Enhancing the pharmaceutical properties of peptides

To begin the discussion about enhancing or improving pharmaceutical properties, one must first understand "the good, the bad, and the ugly" of peptides (1). The good. Peptides are generally highly potent, selective, and have a low potential for toxicity and low risk of drug-drug interaction. The bad. Peptides are generally not terribly stable in biological matrices, susceptible to protease degradation. The ugly. The polar nature of the peptide bond and the size of peptide molecules makes permeability across cell membranes challenging.

Keywords

PEGylation, lipidation, glycosylation, cyclization, non-natural amino acid substitution

In small molecule drug development, we commonly think about Lipinski's rule of five (2), which is based on the observation that most orally administered drugs have common physicochemical characteristics, namely,

1. a molecular mass less than 500 daltons
2. a logP (octanol-water partition coefficient) less than 5
3. no more than 5 hydrogen bond donors
4. no more than 10 (2 x 5) hydrogen bond acceptors.

Peptides violate each and every one of these rules, and hence the need to improve their pharmaceutical properties.

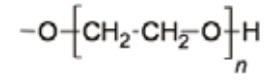
The focus of this paper is to summarize strategies that can make "the bad" into "the good" and perhaps even make "the ugly" into good or bad—enhancing the pharmaceutical properties of peptides.

FROM "THE BAD" TO "THE GOOD"

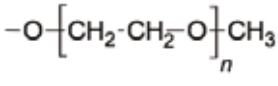
There are numerous strategies to increase peptide stability including peptide conjugates with various polymer molecules (3).

PEGylation

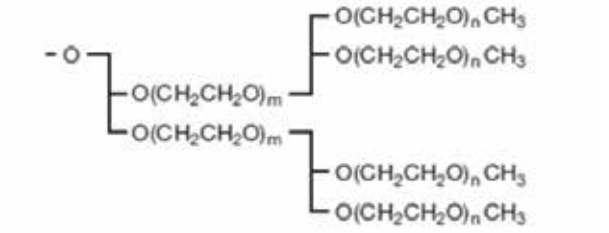
PEGylation refers to the attachment of poly(ethylene glycol) or PEG to peptides or proteins and is able to improve the pharmacokinetic properties of these molecules. PEG increases the hydration shell of a peptide, making the peptide less susceptible to renal clearance and protease degradation. PEGylation can also decrease the immunogenicity potential. There are many different PEG molecules that can be covalently attached to peptides including linear or branched, low molecular weight or high molecular weight.



Linear PEG



Methoxy-PEG (mPEG)



Branched PEG

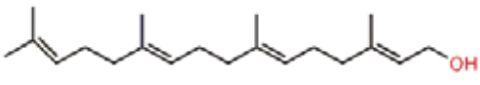
There are many examples of PEGylated pharmaceuticals (4) ranging from small molecules like Naloxegol (PEGylated opioid antagonist) to biologics like Pegfilgrastim (PEGylated GCSF). Although PEGylation can improve solubility, stability, and circulating half-life, the PEG moiety itself is not readily metabolized by the body. Therefore, removal or clearance of PEG from the body can be problematic.

Lipidation

Post-translational modification of proteins with lipid moieties is a well-known, natural modification. Prenylation of cysteine residues occurs on the gamma subunit of the heterotrimeric G-proteins as well as on low-molecular weight G-proteins like *ras* (5). This lipidation is critical for membrane association and appropriate signal transduction. Although common in nature, prenylation is not a strategy used for therapeutic peptides. Instead, a more commonly used strategy is to attach a fatty acid to a peptide using either a labile ester bond or a more stable amide bond (N-acylation). The attachment can be direct to the peptide including the N-terminus or with a spacer molecule between the lipid and the peptide.

The addition of a lipid to a peptide increases its hydrophobicity which can aid in the association of the peptide to cellular membranes. Lipidated peptides can also bind to serum albumin which can facilitate the transport and provide a reservoir.

The addition of lipid to peptides is a 20-year old strategy to increase stability of the peptide and extend the half-life, first used in the 1990s with various insulin analogs. The once-daily liraglutide developed by Novo Nordisk is a success story for acylated peptides. Liraglutide (marketed in the US under the tradename Victoza) is a GLP-1 analog approved for the treatment of type 2 diabetes (6). A palmitic acid (C16 fatty acid) is attached to the peptide via the amino group on a side chain lysine using a gamma-glutamic acid spacer. The result is a soluble and albumin-bound GLP-1 analog with a nearly 10-fold increase in half-life (10-15 hours compared to 1-1.5 hours).

