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Feature article

Synthesis of well-defined protein–polymer conjugates for biomedicine

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ABSTRACT

Covalently conjugating proteins with synthetic polymers, particularly poly(ethylene glycol) (PEG) is widely used as a means to improve protein solubility and stability, prolong their circulating half-lives, and lower their immunogenicity. Conventionally, these polymers are attached to random locations on the protein surfaces through the modification of the reactive side chains of amino acid residues such as lysine and cysteine. The "grafting to" polymer conjugation usually leads to heterogeneous products with reduced activity and low yield, which may not be compatible with the intended applications. Therefore, it is highly desirable to synthesize well-defined protein–polymer conjugates by site-specific polymer conjugation. Recently, in situ growth of polymer conjugates from proteins ("grafting from") has emerged as an alternative to the "grafting to" method. Particularly, site-specific in situ growth of polymer bioconjugates (SIP) is promising in overcoming the limitations of the "grafting to" method. In this review, we introduce the chemistry for synthesis of well-defined protein–polymer conjugates, and emphasize the SIP method as the next-generation platform for synthesis of well-defined protein–polymer conjugates. Furthermore, we exemplify biomedical applications of well-defined protein–polymer conjugates. In the end, we come up potential directions in this research field.

1. Introduction

Proteins and peptides play important roles as drugs in the pharmaceutical industry and as reagents in biomedical research. To date, more than one hundred different proteins or peptides have been approved for clinical use by the US Food and Drug Administration (FDA), and many more are in development. Therapeutic proteins have frequently been reviewed in the past decade [1–22]. While therapeutic proteins have advantages in the treatment of diseases due to their high activity and specificity, they suffer some shortcomings such as short *in vivo* half-life, poor stability, low solubility [23–25], and immunogenicity. One solution to these shortcomings is conjugating therapeutic proteins with polymers. Conjugation of a protein with a polymer results in a new macromolecule with significantly changed physicochemical characteristics. These changes are typically reflected in alterations of solubility, stability, *in vitro* activity, biodistribution, pharmacokinetic and pharmacodynamic profiles, as well as reduced immunogenicity and toxicity.

The first protein–polymer conjugate was reported in 1970's, where poly(ethylene glycol) (PEG) was conjugated to bovine serum albumin (BSA) [26,27] (Fig. 1a). This polymer conjugation represented a typical "grafting to" methodology and opened a new area in protein post-translation modification, which has led to an explosion of protein–polymer conjugate population. Conjugating with PEG is often named PEGylation. The clinical success of PEGylation has led to a number of FDA approved PEGylated drugs on the market. As an alternative to the "grafting to" method, in situ growth of polymer conjugates from proteins ("grafting from") has recently emerged, in which polymerization initiators are attached to proteins to form macroinitiators, followed by growing polymer conjugates from the macroinitiators through controlled radical polymerization technologies (Fig. 1b) [28]. In brief, "grafting to" is to attach a pre-prepared polymer to a protein, while "grafting from" is to in situ grow a polymer from a protein. Typically, "grafting from" does have two major benefits over "grafting to". First, "grafting from" usually results in high yield due to the high efficiency of the two-step reactions between small molecules (initiator and monomer) and a protein, while "grafting to" often leads to low yield because of the low efficiency of the reaction between two

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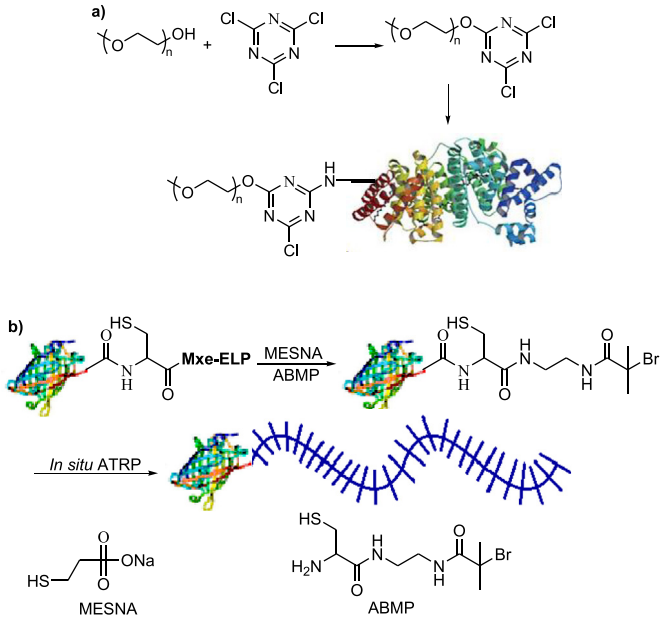

Fig. 1. a) Conventional conjugation of PEG to a protein. Ref. [26]. Reprinted with permission; Copyright© 1977, by the American Society for Biochemistry and Molecular Biology. b) In situ growth of a PEG-like polymer from a protein-based macroinitiator. Ref. [28].

large macromolecules (a polymer and a protein). Second, "grafting from" usually yields products with ease of purification because of the absence of free polymers and high yield.

