

PEG-ging peptides

Gary Hu of American Peptide Company reviews the progress of PEGylation technology

Peptides seem so promising as drug candidates. However, their small size makes delivery difficult and gives them an extremely short half-life; they are often cleared by the kidneys or the reticuloendothelial system only minutes after being administered and are susceptible to degradation by proteolytic enzymes. These problems can be solved by linking them to polyethylene glycol (PEG).

PEG consists of a repeating chain of ethylene oxide ($\text{CH}_2\text{CH}_2\text{O}$)_x. The molecules can be long or short, straight or branched. PEG groups are linked to a reactive group on the peptide, which is usually lysine but can also be aspartic acid, glutamic acid, free cysteine, serine, threonine, the N-terminal amine or the C-terminal carboxylic acid.

Once linked to a peptide, each ethylene glycol sub-unit becomes tightly associated with two or three water molecules. This has the dual function of rendering the peptide more soluble in water and increasing its size.

Since the kidneys filter substances according to their size, the addition of PEG's molecular weight alleviates the renal clearance undergone by small peptides. PEG's globular structure acts as a shield to protect the peptide from proteolytic degradation and reduces the immunogenicity of foreign peptides by reducing uptake by dendritic cells.

PEG itself is not immunogenic or toxic. By increasing the circulating half life of peptide drugs, sometimes by factors of 100, PEGylation allows for lower doses and less frequent administrations, saving money and resources, promoting patient compliance and reducing the development of toxicity, tolerance or allergic reactions. In addition to improving the pharmacokinetic and pharmacodynamic properties of peptide drugs once inside the body, PEGylation can also aid in drug delivery.

The first PEGylated drug to be approved by the FDA was Enzon Pharmaceuticals's Adagen in March 1990.¹ This is the first successful enzyme replacement therapy for an inherited disease. Adagen is PEGylated bovine adenosine deaminase and is used to treat people with the type of X-linked Severe Combined Immunodeficiency Disease (SCID) that was first brought to public attention by the 'bubble boy'.

Another drug using Enzon's proprietary PEG platform is Oncaspar. This is the PEGylated version of L-asparaginase, the enzyme that degrades the amino acid asparagine.¹ Oncaspar is given to people with leukemia to kill leukemic cells by starving these cells of the asparagine that they cannot produce on their own and must get from diet. PEGylation extends the half life of this enzyme from 20 to 357 hours and reduces adverse immune reactions.

Amgen's Neulasta (pegfilgrastim) is currently being used to fight chemotherapy-induced neutropenia in cancer patients.¹ It is a PEGylated form of recombinant granulocyte colony-stimulating factor, a cytokine that stimulates the survival of the neutrophils that are vital to fighting infection but are unfortunately destroyed by chemotherapy. Pegfilgrastim requires only one injection per chemotherapy trial, whereas the original drug, filgrastim, had to be injected daily for two weeks.

A recent trend in cancer therapeutics focuses on metastases and the angiogenesis that enables them to occur. The urokinase-mediated plasminogen activation system plays a central role in breaking down the extracellular matrix and inhibiting this process might be a valuable anti-cancer strategy.

Although an inhibitor, DX-1000, was identified using phage display, it was cleared so rapidly by the



Solid phase synthesis is the company speciality

kidneys because of its low molecular weight that it was clinically useless. Site-specific PEGylation enhanced its *in vivo* stability and slowed its clearance yet did not significantly diminish its antiangiogenic capabilities.²

Sometimes the lysine residues are in an inaccessible region of the peptide, or in the active or binding site. In these cases, site-directed mutagenesis can engineer free cysteine residues into a chosen place in the peptide to link with PEG.

This approach was used to PEGylate recombinant GM-CSF, a cytokine that stimulates the proliferation of macrophages and is used clinically to treat melanoma, Crohn's disease, and myeloid and hematopoietic disorders, including neutropenia.³ Because of its short circulating half-life GM-CSF must be injected daily. PEGylation increased this half-life from only one hour to 22.

The hepatitis C and hepatitis B viruses are being successfully treated with PEGylated interferon α -2a, known as both Pegasys (Hoffman-LaRoche) and Peginteron (Schering-Plough).⁴ PEGylation prolongs the serum half-life of interferon α -2a and -2b, from 6-9 hours to 72-96 and 40 respectively. Once-weekly dosing with any of these PEGylated interferon α s produced higher rates of viral eradication than the standard three times weekly dosing and had a comparable safety profile.

A number of other PEGylated peptides are in various stages of development for a range of maladies. Somavert is a PEGylated human growth hormone antagonist marketed by Pfizer to treat acromegaly, a



Laboratory at the American Peptide Company site

rare hormonal disease in which excessive insulin-like growth factor -1 causes soft-tissue enlargement.¹

Meanwhile, researchers at Bayer Healthcare developed a reproducible method for PEGylating a peptide they hope to use to treat Type II diabetes.⁵ Branched PEGs were linked to lysostaphin, an antibacterial endopeptidase that can be used to fight multi-drug-resistant strains of *Staphylococcus aureus*, to increase its serum half-life from less than one hour to 24. PEGylation also reduced the generation of antibodies against lysostaphin.⁶

PEGylation can clearly make drugs more effective by granting them more time in the body to work. Moreover, it can also help by more efficiently delivering these drugs where they need to go.

