improve stability of peptides.
- Glycopeptides exhibit enhanced penetration of the blood-brain barrier (BBB) compared non-glycosylated peptides.
- Glycopeptide analogues of their native neuropeptides generally retain potency and efficacy at their target receptors.

Abstract

Peptides are an important class of molecules with diverse biological activities. Many endogenous peptides, especially neuropeptides and peptide hormones, play critical roles in development and regulating homeostasis. Furthermore, as drug candidates their high receptor selectivity and potent binding leads to reduced off-target interactions and potential negative side effects. However, the therapeutic potential of peptides is severely hampered by their poor stability *in vivo* and low permeability across biological membranes. Several strategies have been successfully employed over the decades to address these concerns, and one of the most promising strategies is glycosylation. It has been demonstrated in numerous cases that glycosylation is an effective synthetic approach to improve the pharmacokinetic profiles and membrane permeability of peptides. The effects of glycosylation on peptide stability and peptide-membrane interactions in the context of blood-brain barrier penetration will be explored. Numerous examples of glycosylated analogues of endogenous peptides targeting class A and B G-protein coupled receptors (GPCRs) with an emphasis on O-linked glycopeptides will be reviewed. Notable examples of N-, S-, and C-linked glycopeptides will also be discussed. A small section is devoted to synthetic methods for the preparation of glycopeptides and requisite amino acid glycoside building blocks.

Introduction

Signaling and neuromodulatory peptides are a major class of biomolecules that play a myriad of important physiological roles. The ability of peptides to modulate diverse processes necessary for life has drawn the attention of a large number of research groups who aim to develop peptides for potential therapeutics to address a variety of medical disorders. Endogenous peptides act as neurotransmitters and signaling molecules that regulate and modulate intracellular communication, sometimes between adjacent cells and sometimes between remote organ systems [1,2]. Peptides exhibit high receptor affinity and low toxicity compared to small molecule drugs. Despite their immense therapeutic potential peptides suffer from low bioavailability and poor pharmacokinetic properties (PK/PD), as they are often metabolized by numerous proteases and peptidases. Various strategies have been employed to make peptides more effective drug candidates, including the use of non-natural amino acids, D-amino acids β-amino acids, cyclization, *N*-methylation, conjugation to drug delivery vectors, lipidation, and PEGylation [[3], [4], [5], [6], [7]]. Although these modifications have shown success in improving stability, many of the resulting stable peptide-based drugs are still incapable of crossing cellular barriers, such as ocular compartments, the blood nerve barrier (BNB), and the blood brain barrier (BBB). Cyclization and lipidation have shown enhancement of BBB penetration for some peptides by increasing their lipophilicity [7]. However, lipidation may pose a disadvantage because the introduction of highly lipophilic moieties can decrease water solubility, resulting in the reduction of overall bioavailability. Glycosylation provides an attractive alternative strategy for improving penetration of the BBB and enhancing stability while also increasing water solubility. Glycosylation of proteins *in vivo* is an essential post-translational modification for membrane proteins. Endogenous glycoproteins

and glycopeptide hormones play critical roles in cell-cell adhesion, recognition processes, immune responses, ovulation, and more [[8], [9], [10]]. As interest in endogenous glycopeptides and glycoproteins has grown over the decades, novel methods detailing their isolation and characterization by nanoflow liquid chromatography coupled to mass spectrometry have been extensively developed. [11]. Glycosylation of endogenous neuropeptides has resulted in enhanced resistance to proteolytic degradation and, perhaps most importantly for our purposes, can modulate interactions with biological membranes, ultimately inducing penetration of the BBB [[12], [13], [14]].

