Original Article

A Novel Derivatization Reagent in the Determination of the Number of OH End Groups in Poly(ethylene glycol) by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

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The potential of a novel derivatization reagent, trifluoroacetic anhydride (TFAA), in determining the number of OH groups in poly(ethylene glycol) (PEG) was investigated by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The MALDI mass spectra of the products revealed peaks of sodiated derivative cations, whose shift by the respective increments, $\Delta m/z$: $96 \times$ number of OH, allowed for the determination of the number of end functional groups with active hydrogens. In the present study, complete acylation of OH groups by TFAA proceeded rapidly, and only required mixing in acetonitrile solvent without purification. As a result, the number of OH end groups of PEG could be determined rapidly.

Keywords: MALDI-MS, poly(ethylene glycol), trifluoroacetic anhydride, end structure group

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INTRODUCTION

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a powerful tool that can be utilized for the analysis of synthetic polymers.^{1–5)} However, MALDI mass spectra only provide information pertaining to molecular-weight, such as protonated, deprotonated or cationized molecules. In an effort to overcome this limitation, tandem mass spectrometry (collision-induced dissociation, CID, or post source decay) has been employed to provide some additional structural information. There is, however, a problem in the case of non-polar polymers whose constituent molecules are not directly amenable to ionization by popular organic matrices, and are therefore unsuited to analysis by MALDI-MS. In such cases, derivatization may be employed to increase the efficiency of MALDI-MS in the analysis of synthetic polymers.

A number of derivatization methods have been suggested for the determination of the molecular-weight distribution of non-polar polyolefins and polystyrenes.⁶⁾ Partial degradation reactions, such as ozonolysis, ammonolysis, saponification, hydrolysis and photolysis, have been employed as alternative derivatization methods.⁷⁾ The degradation reactions are designed to generate low molecular weight fragments where analysis by MALDI-MS can provide information concerning the microstructure of the various synthetic polymers.

Several simple derivatization methods (silylation, acylation, or alkylation of polar functional groups), widely employed in gas chromatography/mass spectrometry (GC/MS),⁷⁾ have rarely been applied to the analysis of polymers and oligomers by MALDI-MS. In a previous study,⁸⁾ tri-

methylsilylation, methylation and anthrylmethylation were used in an effort to improve the cationization and oligomeric resolution of poly(acrylic acid) under MALDI conditions. Other applications employing derivatization include determination of the number of functional groups possessing active hydrogen atoms. This simple methodology is widely used in GC/MS⁷⁾ and was recently applied in determining the number of OH groups in oligosaccharides by MALDI-MS.⁹⁾ Borisov and co-workers reported the potential of trimethylsilylation and acylation methods in determining the number of OH groups (or other groups with active hydrogens) and for the structural elucidation of poly(ethylene glycol) (PEG) and poly(1,2-propylene glycol) by MALDI-MS.¹⁰⁾ However, complete trimethylsilylation or acylation required ten hours incubation in an ultrasonic bath, and tetrahydrofuran solvent was needed for the dehydration process. In the present study, we investigated the potential of a novel derivatization reagent, trifluoroacetic anhydride (TFAA), for the determination of the number of OH end groups in PEG by MALDI-MS.

EXPERIMENTAL

PEG (average molecular weight: *ca.* 1,000 and 3,000),

Fig. 1. Structure of trifluoroacetic anhydride (TFAA).

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Fig. 2. Comparison of MALDI mass spectra of initial (a) and acylated (b) PEG-1,000.

acetonitrile, tetrahydrofuran (THF) with about 0.03% antioxidants, 2,6-di-butyl-4-methylphenol, and 2,5-dihydroxybenzoic acid (2,5-DHB) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Thermo Fischer Scientific (Yokohama, Japan). Trifluoroacetic anhydride (TFAA^{*1}; Fig. 1) was purchased from Tokyo Kasei (Tokyo, Japan). Sodium trifluoroacetate (NaTFA) was purchased from Aldrich (Tokyo, Japan). Acetonitrile and THF were used without purification.