Intranasal delivery is a very attractive route for administering biologically active peptides and scientists at MDRNA, formerly Nastech Pharmaceutical, demonstrated that PEGylated peptides can act as permeation enhancers for

nasal drug delivery. Furthermore, synthetic PEGylated glycoproteins have been used in lieu of viruses for targeted gene delivery.⁷ Genes delivered in this manner could be expressed *in vivo*.

PEG can not only help peptides achieve their goals, the converse is also true. PEG has been shown to act as a cell repellent, preventing mammalian and bacterial cell growth on substances used as medical implants such as plastics and metals.

Grafting PEG to these materials has proven to be a challenge but linking it to a hydrophilic molecule created a coating on polystyrene that

reduced the attachment of both human umbilical vein endothelial cells and *S. aureus*.⁸ Other PEGylated peptides had affinities for other implant materials like titanium. These cytophobic coatings may find other uses, for example in proteomic studies and cell culture technologies.

Sometimes PEGylation can cause a decrease in the binding affinity or activity compared to the unconjugated peptide. However in all cases, the extended half life more than compensates for this effect. So too the slight increase in manufacturing cost incurred by PEGylation will certainly be deemed worthwhile.

For more information, please contact:

Gary Hu
American Peptide Company
777 E. Evelyn Avenue
Sunnyvale
CA 94083
USA
Tel: +1 408 733 7604
E-mail: gary@americanpeptide.com
Website: www.americanpeptide.com

References:

1. J.M. Chess & R.B. Harris, Effect of PEGylation on Pharmaceuticals, *Nat. Rev. Drug Discov.* **2003**, 2(3), 214-21
2. L. Devy, S.A. Rabbani, M. Stochl, M. Kuschowski, L. Naa, M. Toews, R. Van Gool, J. Chen, A. Ley, R.C. Ladner, D.T. Dransfield & P. Henderix, PEGylated DX-1000: Pharmacokinetics & Antineoplastic Activity of a Specific Plasmin Inhibitor, *Neoplasia* **2007**, 9(11), 927-37
3. D.H. Doherty, M.S. Rosendahl, D.J. Smith, J.M. Hughes, E.A. Chlipala & G.N. Cox, Site-Specific PEGylation of Engineered Cysteine Analogues of Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor, *Bioconjug. Chem.* **2005**, 16(5), 1291-8
4. J. Shepherd, H. Brodin, C. Cave, N. Vaughn, A. Price & J. Gabbay, Pegylated Interferon α -2a & -2b in Combination with Ribavirin in the Treatment of Chronic Hepatitis C: A Systematic Review & Economic Evaluation, *Health Technol. Assess.* **2004** 8(39):3-4, 1-125

5. I. Tom, V. Lee, M. Dumas, M. Madanat, J. Ouyang, J. Severs, J. Andersen, J.M. Buxton, J.P. Whelan & C.-Q. Pan, Reproducible Production of a PEGylated Dual-Acting Peptide for Diabetes, *AAPS J* **2007**, 9(2), E227-34
6. S. Walsh, A. Shah & J. Mond, Improved Pharmacokinetics & Reduced Antibody Reactivity of Lysostaphin Conjugated to Polyethylene Glycol, *Antimicrob. Agents Chemother.* **2003**, 47(2), 554-8.
7. C.P. Chen, J.S. Kim, D. Liu, G.R. Rettig, M.A. McAnuff, M.A. Martin & K.G. Rice, Synthetic PEGylated Glycoproteins & Their Utility in Gene Delivery, *Bioconjug. Chem.* **2007**, 18(2), 371-8
8. D.J. Kenan, E.B. Walsh, S.R. Meyers, G.A. O'Toole, E.G. Carruthers, W.K. Lee, S. Zauscher, C.A. Prata & M.W. Grinstaff, Peptide-PEG Amphiphiles as Cytophobic Coatings for Mammalian & Bacterial cells, *Chem. Biol.* **2006**, 13(7), 695-700



MERCACHEM

Partner in Innovation

We conduct R&D programs for research-driven, innovative companies across the globe. Our activities span from early discovery to late-stage chemical development and improvement of commercialized processes.

Our Process Research Services include:

- Synthetic route development
- Material supply (~1 kg)
- Catalyst screening and reaction optimization
- Optimization of down-stream processing

Mercachem Process Research BV
Kerkenbos 1013 | 6546 BB Nijmegen
PO Box 6747 | 6503 GE Nijmegen
The Netherlands
Phone +31 (0)24 372 33 00
Fax +31 (0)24 372 33 05
E-mail info@mercachem.com
Internet www.mercachem.com