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Why synthesize protein–polymer conjugates? The stability and activity of chymotrypsin-polymer bioconjugates synthesized by RAFT

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ABSTRACT

α-Chymotrypsin, a commonly used protease, was modified with well-defined oligomers synthesized by RAFT. The well defined polymers were synthesized based on the monomers N,N-dimethylacrylamide (DMAm) or oligo(ethylene oxide) methyl ether acrylate (OEOA). The polymers were conjugated to free amine groups on chymotrypsin through an *in-situ* 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) coupling approach. The protein–polymer conjugates retained enzymatic activity, and the higher molecular weight DMAm and OEOA polymer, created protein–polymer conjugates with significantly enhanced stability, presumably due to the high molecular weight polymer preventing autolysis of the α-chymotrypsin.

1. Introduction

The ability to precisely synthesize a macromolecule with controlled functionality and molecular architecture for a given application is an ongoing target in the field of polymer chemistry. One area where this synthetic ability is particularly important is in the preparation of bioconjugates, or materials that combine a biologically relevant molecule with a synthetic compound [1]. Bioconjugates are an emerging class of materials that offer the benefits of activity and function in biological applications with the flexibility of chemical functionality and structure possible through synthetic chemistry [1,2]. A particularly interesting group of bioconjugates are protein-polymer hybrids, wherein the attached polymer can provide a synthetic handle to modulate the performance of the biomaterial [3–6]. The polymer attached to the protein can serve multiple roles including, stabilizing the protein–polymer conjugate [3], shifting the optimal pH and temperature for the enzyme [7], leading to responsive or "smart" biomaterials [7,8].

The synthesis of bioconjugates, including protein–polymer conjugates, typically involves one of two strategies, the "grafting-to" and the "grafting-from" approaches [1,9]. In grafting-to, a polymer is first synthesized, and subsequently attached to the protein, or other biomolecule, using an efficient organic reaction [9]. In contrast, the grafting-from approach first attaches a small molecule initiator or chain transfer agent (CTA) to the protein, or biomolecule of interest, and then directly grows the polymer from the protein in an aqueous solution [10]. The advantages of grafting-from include simple purification, and in many cases a higher grafting-density [9–11]. However, the difficulties with grafting-from include potential loss of protein stability upon attaching the initiator or CTA [12], and choosing reaction conditions that preserve protein stability while giving well controlled polymers [11]. In contrast, grafting-to offers the advantages of simple synthesis and characterization of the polymer and protein before conjugation, and that the polymerization conditions do not affect protein stability [9,13,14]. The disadvantages of grafting-to include difficulty achieving high graft density, particularly with high molecular weight polymers, and difficulty purifying the polymer from the conjugate after synthesis [10–12]. A representation of the grafting-from and grafting-to strategies is given in Scheme 1.

Reversible deactivation radical polymerization (RDRP) methods have revolutionized the fields of polymer chemistry and material science [15]. Nitroxide mediated polymerization (NMP) [16], atom

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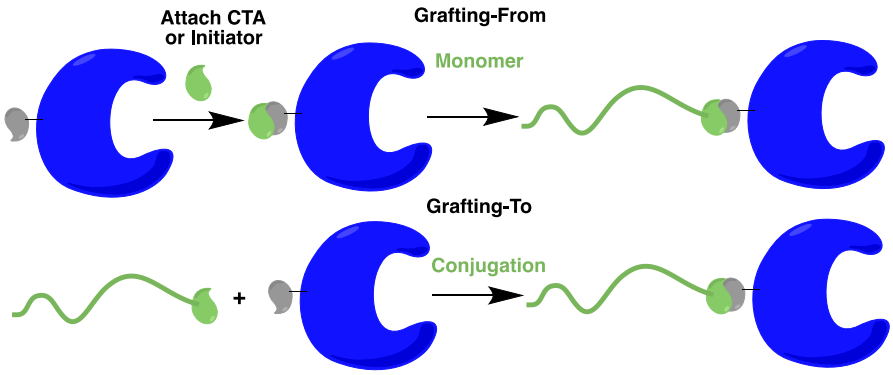


Scheme 1. Top shows a grafting-from strategy for synthesizing a protein-polymer bioconjugate, and bottom shows a grafting-to strategy for bioconjugate synthesis.

transfer radical polymerization (ATRP) [17,18], and reversible addition-fragmentation chain transfer polymerization (RAFT) [19], are the three most commonly used RDRP methods. Each of these three RDRP methods has been used to create well controlled protein–polymer conjugates [8,20–29]. RDRP methods are particularly well suited to protein–polymer conjugate synthesis since both the grafting-from and grafting-to methods can be used to create well defined biohybrids [1]. This manuscript focuses on RAFT polymerization, as a tool to synthesize well-defined protein–polymer conjugates. RAFT is well suited to the synthesis of bioconjugates [30,31], including protein–polymer conjugates [8,12,23,25,32,33], since it creates living polymers from a wide variety of functional groups, and offers excellent control over short chains [34,35].