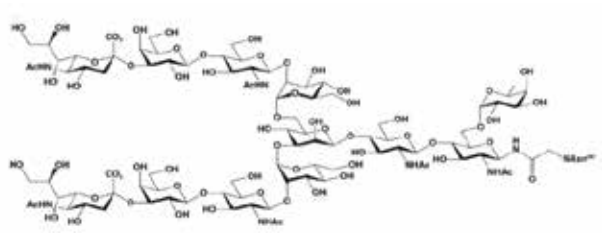


Geranylgeraniol (prenyl-type lipid)

palmitic acid (C16 fatty acid)

Glycosylation

Glycosylation, the attachment of sugar residues to molecules, is very common in nature with nearly 50% of all proteins having this modification. These glycosyl chains are typically attached to amino acid side chains, namely serine/threonine (O-linkage) and asparagine/glutamine (N-linkage). The complex stereochemistry of the glycosyl chains have previously limited the wide spread introduction of glycosylation into chemically-defined, therapeutic peptides. However, this is now changing with commercially available libraries of chemically-defined glycans (see www.glytech.jp) which allows for an empirical approach to optimize pharmaceutical properties of peptides with the selective introduction of glycosyl chains in solid-phase peptide synthesis (7, 8).



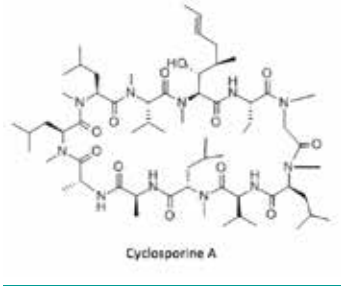
Example of a complex biantennary glycan (9). From Qasba, PK (2015) Bioconjug Chem 26 (11), 2170-5

FROM "THE UGLY" TO "THE BAD/GOOD"?

"The ugly" for peptides is permeability, getting across the various cell membranes in the body. From the gastro intestinal tract (oral bioavailability) to intracellular targets to crossing the endothelial cells of the blood-brain-barrier, each a challenge and general limitation for peptides.

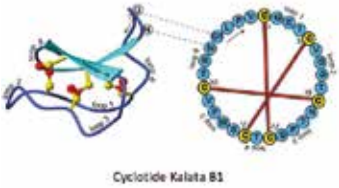
A notable exception to "the ugly" comes from the natural product cyclosporine A. Cyclosporine A is a potent, orally bioavailable immunosuppressant isolated from the fungus, *Tolypocladim inflatum*. It is an eleven-amino acid, macrocyclic peptide with some unique chemical features, including its cyclic nature, N-methylation of the peptide amide nitrogen, and the incorporation of non-natural and D-amino acids. Collectively, these features improve the physico-chemical properties of the peptide and allow gut and membrane permeation as well as increased stability.

Peptide chemists have attempted to incorporate cyclosporine-like features into other peptide structures such as cyclization, intramolecular disulfide bonds and



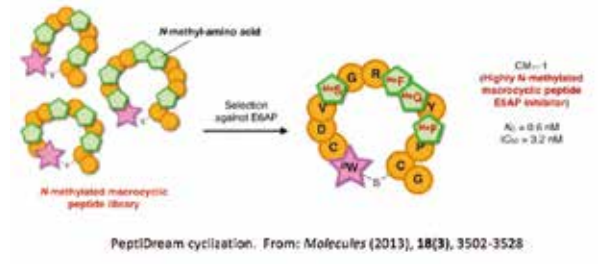
Cyclosporine A

the use of stapled peptides to generate stable alpha helical structures. These conformationally restricted structures are less prone to degradation possibly due to the introduction of more rigid secondary and tertiary structure. Cyclotides represent a class of natural products found in many plants in which intramolecular disulfide bonds stabilize the peptide (10, 11).



Cyclotide Kalata B1

Taking the learning from cyclosporine A one step further, the PeptiDream technology not only creates cyclic peptides but can also include N-methyl, D-amino acids, and non-natural amino acids (12, 13). An example is shown below.



PeptiDream cyclization. From: *Molecules* (2013), **18(3)**, 3502-3528

SUMMARY

For years, peptide therapeutics have been relegated to making better natural hormones. Now with the advent and application of various chemical modifications to enhance the pharmaceutical properties of peptides, novel peptide-based therapeutics are being developed. So just like in the 1966 Clint Eastwood western, "the good" prevails over "the bad" and "the ugly", peptides too have the potential to overcome these challenges.

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