SAMPLE PREPARATION

- 1) Trimethylsilylation was performed by placing 1 mL of polymer solution in acetonitrile (2 mg/mL), 100 μL BSTFA and 50 μL THF in a sealed glass sample tube and incubating the mixture in a hot bath for 2 h at 60°.
- 2) Acylation was performed by placing 1 mL of polymer solution in acetonitrile (2 mg/mL) and 100 μL TFAA in a sealed glass sample tube and mixing gently by hand.^{*2}

MALDI-MS

Initial polymers and derivatized products were analyzed by MALDI-MS using a Voyager DE-STR mass spectrometer (Applied Biosystems, Framingham, MA, USA), equipped with a nitrogen laser ($\lambda=337\text{ nm}$ and 3 ns pulse width), in positive ion reflectron mode. 2,5-DHB (10 mg/mL in ace-

tonitrile) and NaTFA (1 mg/mL in acetonitrile) were mixed with each sample, and analyte solution (0.2 μL) was spotted onto the MALDI plate using an Eppendorf pipette and then dried under ambient conditions.

RESULTS AND DISCUSSION

Two derivatization methods, trimethylsilylation (BSTFA) and acylation (TFAA), were evaluated. Figure 2 shows MALDI mass spectra of initial and acylated products of PEG-1,000. Analysis of PEG-1,000 by MALDI-MS indicated that the trimethylsilylated sample failed to yield detectable derivatized product ions (data not shown) unlike the case with the acylated sample where sensitive detection of derivatized product ions was achieved. The same trend was observed with PEG-3,000 (Fig. 3). It was supposed that trimethylsilylation could not proceed successfully due to the presence of moisture in any of the solvents employed, or that any derivatized products generated decomposed during preparation of the analyte solutions for MALDI-MS. This phenomenon indicated that the complete dehydration process of all solvents to use was necessary for proceeding trimethylsilylation successfully. On the other hand, the results revealed that acylation proceeded rapidly to form stable products, and that this process was not affected by the presence of any moisture in the solvents employed.

The mass spectra of acylated PEG do not exhibit peaks representing underivatized oligomers, thus confirming the

^{*1} Hazard statements of TFAA

TFAA causes severe skin burns, eye damage and respiratory irritation. At the time of use of the TFAA, please wear tools for protection, protective gloves/protective clothing/eye protection/face protection as needed. When it gets into eyes, please rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

^{*2} Instructions

While mixing reagents, please be careful a sealed glass sample tube to be hot, because it causes exothermic reaction. The reactivity of the TFAA is extremely high, the acylation is completed momentarily.

Fig. 3. Comparison of MALDI mass spectra of initial (a) and acylated (b) PEG-3,000.

Scheme 1. Acylated reaction process for the formation of trifluoroacetate end groups in the presence of TFAA.

quantitative derivatization of the samples. The shift in molecular mass by an increment due to the derivatizing agent ($\Delta m/z$: $96 \times n$ Da for the acyl group, $\text{CF}_3\text{C}(=\text{O})$; (Scheme 1)) demonstrated that the major oligomers contained the two expected residual OH groups ($\Delta m/z$ increment; 192 Da).

The MALDI mass spectra of PEG-1,000 showed the presence of minor components, possessing molecular masses 16 Da lower than those of the regular oligomers. At the same time the mass shift of the acylated minor oligomer peaks by an increment of 96 Da indicated the presence of only one OH group in the molecules (Fig. 2). The results indicated that the minor oligomers possess only one OH group and probably one ethoxy group. This was estimated that the residual vinyl end group was hydrogenated, and formed byproduct of PEG origin. As a result, the number of OH end groups of PEG could be determined rapidly by acylation using TFAA, with PEG-1,000 having one or two OH end groups, and PEG-3,000 having two OH end groups.

When two OH groups of 2,5-DHB were derivatized by TFAA, as mixed sample solutions with TFAA, volatility of acylated 2,5-DHB increased, and the rapid loss of the matrix was concerned about of a high vacuum, however, in this experiment, it was not confirmed. Because the carboxylic acid group of 2,5-DHB could not be derivatized by TFAA, the

polarity of the matrix was maintained and estimated that the matrix could be avoided a loss caused by the volatilization.

CONCLUSION

The present study indicated that acylation by TFAA may be employed for the rapid analysis of end and side-chain groups possessing OH units, and can provide structural information of individual oligomeric macromolecules containing such functional groups.

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