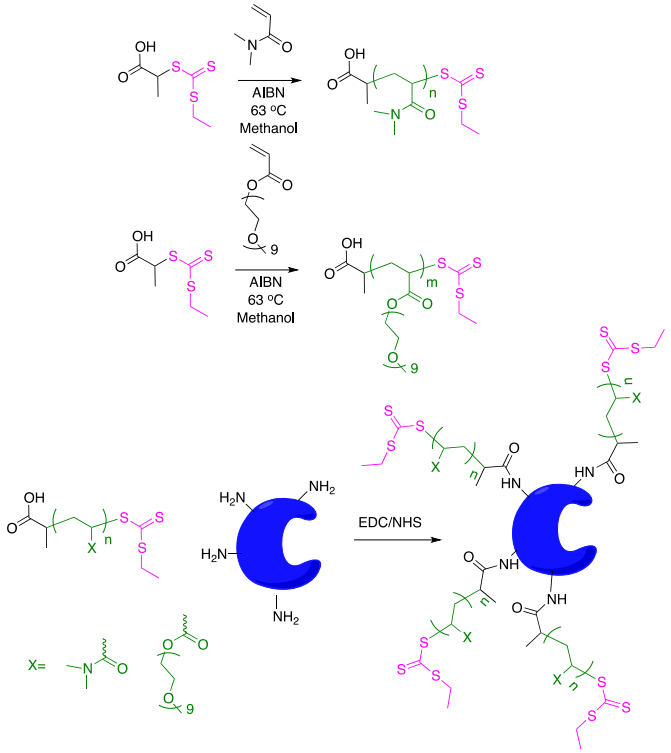
This paper uses α-chymotrypsin as the enzyme to be conjugated with synthetic polymers made by RAFT. Chymotrypsin is a protease, an enzyme that digests other proteins, including other α-chymotrypsin molecules (autolysis), by catalyzing peptide bond hydrolysis [36,37]. Due to promiscuous activities, conjugation with synthetic polymers can dramatically improve the stability and useful lifetime of proteases such as trypsin and chymotrypsin [7,38–40]. Although chymotrypsin polymer bioconjugates have been synthesized by ATRP [7,20], to the best of our knowledge there are no examples of chymotrypsin–polymer conjugates with the polymer synthesized by RAFT.

2. Results and discussion

In this paper RAFT polymerization was used to synthesize polymers containing a single carboxylic acid group, from the R group of the CTA. RAFT was used to synthesize the polymers of N,N-dimethylacrylamide (DMAm) and oligo(ethylene oxide)methyl ether acrylate (OEOA) of average molecular weight = 480. Polymers with number average molecular weight below ~5000 were chosen since the short chain facilitates grafting-to processes. Subsequently, each polymer was conjugated to free amine groups on chymotrypsin to create amide bonds through an *in-situ* 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) coupling strategy. Subsequently the activity and stability of these bioconjugates was determined and compared to that of the unmodified chymotrypsin. A summary of this approach is given in Scheme 2.

2.1. Synthesis and characterization of polymers made by RAFT

In this approach, three polymers were synthesized, and subsequently attached to chymotrypsin. 2-(((ethylthio)carbonothioyl)thio)propanoic acid (PAETC) was used as the chain transfer agent. RAFT polymerization was used to create the poly(DMAm) (pDMAm) and the poly(OEOA) (pOEOA) based chains, using AIBN (0.2 mol equivalents to CTA) as the initiator, at 63 °C, with methanol being the solvent. The temperature of 63 °C was chosen to be below methanol's boiling point of 64.65 °C [41], and at 63 °C AIBN has a half life of approximately 12.4 h [42]. The three polymers synthesized are labeled pDMAm-low MW for a polymer with a target of 10 repeat units of DMAm giving a targeted molecular weight of ~1200, pDMAm-high MW for a polymer with a target of 48 repeat units of DMAm giving a targeted molecular weight of ~5000, and pOEOA for a polymer with a target of 10 repeat units of OEOA giving a targeted molecular weight of ~5000. In all cases the monomer conversion after 24 h of reaction time was over 95%, the limit of NMR measurement.



Scheme 2. Synthesis of pDMAm and pOEOA chains containing a single carboxylic acid by RAFT polymerization, followed by the subsequent conjugation of the oligomers to the protein through EDC/NHS coupling.