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Exploring the post-polymerization modification of side-chain amino acid containing polymers *via* Michael addition reactions

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ABSTRACT

An efficacious protocol for the aza-Michael addition of a *C*-terminus modified phenylalanine side-chain containing polymer (Michael donor) was demonstrated with various Michael acceptors. The aza-Michael addition reactions were carried out at 50 °C in anhydrous methanol, which is a protic solvent, to enhance the advancement of the reactions. ^{1}H and inverse gated ^{13}C NMR spectroscopy were utilized to qualitatively deliver comprehensive data on the reaction progress. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) provided quantitative information about the modification of the polymer. The extent of change in the aza-Michael addition varied with different Michael acceptors and decreased in the order of acrylate > acrylamide > methacrylate. The present study opens up a library of polymers with functional modifications in the side-chain.

1. Introduction

Michael addition reactions, one of the most popular types of organic reactions, have received more attention in the present decade from polymer chemists due to their mild reaction conditions, high functional group tolerance, good conversion, and absence of side products and toxic metal catalysts, which makes them fit for post-polymerization modification reactions [1]. The general reaction chemistry is the addition of a Michael donor and an acceptor towards the formation of a Michael adduct [2]. Michael donors are mostly nucleophilic functional groups with an electronegative heteroatom bearing lone pair electrons, such as amines, thiols and phosphines. Other contributing reactants are the Michael acceptors, which are mostly electrophilic olefins. Among the two steps in a general Michael addition reaction, the rate-determining step is the generation of an anionic intermediate, which explains the significance of the electronic effects around the electrophilic center of the acceptor for the progress of the reaction [3]. Aza-Michael addition is a subclass of Michael addition reactions where an amine group is the Michael donor and α, β-unsaturated carbonyl compounds act as Michael acceptors [4]. The particular importance of aza-Michael addition rises from the presence of amine nucleophiles in biological systems. Carbon- and thia-Michael additions are relatively slow and require the presence of an external base for the deprotonation of the donor. In contrast, aza-Michael addition does not require any intervention from a base, because the amine group can work as a base and nucleophile simultaneously. Therefore, aza-Michael addition has been utilized in the field of polymer chemistry for various purposes. Initially, linear step growth polymerization by sequential Michael addition was reported [5,6]. Modern controlled radical polymerization (CRP) methods were also enriched by employing this type of reaction in the synthesis of macroinitiators in atom transfer radical polymerization (ATRP) [7], chain end functionalization in reversible addition-fragmentation chain transfer polymerization (RAFT) [8], and as a post-polymerization modification tool [9]. The foremost report about tailoring higher order topological polymer networks was from Tomalia's group. They reported the synthesis of poly(amidoamine) *via* Michael addition of bisacrylamides and diamines, which was further utilized as a nonviral transfection agent [10]. In the field of bioconjugate chemistry, aza-Michael addition has been substantially investigated for surface modifications [11,12], tissue engineering [13], etc. Very few reports are available regarding the utilization of aza-Michael addition as a post-polymerization tool. Studies comparing the reactivity of poly(2-aminoethyl methacrylate) with different acrylates and acrylamides were carried out by Armes et al. [9]. Recently, aza and thia-Michael additions were used in combination for framing fifth generation dendrimers [14]. Hoffman and coworkers performed functionalization of polyesters prepared through an enzymatic pathway by aza-Michael addition reaction [15].

Chiral *C*-terminus modified amino acid containing polymers are of special interest due to their dexterity in showing secondary

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structures [16], supramolecular network formation [17], chiral recognition [18], responsiveness towards a physical stimulus [19], high ion conducting polyelectrolytes [20], ability to act as a drug/gene delivery vehicle [21], and capability in forming potential three-dimensional networks [22]. Post-polymerization modifications of these important polymers are necessary to incorporate molecules that often cannot be included before polymerization but impart novel properties to the polymers [23–25]. Therefore, in the present study, we aimed to understand and develop protocol chemistry for the aza-Michael addition reaction of amino acid containing polymers with popular acrylates and methacrylates in methanol. The solvent choice is crucial in performing Michael addition reactions because aprotic solvents can lead to incomplete alkylation of the —NH_2 group [26]. Smith and coworkers performed Michael addition in different solvents and found that the reaction yields monodisperse dendrimers in methanol [27]. In addition to the post-polymerization modification of the polymer, the electronic effects on and kinetics of the aza-Michael addition were also investigated by varying the electron density around the nucleophilic center of the olefins. Specifically, the phenylalanine containing amino acid was designated because the side chain carried aromatic protons, which would not be affected during the aza-Michael addition reaction.

2. Experimental section

2.1. Materials

Tert-butyloxycarbonyl-L-phenylalanine methacryloyloxyethyl ester (Boc-Phe-HEMA) [28] and the chain transfer agent 4-cyano-4-(dodecylsulfanylthiocarbonyl) sulfanylpentanoic acid (CDP) [29] were synthesized as per previous reports. Recrystallized 2,2-azobisisobutyronitrile (AIBN, Sigma, 98%) was used as the initiator for the polymerization. Trifluoroacetic acid (TFA, 99.5%) was purchased from Sisco Research Laboratories Pvt. Ltd., India. The Michael acceptor units methylacrylate (MA), *N*,*N*-dimethylacrylamide (DMA), polyethylene glycol methyl ether acrylate (PEGA, M_n = 480 g/mol), methyl methacrylate (MMA), *N*,*N*-(dimethylamino)ethylmethacrylate (DMAEMA) and polyethylene glycol methyl ether methacrylate (PEGMA, M_n = 300 g/mol) were purchased from Sigma–Aldrich and used without removing the inhibitor to exclude the chance of homopolymerization during the Michael addition reactions. Anhydrous *N*,*N*-dimethylformamide (DMF, 99.9%), and anhydrous methanol (99.8%) were purchased from Sigma–Aldrich and used without any further purification. DMSO-d_6 (99.8% D) was purchased from Cambridge Isotope Laboratories, Inc., USA for the nuclear magnetic responance (NMR) study. The solvents such as hexanes (mixture of isomers), acetone, ethyl acetate and dichloromethane (DCM) were purified using standard procedures.

