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COMMUNICATIONS

Published on Web 04/16/2005

Streptavidin as a Macroinitiator for Polymerization: In Situ Protein−Polymer Conjugate Formation

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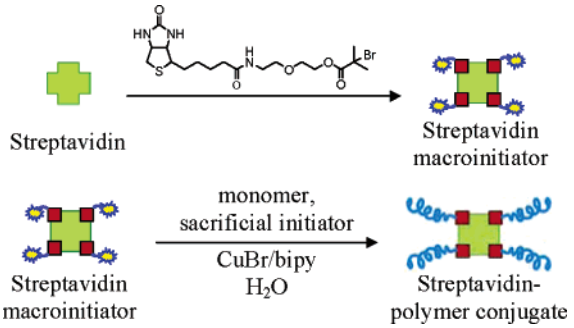
Protein−polymer conjugates are employed in numerous applications in medicine,[1] nanotechnology,[2] and bioengineering.[3] As a result, exploration of synthetic strategies to obtain these bioconjugates has been an active area of research for many years. Protocols have been established for the preparation of polymers reactive to amino acid side chains.[4] Typically, post-polymerization modification of polymer chain ends is undertaken, requiring numerous steps to form the reactive end group. Recently, straightforward routes to semi-telechelic polymers using protein-reactive initiators to prepare the polymers have been established by our group[5] and others.[6] In these cases, polymers react with proteins without further modification, simplifying the approach. Nonetheless, all of these strategies require first preparing the polymer and then conjugating to the protein. Herein we introduce a synthetic approach that employs the protein as a macroinitiator for polymerization, thereby preparing the protein−polymer conjugate in situ.

We envisioned that modifying a protein with a small molecule capable of initiating polymerization and then forming the polymer−protein conjugate would have many advantages. For example, polymer would be synthesized already attached to the protein, and removal of residual monomer is easier then purification from excess polymer. Polymers have been grafted from proteins by generating radicals on random amino acid side chains through redox processes promoted, for example, by peroxides, persulfates, or ceric ion with limited success.[7] These approaches do not allow control over the number and sites of polymerization, resulting in poorly defined conjugates. Additionally, free polymer chains are usually obtained in solution, complicating the purification of the final product. To our knowledge, our scheme is the first example of a polymerization that is initiated from specific domains of a protein and represents a straightforward strategy that promises to simplify the synthesis and purification of the target product, while controlling the exact site of modification.

Streptavidin (SAv) was chosen as the protein to demonstrate the technique. SAv is a well-studied protein that consists of four subunits, each of which is capable of binding one molecule of biotin with very high affinity ($K_d = 10^{-15}$ M).[8] Our general procedure for conducting polymerizations from proteins is outlined in Scheme 1. Modification of SAv with an opportune initiator is a prerequisite to achieve polymer formation. Atom transfer radical polymerization (ATRP)[9] is well-known for its compatibility with functional groups and has allowed the polymerization of 2-hydroxyethyl methacrylate from a peptide on solid support.[10] ATRP's tolerance of aqueous conditions at ambient temperature is also an important feature for polymerization in the presence of proteous materials and has been demonstrated for a variety of monomers.[11] Even more importantly, the use of an ATRP initiator would allow us to localize the initiation on specific domains of the protein. A known biotinylated ATRP initiator[6c] was therefore prepared and interacted with SAv to generate the protein macroinitiator.

Scheme 1. Streptavidin-Initiated Polymerization



We explored the polymerization of *N*-isopropylacrylamide (NIPAAm), because poly(NIPAAm) is well-known for its characteristic lower critical solution temperature (LCST) in water at 32 °C, above which it precipitates out of solution, making it a valuable material for applications in bioanalysis and microfluidics.[12] The same property was of interest to us also to quickly assess whether polymer chains were formed by raising the temperature above the LCST. The SAv−biotin initiator complex (2.7 mg/mL) was exposed to an aqueous solution of monomer and CuBr/2,2′-bipyridine catalyst (CuBr/bipy) at room temperature to start the polymerization. We initially attempted SAv−biotin-initiated polymerization of NIPAAm using a ratio of 100:1 between monomer and initiating sites, which resulted in a NIPAAm concentration of 14.6 mM. Little or no modification of the protein was observed by SDS-PAGE or size exclusion chromatography (SEC), most likely because of the very low concentration of the components as a consequence of the high molecular weight of the protein−macroinitiator and the small amounts of SAv present.[13]

To increase the concentration of initiating sites and monomer, polymerization from the SAv macroinitiator was performed in the presence of a sacrificial initiator. We employed a bromoisobutyrate-modified Wang resin as the sacrificial initiator because the resulting polymer-modified Wang resin could be readily removed, leaving the streptavidin−polymer conjugate in solution. A similar, but reversed, concept has been employed in surface-initiated ATRP, where additional soluble initiator has been successfully employed to increase the concentration of initiating sites and thus allow the system to self-equilibrate via persistent radical effect.[14] The polymerization was performed on 1.0 mg of SAv macroinitiator using a NIPAAm to total initiator ratio of 100:1. Conjugate formation was evident by shifts to higher molecular weight in both the SDS-PAGE gel (Figure 1, lane C) and SEC chromatograph (Figure 2). Remarkably, no unmodified SAv was detected, meaning at least one site per protein had initiated. In addition, the conjugate precipitated when the solution was heated, behavior typical of poly(NIPAAm)−protein conjugates.

