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Site-selective protein modification with polymers for advanced biomedical applications

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ABSTRACT

Protein modification with polymers has led to intriguing and new types of bioconjugates. They combine the tunable physicochemical properties of the polymers with the specific biological activity of the proteins. These unique attributes of protein-polymer conjugates render them interesting and useful in biomedicine. However, the application potential of protein-polymer conjugates is limited by the mostly non-selective protein modification with polymers due to the lack of site-selective protein modification technology. Recent advances in site-selective protein modification and controlled polymerization have made it possible to modify proteins with polymers in a site-selective and controlled manner. In this review, recent advances in site-selective protein modification with polymers are depicted in five parts as follows: site-selective protein modification; site-selective polymer modification; site-selective in situ growth of polymers from proteins; biosafety of polymers; and biomedical applications. Site-selective protein-polymer conjugates are superior to non-selective ones in precise control of structures and functions, which makes them more interesting for advanced biomedical applications ranging from protein delivery to diagnostics. Particularly, important examples in this regard are highlighted in this review. Additionally, major challenges and future directions in this emerging research field are also discussed in the perspective section of this review.

1. Introduction

A range of biomaterials can be produced by covalent conjugation of synthetic polymers to proteins. These biomaterials combine the tunable physicochemical properties of polymers with the specific biological activity of proteins, which exhibit precise control over biological activity, function, and molecular weight for biomedical applications. For example, attachment of poly(ethylene glycol) (PEG) to proteins, called PEGylation [1], can increase the solubility and stability of the target proteins, prolong the half-life in circulating blood, and reduce the potential immunogenicity. As a result, a dozen of FDA-approved PEGylated protein therapeutics are used to treat cancer, hepatitis and diabetes in clinic [2]. Typically, preformed polymers are attached to random locations on the protein surface through the modification of the reactive side chains of protein residues such as lysine and cysteine (“grafting to” method). The non-selective nature of the modification usually leads to heterogeneous products composed of positional isomers with reduced activity. Particularly, the positional isomers are difficult to separate and purify, which brings about batch-to-batch variations; and each exhibits distinct physicochemical property, biological activity, pharmacology, and biosafety. Additionally, the protein-polymer conjugation efficiency is low due to the steric hindrance between the two macromolecules, which means a high cost for the production of a protein-polymer conjugate therapeutic. These drawbacks are incompatible with the intended applications.

Recent advances in site-selective protein modification have made it possible to modify proteins with polymers in a site-selective and controlled manner [3]. Site-selective protein modification with polymers usually involves two-step reactions: a unique chemical group is incorporated into the polypeptide chain of a protein by either recombinant or chemical means, followed by chemoselective modification of the protein with a polymer carrying a complementary chemical group that can chemoselectively react with the new chemical group incorporated into the proteins. Consequently, the structure of the resulting protein-polymer conjugate can be well controlled. The precise control in reaction and structure enables the rational design of protein-polymer conjugates with optimized properties for advanced biomedical applications.

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Over the past two decades, controlled radical polymerization (CRP) techniques [4] and ring opening metathesis polymerization (ROMP) [5] have emerged as powerful technology platforms to synthesize functional and rationally designed polymers for advanced application [6]. These techniques are highly robust and flexible, well tolerable to functional groups, mild in reaction condition, and water-tolerable as compared to other controlled polymerization techniques such as ring opening polymerization (ROP) [7], and anionic polymerization [8]. These unique advantages have made CRP and ROMP techniques more interesting for the development of protein-polymer conjugates. Particularly, the two most widely used CRP techniques atom transfer radical polymerization (ATRP) [9] and reversible addition-fragmentation chain transfer (RAFT) [10] polymerization along with ROMP have made it possible to directly grow a polymer from a protein attached with initiators in a controlled manner to form protein-polymer conjugates with high efficiency ("grafting from" method). The "grafting from" method not only simplifies the purification of the resulting protein-polymer conjugates but also remarkably increases the product yield as compared to the "grafting to" method. More importantly, the combination of CRP techniques with site-selective protein modification has significantly advanced the progress of designing site-selective protein-polymer conjugates for advanced biomedical applications.

In this review, we introduce the development of site-selective "grafting to" and "grafting from" methods for the preparation of site-selective protein-polymer conjugates (Fig. 1). We highlight examples in which the combination of site-selective protein modification and controlled polymerization techniques leads to next-generation protein-polymer conjugates for advanced biomedical applications ranging from protein delivery to disease diagnosis. Notably, hydrophilic polymers beyond PEG are discussed, especially in the aspects of immunogenicity, biodegradability, and toxicity. Furthermore, future directions and challenges in this emerging research field are discussed in the perspective section. It should be pointed out that this review is not meant to exhaustively list all available chemical protein modification methodologies [3] or controlled polymerization techniques [11] as both have comprehensively been reviewed in the literature separately.