2.2. Instrumentation

^{1}H and ^{13}C NMR spectroscopy were performed on a Bruker Avance^{III} spectrometer operating at 500 and 125 MHz, respectively. The ^{13}C NMR spectra were recorded under inverse gated decoupling with a 5 s delay time between the pulses and a line broadening of 1.0 Hz. The FT-IR spectrum was obtained on potassium bromide pellets using a Perkin–Elmer Spectrum 100 FT-IR Spectrometer. Gel permeation chromatography (GPC) was performed on a Viscotek instrument using two Visco Gel I-Series G4000 columns, where the flow rate of the eluent (DMF) was 1.0 mL/min at 35 °C. The detectors used were a Viscotek refractive index (RI) detector operating at λ = 660 nm and a Viscotek model 270 series platform consisting of a laser light scattering detector (operating at 3 mW, λ = 670 nm with detection angles of 7° and 90°) and a four-capillary viscometer. Calibration of the system was performed using poly(-methyl methacrylate) (PMMA) standards of narrow molecular weight distribution (dispersity, $Đ$). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was carried out on a Bruker ultrafleXtreme™ instrument equipped with a smart beam-II laser in the reflector mode and an acceleration voltage of 22 kV. 2,5-Dihydroxybenzoic acid (DHB, Bruker) was used as the matrix. Thermogravimetric analysis (TGA) was accomplished on a Mettler Toledo TG/SDTA 851e instrument at a heating rate of 10 °C min^{-1} with a sample of mass of 5–10 mg in N_2 atmosphere. Thermal analysis was carried out using a Mettler Toledo DSC1 STARe differential scanning calorimeter (DSC) under N_2 atmosphere. The polymers were first cooled from room temperature to −50 °C, then heated to 125 °C and again cooled to −50 °C at 10 °C min^{-1}. The glass transition temperature (T_g) was taken from the third segment of the run, i.e., from 125 to −50 °C, because the solvent or other volatile impurities would have evaporated during heating from −50 to 125 °C [30].

2.3. General RAFT polymerization procedure

The poly(Boc-phenylalanine methacryloyloxyethyl ester) (P(Boc-Phe-HEMA)) polymer was prepared by following a previously reported RAFT polymerization of Boc-Phe-HEMA [28]. Typically, to a 20 mL septum-sealed glass vial equipped with a magnetic spin bar, Boc-Phe-HEMA (2.0 g, 5.3 mmol), CDP (70.72 mg, 0.176 mmol) and AIBN (2.9 mg, 0.17 μmol) were subsequently added and homogenized in anhydrous DMF (4.0 g). A ratio of monomer to solvent of 1:2 (weight/weight) was maintained during the polymerization reaction. The vial was subjected to degassing for 20 min using dry N_2 and then kept for 3 h in a pre-heated reaction block at 70 °C. The reaction was stopped by cooling the vial in an ice-water bath. Then, acetone (2.0 mL) was added to the vial, and the polymer, P(Boc-Phe-HEMA), was precipitated in cold hexanes. Further purification was achieved through dilution of the polymer in acetone and reprecipitation (5×) in hexanes. The precipitate was then dried under vacuum overnight to give a yellow powder. The P(Boc-Phe-HEMA) was then structurally characterized by ^{1}H NMR spectroscopy [28]. For the P(Boc-Phe-HEMA), number-average molecular weight was determined from GPC ($M_{n,GPC}$ = 10,100 g/mol, $Đ$ = 1.30) and NMR spectroscopy ($M_{n,NMR}$ = 8900 g/mol). These values matched nicely with the molecular weight theoretically calculated based on conversion ($M_{n,theo}$ = 9100 g/mol) [18] and confirmed the successful controlled synthesis of P(Boc-Phe-HEMA) (**1**).

2.4. Deprotection of Boc groups

In a 20 mL glass vial (equipped with a magnetic bar), 1.0 g of **1** was dissolved in 1.5 mL of DCM and was allowed to homogenize by stirring at room temperature for 10 min. After keeping the vial in an ice-water bath, 3.0 mL of TFA was added and stirred for 90 min at room temperature. After completion of the reaction, isolation of the desired compound was acquired through precipitation in excess diethyl ether. The precipitate was then dried under vacuum overnight. This procedure generated P(CF_3COO^- NH_3^+-Phe-HEMA) (**2**), which was pale yellow in color, in 95% yield. Successful Boc group detachment was revealed by the disappearance of Boc (—C(C***H***$_3$)$_3$) protons from 1.44 ppm [18] (data not shown here). Furthermore, from the FT-IR spectrum, we observed that the absorption due to the amide (—NH) bond stretching at 1498 cm^{-1} was no longer present in **2**, and 1° amine (—NH_2) bending frequency at 1558 cm^{-1} was generated after deprotection of the Boc group [31].