In the late 1980s several research groups began the investigation of glycosylated analogues of various endogenous peptides and their therapeutic potential, particularly those with a role in the CNS. Several of the initial studies on therapeutic glycopeptides focused on the different subgroups of endogenous opioid peptides. Both the *in vitro* stability and *in vivo* biological activity of many of these analogues were improved significantly compared to the native un-glycosylated peptides. Given the success of these pioneering studies, other endogenous peptides targeting both class A and class B G-protein coupled receptors (GPCRs) have been subjected to glycosylation and subsequent *in vitro* and *in vivo* studies. In this review we will survey past and current examples of potentially therapeutic glycopeptides that target both class A and class B GPCRs. Some examples of non-therapeutic glycopeptides will also be discussed. The focus of this review will primarily be on O-linked glycopeptides, but notable examples of glycopeptides containing N-, S-, and C-linked carbohydrate moieties will be briefly discussed. Additionally, a small section will be devoted to the synthetic methodology employed for convenient introduction of carbohydrate moieties into peptide backbones using Fmoc-based solid phase peptide synthesis (SPPS).

When performing structure-activity relationship (SAR) studies on a peptide it is essential to determine what changes to the amino acid sequence will be produce an ideal drug candidate. It is important to identify regions within the peptide sequence that are susceptible to enzymatic cleavage and amino acid residues which are responsible for pharmacological efficacy. Residues that do not greatly affect the overall biological activity of the peptide but are enzymatically labile can be replaced by more stable moieties. Additionally, amino acid motifs that are pharmacophoric elements can be appropriately substituted by an amino acid residue that can modify the receptor binding and biological activity of the peptide, and simultaneously improve stability. One notable example of this strategy is the replacement of Tyr in opioid peptides with 2,5-dimethyltyrosine (χ-space) [15]. Peptide cyclization offers several advantages including stabilizing the peptide’s bioactive conformation and improving penetration of biological membranes. This is especially true for the hydrocarbon-stapled class of cyclic peptides that can penetrate cell membranes. A second notable example comes from Sawyer and coworkers at Merck. They designed and synthesized a number of novel cyclic peptides with an olefin bridge that exhibit potent and selective anticancer activity *in vitro* and *in vivo* [16]. Peptide lipidation has also been extensively explored as a method for enhanced membrane permeability, which is rationalized by the hypothesis that increasing the lipophilicity of the peptide will promote interactions with the lipid bilayer. Typically, a lipoamino acid moiety containing a linear alkyl side chain is introduced during solid phase synthesis [17]. This approach has been applied to the development of potent glucagon analogues and to human neutrophil elastase inhibitors with improved transport across the human epidermis *in vitro* [18,19]. PEGylation of the peptide backbone has also been widely explored as a means of improving the water solubility of the peptide, increasing its plasma half-life, and reducing renal clearance. However, further investigation of these proteins has indicated potential health concerns from introducing PEGylated drug conjugates into the human body, including pronounced cellular vacuolization, making this strategy a less attractive alternative [20,21].· Glycosylation is a strategy that improves membrane permeability and stability, increases water solubility, and causes minimal side effects. Glycosylation of peptides and the resultant effects on physical and chemical properties of peptides will be discussed in the next section.

Glycosylation of the peptide backbone increases steric bulk around the amide bond, providing a shield from certain proteases and peptidases (Fig. 1A). Additionally, the steric bulk helps prevent aggregation of individual peptide monomers and the hydroxyl groups of the sugar moiety improve water solubility. This translates into increased *in vitro* and *in vivo* half-lives and improved stability. It has also been postulated that the presence of the carbohydrate moiety influences the interactions a peptide has with biological membranes. Polt and coworkers have suggested that glycopeptides have *biousian* character, literally meaning “two essences.” These “essences” refer to the two different conformational states glycopeptides can adopt in H_2O (bulk) and when bound to a membrane. Glycopeptides have a relatively lipophilic backbone that interacts with the membrane surface in an α-helical conformation, while the carbohydrate moiety increases the water solubility and allows the glycopeptide to interact with the aqueous compartment in an ensemble of random coil conformations. This allows the peptide to interact with both the membrane surface and aqueous compartment in a “hopping” motion (Fig. 1B), improving bioavailability by reducing an inefficient three-dimensional search for its target receptor to a simpler two-dimensional search, ultimately reducing the amount of space the peptide must survey.

