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Site-specific PEGylation of Peptide Drugs

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Problems of bioactive peptide drugs

Bioactive peptides are known to play key pharmacological roles and have found widespread application in a variety of fields. Although many peptides have found application for a variety of clinical indications, their therapeutic use has been limited due to the problems of short circulating half-life and rapid proteolytic degradation. Chemists have therefore developed ingenious methods for the modification of peptides, e.g. peptidomimetic design, cyclization etc., with the goal of maintaining or improving their receptor selectivity while stabilizing them to enzymatic degradation. Although these efforts have resulted in important progress, the problems of poor absorption of peptides, rapid proteolytic degradation and potential immunogenicity have severely hampered the application of peptides as potential therapeutics.

PEGylation technology

PEGylation, covalent attachment of polyethylene glycol (PEG) to therapeutic agents, is a procedure of growing interest for enhancing the therapeutic and biotechnological potential of peptides and proteins. When PEG is properly linked to a drug molecule, it modifies many of its features while the main biological functions, such as enzymatic activity or receptor recognition, may be maintained. PEGylation masks the surface of proteins and increases the molecular size of the drugs, thus reducing its renal filtration, preventing the approach of antibodies or antigen processing cells and reducing the degradation by proteolytic enzymes. Finally, PEG conveys molecules to its physico-chemical properties and therefore modifies also biodistribution and solubility of peptide and non-peptide drugs. An additional factor leading to industry acceptance of PEG is that the polymer has been approved for internal and topical applications by the drug regulatory agencies.

Since the initial demonstration for utility of PEGylated proteins as therapeutics, several therapeutic agents have been PEGylated and have been shown to have properties of use in clinical applications. These improved clinical properties include better physical and thermal stability, protection against susceptibility to enzymatic degradation, increased solubility, reduced immunogenicity and antigenicity, decreased toxicity, longer in vivo circulating half-life, decreased clearance and enhanced potency. FDA approval for four PEG-protein drugs, PEG-

adenosine deaminase, PEG-asparaginase, PEG-interferon alpha and PEG-granulocyte-colony stimulating factor (PEG-G-CSF), and several other PEG-drugs in clinical development are clear indication of the tremendous utility and the maturity of this technology.

Several important PEG-proteins are currently in advanced-stage clinical trials, and it is expected that some of these conjugates will receive approval within the next year. Molecules in trials include PEG-growth hormone receptor antagonist for treatment of acromegaly, soluble PEG-TNF receptor for treatment of rheumatoid arthritis, and a PEGylated humanized antibody fragment that binds TNF-alpha and also targets rheumatoid arthritis.

Site-specific PEGylation

The most frequently used PEGylation reaction is the conjugation of an electrophilically activated PEG to the ε-amino group of lysine or N-terminal amino group on the surface of a protein. Due to the nature of most proteins, several of these groups are normally found on the surface and therefore the conjugation can be considered to be nonspecific. This type of protein PEGylation results in several degrees of heterogeneity. The heterogeneity is due to the number of PEG molecules attached per protein molecule, the site of PEG conjugation, and the polydisperse nature of PEG. Recently, a considerable effort has addressed the need for PEG-protein conjugates with decreased heterogeneity.

Several advantages can be obtained when the site-specific PEGylation is done. First of all, preclinical development of a PEG-protein with the desired properties must show reproducibility in the number and location of PEG conjugation. This reproducibility results in a purification strategy that is less complicated. Site-specific PEGylation may also result in a conjugate where the PEGylation site is far removed from the site where the protein binds to surface cell receptors or far removed from an enzyme's active site. In this instance the protein may retain much or all of its biological activity. Site-specific PEGylation of a protein epitope responsible for rapid clearance or immunogenic reaction may result in an extension of plasma half-life and reduced immunogenicity without loss of biological activity.

However, the specific position of PEGylation in proteins usually cannot be controlled using the conditions of the present protein PEGylation methods. Although many approaches for site-specific PEGylation have been reported, none of the methods have completely prepared the site-specifically PEGylated proteins. In contrast to proteins, peptides are ideal targets for PEGylation since they may be specifically PEGylated using modern orthogonal protection strategies. The enormous progress that has been made in peptide synthetic methodology has provided an important basis for the site-specific PEGylation of peptides.

We have developed site-specific PEGylation method of peptide drugs using site-protection strategy by solid-phase synthesis. This approach has advantages of increased stability,

preserved bioactivity, and high production yield for the scale-up.

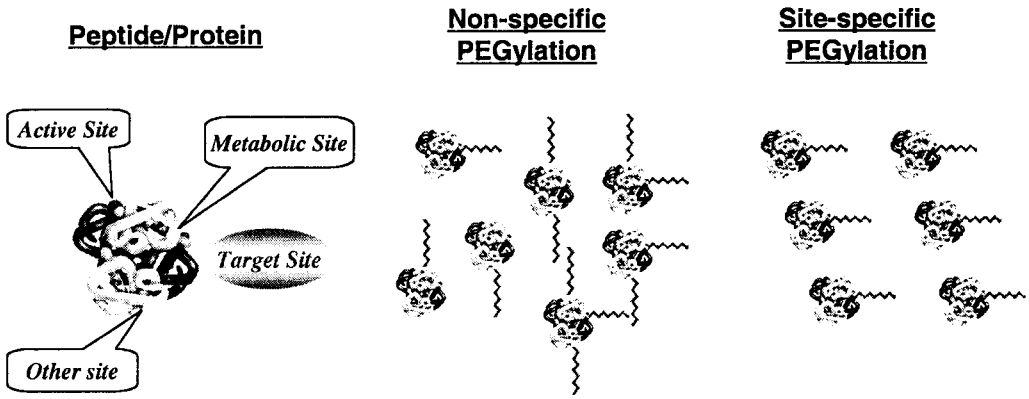

Figure 1. Non-specific and site-specific PEGylation. While non-specific PEGylation yields the mixture of various positional isomers of mono- and multi-PEG conjugates, site-specific PEGylation results in a homogeneous PEG conjugate.

Conclusion

PEGylation can be a powerful tool for peptide delivery system by enhancing enzyme stability and chemical stability of peptide drugs. Biological activities and enzyme stability vary according to not only the number of PEG attached but also the PEGylation site. Site-specific PEGylation is essential for high activity yield, simple purification process, quality control, and low manufacturing cost. Almost absolute control of PEGylation site was achieved by a new PEGylation method using site-protected peptide.

References

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