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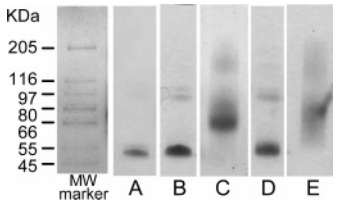

Figure 1. SDS-PAGE gel. Lane A: SAv. Lane B: SAv macroinitiator. Lane C: SAv−polyNIPAAm (obtained in the presence of sacrificial initiator). Lane D: SAv−biotin control polymerization. Lane E: SAv−polyPEGMA (obtained in the presence of sacrificial initiator).

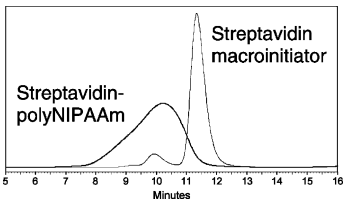

Figure 2. SEC chromatograph of the SAv macroinitiator (dash−dot line) and the SAv−polyNIPAAm conjugate obtained in the presence of sacrificial initiator. (10 mM ammonium acetate and 100 mM sodium chloride buffer, pH = 6.6; flow rate: 0.25 mL/min; λ = 290 nm.)

Control experiments were performed by exposing SAv or SAv−biotin not modified with the ATRP initiator to identical polymerization conditions in the presence of the sacrificial initiator. Polymerization proceeded from the solid resin as evidenced by FT-IR, but as expected, no protein−polymer conjugates were detected by SDS-PAGE (Figure 1, lane D), mass spectrometry, or SEC. These results illustrate that polymerization proceeds only when the protein is modified with initiator.

To further confirm that poly(NIPAAm) is formed and is attached to SAv at the biotin binding sites only, the polymerization was repeated with 5 mg of macroinitiator, and the conjugate was isolated. Treatment of the SAv−polymer conjugate with DMF/water at 90 °C for 1 h resulted in dissociation of the protein into monomeric subunits and simultaneous release of the polyNIPAAM. SDS-PAGE demonstrated that all SAv had dissociated, and the shift in the gel was identifical to the monomeric subunits of unmodified SAv. The latter indicates that polymer is not covalently attached to amino acid residues.

The identity of the isolated polymer was confirmed by ^{1}H NMR. Gel permeation chromatography (GPC) resulted in a monomodal peak with a number-average molecular weight (M_n) of 27 000 and polydispersity index (PDI) = 1.7; a broad PDI is not uncommon for ATRP in pure water[11b,c] and indicates poor control over the polymerization. Although the presence of biotin was difficult to observe by ^{1}H NMR, biotinylation was confirmed using surface plasmon resonance (SPR) by passing the polymer solution over a SAv-coated chip. Binding of the polymer to the surface was clearly observed, demonstrating that the polyNIPAAm is biotinylated. Taken together, these results show that bioconjugate formation results from polymerization initiated at the biotin binding sites only of the macroinitiator.

The strategy is flexible, and additional monomers were successfully polymerized from the SAv initiator. In particular, conjugates of poly(ethylene glycol) methyl ether methacrylate (PEGMA) polymers, which are of interest in the context of drug delivery systems and protein-repellent materials, were easily obtained from 1.0 mg of SAv initiator in the presence of sacrificial initiator (Figure 1, lane E, PEGMA/total initiator $\approx$ 40:1).

In conclusion, we report a versatile new strategy to prepare protein conjugates with synthetic polymers using modified SAv as an initiator for the polymerization of NIPAAm. We demonstrated that the protein is quantitatively modified with polymer and that the resulting polymer is conjugated to the SAv at the biotin binding sites only. Streptavidin retains its bioactivity after subjection to polymerization; no monomeric subunits were observed, and the protein still binds biotin. This straightforward approach can be applied to a variety of monomers, including the biologically relevant PEGMA. The extension of this technique to other proteins is currently being explored.

Acknowledgment. This work was supported by the NSF (Grant No. CHE-0416359). Prof. Thomas Ward, U. de Neuchâtel, is acknowledged for kindly donating recombinant streptavidin. Dr. Julian P. Whitelegge in the Pasarow Mass Spectrometry Laboratory is thanked for protein mass spectrometry.

Supporting Information Available: Experimental details, additional SEC traces and SDS-PAGE gels, mass spectroscopy data, ^{1}H NMR, GPC, and SPR of the polyNIPAAm grown from streptavidin. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA042230+