Analytica Chimica Acta 816 (2014) 28–40

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Determination of polyethylene glycol end group functionalities by combination of selective reactions and characterization by matrix assisted laser desorption/ionization time-of-flight mass spectrometry

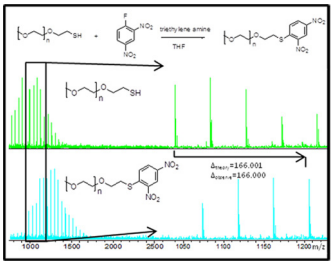
Boyu Zhang, Hong Zhang, Brittany K. Myers, Ravinder Elupula, Janarthanan Jayawickramarajah, Scott M. Grayson*

Tulane University, Department of Chemistry, <New Orleans, LA 70118, United States

HIGHLIGHTS

- MALDI-TOF MS examined as a tool for end group identification.
- Reactions identified that differentiate alcohol, amine and thiol groups.
- Rapid click conjugation reactions also confirmed by MALDI-TOF MS.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 December 2013
Accepted 13 January 2014
Available online 22 January 2014

Keywords:
Matrix-assisted laser desorption/ionization time-of-fight mass spectrometry
Polyethylene glycol
End group determination
Click chemistry
Polymer characterization

ABSTRACT

End groups play a critical role in macromolecular coupling reactions for building complex polymer architectures, yet their identity and purity can be difficult to ascertain using traditional analytical technique. Recent advances in mass spectrometry techniques have made matrix-assisted laser desorption/ionization time-of-fight (MALDI-TOF) mass spectrometry a rapid and powerful tool for providing detailed information about the identity and purity of homopolymer end groups. In this work, MALDI-TOF mass spectrometry was used to study end groups of linear polyethylene glycols. In particular, the identifications of alcohol, amine and thiol end groups are investigated because these nucleophilic moieties are among the most common within biological and synthetic macromolecules. Through comparative characterization of alcohol, amine, and thiol end groups, the exact identification of these end groups could be confirmed by selective and quantitative modification. The precision of this technique enables the unambiguous differentiation of primary amino groups relative to hydroxyl groups, which differ by only 1 mass unit. In addition, the quantitative conversion of various polyethylene glycol end groups using highly efficient coupling reactions such as the thiol-ene and azide-alkyne click reactions can be confirmed using MALDI-TOF mass spectrometry.

1. Introduction

Matrix-assisted laser desorption/ionization time-of-fight mass spectrometry (MALDI-TOF MS) is a soft ionization technique that allows desorption and ionization of macromolecules from solid state into a gas state with little to no fragmentation in their mass spectra [1–7]. Unlike electrospray ionization mass spectrometry which typically creates numerous multiply–charged species [8], singly charged ions predominate in MALDI-TOF mass spectra, making it is easier to interpret the spectra of polydisperse macromolecules. Within the last few decades, MALDI-TOF has become an important tool in the characterization of many synthetic polymers

* Corresponding author. Tel.: +1 504 862 8135.
E-mail address: sgrayson@tulane.edu (S.M. Grayson).

0003-2670/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2014.01.039

providing useful information such as the average molecular weight [9], the repeat unit molecular weight [10], and polydispersity [11]. It provides several advantages over other traditional polymer characterization techniques such as gel permeation chromatography (GPC) or nuclear magnetic resonance (NMR) spectroscopy including rapid generation of spectra, ease of sample preparation, microgram sample requirements, and tolerance of low molecular weight impurities. It also provides a rapid and straight-forward methodology to monitor macromolecular reactions *via* the analysis of small aliquots taken during the course of a reaction [12,13].

The end groups of linear macromolecules play a critical role in the resulting physical properties of the polymer and can be coupled to other macromolecules to construct more complex polymer architectures [14–17]. Because it is often challenging to isolate products from starting materials or byproducts during macromolecular end group modifications, quantitative functional group transformations are of particular value for polymer chemists. However, traditional characterization techniques such as GPC, NMR, and infrared (IR) spectroscopy have severe limitations when determining the efficiency of end group transformations because any signal that originates from the end groups tends to be overwhelmed by the larger signal from the backbone [18]. As a result, the reaction conversion ratio is difficult to determine accurately, which can lead to substantial synthetic complications. However, in the case of MALDI-TOF MS, each observed signal contains information about the end groups of that species, providing a vast increase in sensitivity that can enable the accurate determination of macromolecular end groups [19–23]. When two polymers vary only in the type and mass of their end groups, their spectra will show two different series of m/z peaks in their MALDI-TOF mass spectra, each of the peaks separated by the mass of repeat unit. The presence of strong signal from the product and the complete loss of signal from the starting material (and any possible intermediates) can be used as strong evidence for the quantitative nature of a reaction. However, different end group can have vastly different ionization efficiencies, requiring the use of controls to verify accurate quantification. Therefore, if proper precautions are taken, MALDI-TOF MS can easily distinguish between polymers with the same repeating unit structure but different end groups and provide a technique for confirming high end group purity [24–26].