Conventionally, polymers are conjugated to proteins typically at the sites of lysine or cysteine residues that are ubiquitously present on the protein surfaces, which makes it difficult to control the site of conjugation and the stoichiometry of the conjugates. The non-specific nature of the conventional polymer conjugation methods, including "grafting to" and "grafting from", often leads to a heterogeneous product mixture of positional isomers with significantly reduced biological activity [29–31]. Furthermore, it is difficult to isolate and purify the conjugate mixture, especially positional isomers. These limitations of the conventional polymer conjugation methods generally complicate the development process of protein–polymer conjugates, limiting their wide-spread applications. Therefore, there is a need to synthesize well-defined protein–polymer conjugates in which both the site of conjugation and the stoichiometry of the conjugates should be controlled [32].

In our opinion, a well-defined protein–polymer conjugate consists of at least two components, a functional protein, and an end-functionalized polymer with designed molecular weight and narrow polydispersity, in which the protein and the polymer are site-specifically conjugated together to form a site-specific and stoichiometric protein–polymer conjugate by "grafting to" or "grafting from" method. Therefore, the structure and properties of a well-defined protein–polymer conjugate can be precisely designed, which is particularly important for advanced applications [28,33–35]. Usually, the protein acts as an active component in biomedicine; meanwhile, the polymer plays a role as drug delivery carrier, targeting moiety, or a co-functional group. Up to date, there are many review papers on synthesis of well-defined protein–polymer conjugates by the "grafting to" method [32,36–45], in which a well-defined end-functionalized polymer is synthesized via a controlled polymerization process, and then attached to a protein at a specific site. In this review, we focus on synthesis of well-defined protein–polymer conjugates by site-specific in situ polymerization (SIP) due to the advantages of the "grafting from" method, such as high yield and simplified purification, over the "grafting to" method. Particularly, we emphasize the chemistry of site-specific protein modifications and biomedical applications of well-defined protein–polymer conjugates.

2. Controlled polymerization

The past decades have witnessed a huge development in controlled polymerization where the polymerization process is controllable to precisely synthesize well-defined polymers with desired structure and properties [46–56]. Particularly, controlled radical polymerization (CRP) techniques, such as atom transfer radical polymerization (ATRP) and reversible addition fragmentation chain transfer (RAFT) polymerization, are powerful to synthesize well-defined polymers, especially under mild aqueous conditions, which is highly desirable for the "grafting from" method. Here we focus on ATRP and RAFT polymerization as both techniques have been used to grow polymer conjugates from proteins.

2.1. Atom transfer radical polymerization

ATRP is one of the most powerful and versatile CRP techniques [57]. It was discovered independently by Sawamoto in 1994 [58] and Matyjaszewski in 1995 [59–61]. In ATRP, a transition-metal complex (generally copper-, ruthenium-, iron-, or nickel-based) is used as a catalyst for initiation and chain growth, which is an extension of the Kharasch reaction [62]. The polymer growth is controlled by a redox equilibrium between macroradicals and dormant species end-capped by a halogen atom (Fig. 2). There are several advantages in ATRP. It enables precise control on molecular weight, polydispersity, and functionality. It can be carried out in a variety of different solvents and conditions, including water at room temperature, and is tolerant of most functional groups. The polymerization conditions and parameters can be tuned, providing control over reaction kinetics. In addition to homogeneous and heterogeneous solution polymerization, polymers can be grown from nearly any surface or material that is attached with ATRP initiators, including proteins, organic materials, and inorganic materials like nanoparticles (NPs). Particularly, ATRP is well suitable for the synthesis of polymer bioconjugates. Peptide sequences, biotin, and proteins such as chymotrypsin, streptavidin and bovine serum albumin (BSA) have been successfully modified to become ATRP initiators [28,39,63–68]. Polymerization from ATRP initiators containing proteins and short peptide sequences offers an attractive route to produce polymer–protein conjugates.

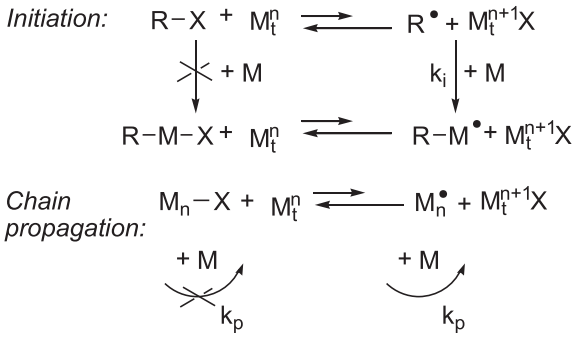

Fig. 2. Proposed mechanism of ATRP.