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Advances in polymer and polymeric nanostructures for protein conjugation

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ABSTRACT

Linear polymers have been considered the best molecular structures for the formation of efficient protein conjugates due to their biological advantages, synthetic convenience and ease of functionalization. In recent years, much attention has been dedicated to develop synthetic strategies that produce the most control over protein conjugation utilizing linear polymers as scaffolds. As a result, different conjugate models, such as semitelechelic, homotelechelic, heterotelechelic and branched or star polymer conjugates, have been obtained that take advantage of these well-controlled synthetic strategies. Development of protein conjugates using nanostructures and the formation of said nanostructures from protein–polymer bioconjugates are other areas in the protein bioconjugation field. Although several polymer–protein technologies have been developed from these discoveries, few review articles have focused on the design and function of these polymers and nanostructures. This review will highlight some recent advances in protein-linear polymer technologies that employ protein covalent conjugation and successful protein-nanostructure bioconjugates (covalent conjugation as well) that have shown great potential for biological applications.

1. Introduction

Polymer science is currently in the process of reinventing itself. Polymers are now not only being developed for the creation of common materials; in recent years, polymers have become an important tool for biological sciences. Synthetic polymers and polymeric assemblies have been designed to interact with biological moieties, such as proteins and nucleic acids, through recognition or hybridization for innovative approaches of interest to biomedicine. In general, research in this area has been focused on creating novel materials to improve the efficiency of therapeutics delivery [1–4], diagnostics and biological assays [5–14].

Although drug development has mainly focused on small molecules that act as activators or inhibitors of proteins, it is also well recognized that there is great potential for proteins themselves to be utilized for therapeutic purposes [15,16]. Therefore, efforts towards protein engineering and discovery of new therapeutic proteins have increased in recent years. Unfortunately, administration of these proteins suffers from a number of limitations including: inability to cross biological barriers, degradation in biological systems, toxicity problems, poor solubility and biodistribution [17]. For these and many other reasons, the conjugation of biocompatible polymeric materials to proteins has garnered interest. Bioconjugation, the covalent (or non-covalent) modification of biomolecules with synthetic molecules or macromolecules [18], is the most common method used to obtain polymer–biomolecule hybrids. The improvement in therapeutic performance of proteins when associated to these materials is the source of substantial growth in this area of research [19].

Many synthetic routes facilitate access to polymers and polymeric macro- and nanostructures to incorporate

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biological moieties, such as proteins. A critical feature of successful protein bioconjugation is that the protein should retain their native properties upon conjugation to the polymer. Therefore, successful conjugation depends on the availability of reactive moieties on protein surfaces, reaction conditions at which the conjugation is performed, and solubility of reagents [20,21]. Amino acid residues such as lysine, glutamate and cysteine are commonly used for protein conjugation with polymeric materials containing complementary functional groups. However, these amino acids can be inaccessible for direct attachment to the artificial polymer scaffolds due to variations in protein structure and conformation. Methods have been developed to circumvent this issue. For instance, if a protein does not contain any accessible amino acids, it can be genetically engineered to conveniently place one within its amino acid sequence [22–24]. Alternatively, artificial amino acids can be incorporated during protein synthesis by modifying cellular molecular machinery [25,26]. These modifications have shown great success in protein structure retention and stabilization. A wide variety of core materials have been conjugated to proteins, such as gold nanoparticles [27], organic small molecules [28] and polymers [29]. Although not as extensively explored, protein conjugation to polymeric nanostructures and formation of amphiphiles from conjugates have also been achieved (*vide infra*).

There has been a significant surge in research activity concerning bioconjugates between proteins and polymers. This has inspired many reviews and notes on the synthesis and application of protein–polymer conjugates [29,30,18,31]. However, to the best of our knowledge, little attention has been paid to the conjugation of proteins to polymeric nanoassemblies. In this review, therefore, we will not dwell on the synthetic techniques utilized for bioconjugation. We will focus on a select few examples of protein conjugate technologies developed so far and highlight two main areas of study within bioconjugation research: (1) protein conjugation with linear polymers (most common) and (2) protein conjugation with polymeric nanostructures or macromolecular assembly formation from conjugates (growing research area). We specifically restrict our analysis to covalent conjugation of polymeric materials, since stabilization of proteins within a complex biological environment is one of the greater advantages of covalent conjugation compared to non-covalent conjugation methods.

2. Protein conjugation with polymers

It is noteworthy that reverse addition fragmentation chain-transfer (RAFT) and atom transfer radical polymerization (ATRP) are understandably the most common synthetic strategies utilized for the preparation of polymers for protein conjugation [29]. These polymeric scaffolds can be covalently attached to proteins through three well-known conjugation strategies: (i) attachment of a preformed polymer to an unmodified or linker-modified protein, also called "grafting to" method; (ii) *in situ* bioconjugation by previously modifying a protein with initiation sites in which a polymer is then "grafted from" the protein microinitiator; and (iii) anchoring of proteins on the pendant side chains of a polymer; the "grafting through" method. For successful bioconjugation, it is essential that the polymeric scaffolds bring certain features to the protein conjugate (e.g. stability to mechanical and proteolytic degradation) without affecting the intrinsic property of the protein. For this reason, it is imperative that we target well-defined polymeric structures and utilize the right conjugation method.

The location at which the protein is conjugated to a polymeric material, conjugation of the protein to a precise single site or to different areas of the protein for instance, can greatly affect the pharmacological behavior of the therapeutic protein. Earlier efforts toward protein conjugation focused on the attachment of poly(ethyleneglycol) based linear polymers to the protein; "PEGylation", as it is known. PEGylation represents the most common and successful conjugation strategy [3,32–36]. PEGylated proteins have demonstrated good bioavailability, thermal stability, enhanced proteolytic resistance and even therapeutic potency [19,30,37]. On the other hand, PEGylation chemistry presents some challenges such as side reactions, poor selectivity in functional group substitutions and lack of homogeneity of the conjugates [31,37]. Efforts have been put into refining conjugation strategies to overcome these issues. Newer technologies, such as "smart" linear polymer–protein conjugates, have been demonstrated to circumvent some of the problems encountered in the PEGylation method. "Smart" polymers are linear polymers synthesized with chemo-specific functional groups having the capability of targeting specific protein sites and are responsive to external stimuli changes, such as pH, light, temperature, and enzymatic cleavage. Many of these polymers are telechelic. Telechelic polymers are those that contain end-functional groups capable of further polymerization [38–41] and are capable of binding to a specific number of proteins (Fig. 1). Methods utilized for achieving this class of linear polymer–protein conjugates will be further discussed in the following sections.

2.1. Monomeric conjugation

2.1.1. Semitelechelic polymer–protein conjugates

Monomeric conjugates are arguably the most common type of conjugates; in this case, the protein is anchored by a single linear polymer (Structure I in

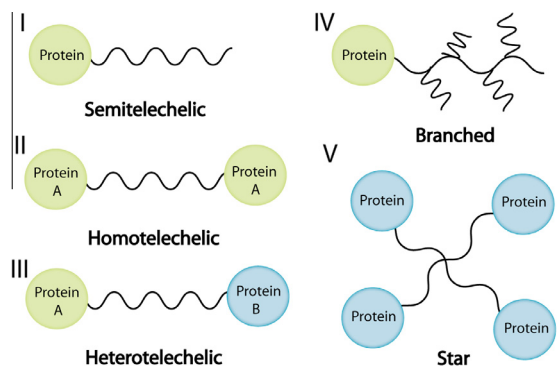

Fig. 1. Representation of different types of polymer–protein conjugates.

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