2. PEGylation

To date, the most successful "grafting to" method is PEGylation. Indeed, more than a dozen of PEGylated protein therapeutics have been approved for clinical use (Table 1) [2]. The first example of the "grafting to" method dates back to 1970s [12,13], in which PEG was conjugated to bovine serum albumin (BSA) to form BSA-PEG conjugates, specifically called PEGylation. Since then, PEGylation has been applied widely in both academia and industry. It is currently the standard method to manufacture Food and Drug Administration (FDA)-approved PEGylated protein therapeutics. The earlier PEGylation was non-selective attachment of PEG to chemically reactive protein residues, such as lysine, serine, tyrosine and histidine, that are ubiquitously located on the surface of proteins in a totally random manner. Several FDA-approved PEGylated protein therapeutics were developed by the earlier PEGylation, such as Adagen [14], Oncaspar [15], and Somavert [16]. In the later several PEGylation examples, such as Krystexxa [17], PEGASYS [18], Mircera [19], and Adynovate [20], attention was paid to site-selective protein modification, where the attachment sites were mainly the ε-amino groups of lysine residues. Nevertheless, due to the relatively high abundance (5.19%) of lysine residues in proteins, the existence of positional isomers cannot be avoided. For example, the isomers isolated from PEGASYS by ion exchange chromatography-high-performance liquid chromatography (IEC-HPLC) were composed of interferon α-2a PEGylated at Lys(31), Lys(134), Lys(70), Lys(83), Lys(121), Lys(131), Lys(49), Lys(112), and Lys(164), respectively [21]. However, such kind of purification was highly costly and time consuming. Furthermore, these isomers had distinct reduced *in vitro* bioactivities and different low yields. Unfortunately, no further *in vivo* investigation was carried out to compare these isomers in pharmacokinetics, pharmacodynamics, biodistribution, and biosafety. Nevertheless, it is generally believed that these isomers should have distinct *in vivo* properties. Overall, the non-selective protein modification with polymers can complicate quality control (*e.g.* batch-to-batch variations) and significantly reduce protein bioactivity (*e.g.* < 10% bioactivity retention for PEGASYS relative to native interferon alpha), which is not beneficial to patients in therapeutic efficacy, biosafety, and treatment cost.

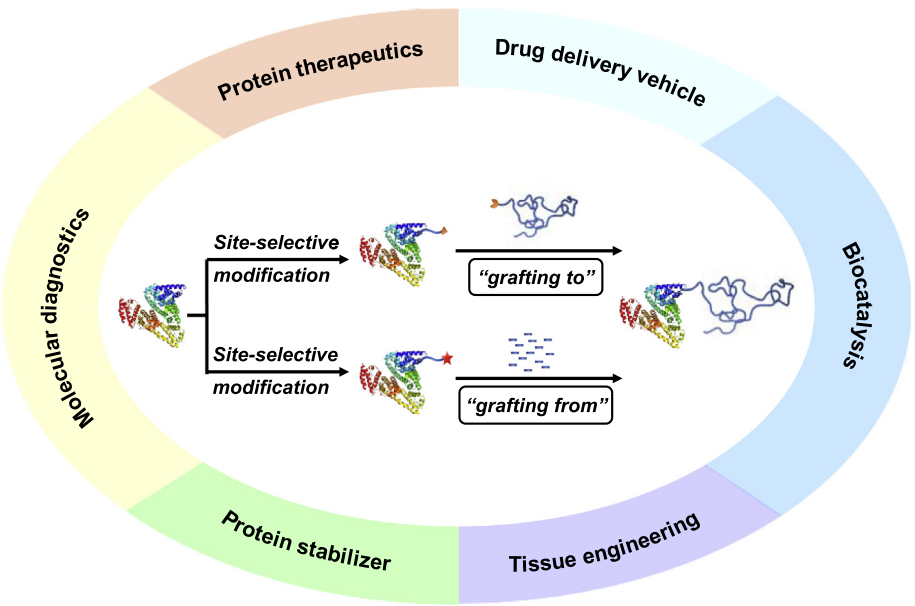

Fig. 1. Site-selective "grafting to" and "grafting from" methods for the preparation of site-selective protein-polymer conjugates and their applications in the biomedical field.

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