Another important characteristic of glycopeptides is their ability to penetrate the BBB. The BBB consists primarily of tightly packed endothelial cells that act as a gatekeeper to the brain, only allowing in key nutrients for brain function (Fig. 2). This includes small ions, water, and biomolecules like glucose and essential amino acids. Many of these species pass through the BBB either by passive diffusion or *via* a transporter protein. Although the exact mechanism for BBB penetration by glycopeptides is not fully understood, several mechanisms have been proposed, and several lines of evidence suggest that the glycopeptides are active in the CNS. One of the proposed mechanisms is adsorptive endocytosis. The “hopping” motions of the glycopeptides induce negative membrane curvature, and once a particular glycopeptide concentration is reached, the membrane will collapse in on itself and form a vesicle that transports the glycopeptide across the BBB.

Section snippets

Chemical methods for O-linked glycosidation and glycopeptide synthesis

In glycopeptide synthesis the carbohydrate moiety can be introduced in a number of ways. The focus of this review is not on synthetic methods for glycopeptide synthesis, so only a few notable examples will be discussed. The most straightforward way to incorporate carbohydrates into peptides is by synthesizing amino acid glycoside building blocks. Historically, this was achieved by the Koenigs-Knorr glycosylation [22]. Protected glycosyl bromides are reacted with the desired amino acid …

Examples of N-, S-, and C-linked glycopeptides

Although the focus of this review is O-linked glycopeptides, notable examples of N-linked, S-linked, and C-linked require some discussion. N-linked carbohydrates are typically appended to an asparagine side chain *via* an amide bond, and the S-linked glycopeptides contain carbohydrates moieties bonded through a cysteine side chain. C-linked glycopeptides are a special case that occur naturally in some antifreeze glycoproteins found in various fish and in human RNase U_S [[40], [41], [42]]. The …

O-linked glycopeptides targeting class A G-protein coupled receptors (GPCRs)

GPCRs comprise the largest class of membrane receptors in humans [69]. They are involved in the regulation of many important physiological functions, ranging from neuroprotection to pain relief. Because of their significance, many small molecule and peptide drugs have been developed to target various GPCRs. Roughly 40 % of drugs currently on the market target a GPCR. [70]. GPCRs can be broken down into several classes based on their overall structure. The largest of these is the class A family …

O-linked and N-Linked glycopeptides targeting class B GPCRs

Class B GPCR peptide ligands are much greater in size than their class A GPCR ligand counterparts and are generally more difficult to synthesize. Due to the difficulty of their synthesis, this class of peptide ligands has not been extensively studied for glycosylation. However, there are a few recent examples including glycosylated vasoactive intestinal peptide (VIP) analogues containing both O- and N-linked carbohydrates, O-glycosylated calcitonin analogues, and GLP-1 analogues containing an …

Considerations for large scale manufacture of glycopeptide drugs

Considering the vast success of various classes of glycopeptides in pre-clinical studies, it is imperative to develop efficient methods for their synthesis on an industrial scale. There are significant challenges in the manufacture of peptide drugs– indeed, as there are with all classes of drugs. Chemists in both academia and industry have devised creative and effective solutions for the synthesis and purification of peptides. One of the world’s leading drugs to treat hypertension is lisinopril …

Clinical implications for the future of glycopeptide drugs

Peptides have high affinities for their binding sites, possess immense chemical and biological diversity, and offer great solutions to a variety of diseases and metabolic syndromes. Over 60 peptide drugs have now been approved for marketing, and more than 150 are now in active clinical development [148]. Glycosylation has proven to be an extremely effective way to improve the pharmacodynamic and pharmacokinetic profiles of biologically active peptides, which are not limited to the peptides …

Declaration of Competing Interest

None. …

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References (150)

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Synthesis of a new conformationally constrained glycoamino acid building block
Tetrahedron Lett. (2003)

S. Manabe *et al.*
Preparation of glycosylated amino acid derivatives for glycoprotein synthesis by in vitro translation system
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A. Baker *et al.*
Improved strategy for the stereoselective synthesis of glycosides using glycosidases as catalysts
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