Poly(ethylene glycol) (PEG) is one of the most important polymers for biomedical and pharmaceutical applications because of its outstanding properties such as high water-solubility, low-toxicity, low immunogenic response, and therefore its acceptance by the FDA for human use *in vivo* [27–29]. Commercially available PEG is an inexpensive material which can be obtained with a well-defined structure and low polydispersity, providing sufficiently homogeneous drug-polymer conjugates to enable their use *in vivo* [30]. The covalent coupling of PEG to a biomolecule or drug can give several advantages for gene or drug delivery systems including increased water-solubility, decreased toxicity, increased blood-circulation times, and increased stability to metabolic enzymes [31]. However, all of these medically relevant applications require quantitative coupling of the PEG end group functionalities to the biologically active species. Three of the most common PEG end group functionalities employed for conjugation are alcohols, amines and thiols, because of their ease of synthesis and their efficient coupling reactions. The importance of these particular functional groups is further supported by their ubiquity within biopolymers, such as proteins and carbohydrates. While evidence for these functional groups can be provided by acquiring well-calibrated MALDI-TOF MS data, and correlating the calculated end group mass to that of the expected end groups [22], uncertainty in the number of repeating units for a given signal and uncertainty in the identity of the other end group can reduce the confidence of an end group assignment. Herein, this study establishes a set of rapid and quantitative end group modifications that will yield an unambiguous conclusion as to the end groups present on a particular polymer chain, as well as provide insight into the purity of the end groups. The precision of this analytical technique enables the rapid and unambiguous differentiation of a primary amino group from a hydroxyl group. In addition because "click" coupling reactions have become a valuable tool for the conjugation of PEG, the thiol-ene [32,33] and azide-alkyne [34,35] click reactions were also monitored, and their rapid and quantitative nature confirmed by using MALDI-TOF mass spectrometry.

2. Experimental

2.1. Materials

PEG monomethyl ether alcohol (poly(ethylene glycol) methyl ether, product no. 202509), PEG monomethyl ether thiol (poly(ethylene glycol) monomethyl ether thiol, product no. 729108), PEG diol, PEG diamine (product no. 753084), benzyl bromine, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB), α-cyano-4-hydroxycinnamic acid, 4-(dimethylamino)pyridine (DMAP), *N*,*N*,*N*′,*N*″,*N*′-pentamethyldiethylenetriamine (PMDETA), copper(I) bromide (CuBr), sodium trifluoroacetate, maleimide, sodium hydride and potassium carbonate were obtained from Sigma-Aldrich. Acetic anhydride was obtained from Fisher Chemical. 2,4-Dinitrofluorobenzene was obtained from TCI America. Copper(I) bromide (CuBr) was purified by washing with acetic acid. Porphyrin-monoalkyne was prepared according to a reported literature procedure [36]. Tetrahydrofuran (THF) was distilled after refluxing with sodium metal overnight. All solvents were reagent grade and used without further distillation or purification.

2.2. Instrumentation

Mass spectrometric analysis was performed using a Bruker Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA). Data were acquired using reflector-positive ion mode, an acceleration voltage of 20 kV, and delayed extraction. Data acquisition was performed using Bruker Daltonics FlexControl 3.0 software, and data analysis was carried out with Bruker Daltonics FlexAnalysis 3.0 software. The mass scale for the MALDI-TOF MS was calibrated using SpheriCal mass standards (Polymer Factory, Sweden). This particular calibrant is used in our laboratories because of its broad compatibility with a wide range of matrices and solvents, yet its limited number of peaks minimizes overlap during internal calibration. All ^{1}H NMR (400 MHz) were obtained using a Varian Mercury spectrometer (Palo Alto, CA), using TMS = 0.00 ppm for ^{1}H calibration. Gel permeation chromatography (GPC) was carried on a Waters model 1515 series pump (Milford, MA) with three column series from Polymer Laboratories, Inc. consisting of PLgel 5 μm Mixed D (300 mm × 7.5 mm, molecular weight range 200–400,000), PLgel 5 μm 500 Å (300 mm × 7.5 mm, molecular weight range 500–30,000), and PLgel 5 μm 50 Å (300 mm × 7.5 mm, molecular weight range up to 2000) columns. The system was fitted with a Model 2487 differential refractometer detector and anhydrous tetrahydrofuran was used as the mobile phase (1 mL min^{-1} flow rate).

2.3. Sample synthesis

2.3.1. Acylation of PEG-diol, PEG-diamine, and PEG monomethyl ether thiol

Acylation of PEG-diol, PEG-diamine and PEG monomethyl ether thiol were achieved using analogous protocols. As a representative