Polymer Bulletin 33, 1–6 (1994)

Polymer Bulletin

Heterobifunctional poly(ethylene oxide

One pot synthesis of poly(ethylene oxide) with a primary amino group at one end and a hydroxyl group at the other end

Yong Joo Kim[1], Yukio Nagasaki[2,*], Kazunori Kataoka[1,2,*], Masao Kato[2], Masayuki Yokoyama[1,3], Teruo Okano[1,3], and Yasuhisa Sakurai[1,3]

[1]International Center for Biomaterials Science (ICBS), Yamazaki 2669, Noda 278, Japan
[2]Department of Materials Science and Techology, Science University of Tokyo, Noda 278, Japan
[3]Institute of Biomedical Engineering, Tokyo Women's Medical College, Shinjuku-ku, Tokyo 162, Japan

Summary

Well-defined poly(ethylene oxide)s with a primary amino group at one end and a hydroxyl group at the other terminus were synthesized with new sila-protected amino functionality initiator, potassium N-[2-(2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentyl)-ethyl]methyl amide [**1b**]. **1b** initiated an anionic polymerization of ethylene oxide (EO) to form a polymer (PEO) without any side reactions such as a cleavage reaction of protective group and a chain transfer reaction. The molecular weights of the PEO determined from GPC and MALDI TOF-MS spectrometry agreed well with those from end group analysis using ^{1}H and ^{13}C NMR and TLC and also with the expected value from EO/initiator ratio. From these results, it was concluded that the polymers thus obtained had a primary amino group at one end and a hydroxy group at the other end and can be regarded as hetrobifunctional PEO.

Introduction

Poly(ethylene oxide) (PEO) chemistries have been widely studied by numerous researchers in terms of synthetic methods and mechanisms, properties and applications (1). Especially, the applications of PEO have been becoming attractive in a variety of fields such as biologies, biomedical sciences, surface chemistries and electrochemistries, owing to their unique properties such as solubility and flexibility of the chains and basicity of ether oxygens in the main chain. For example, PEO can be used in aqueous media for cell fusion to produce hybridoma (2). Recently, end-reactive PEOs have become very important to control their properties. For example, Ohno, et al. reported that cation solubilities in PEO were affected strongly by the end groups of the PEO (3). From the electrochemical point of view, it is quite important that the ionic conductivity in PEO can be controlled by changing the end groups of PEO. Furthermore, reactive groups at the end of PEO chain are essential to control the properties of the PEO as modifier in the field of biochemistry and surface chemistry. A modification of proteins by PEO semitelechelic oligomers reduces an antigenicity of the protein (4). Semitelechelic PEO can also be used as surface modifiers to change surface properties *e. g.* for biocompatible surface (5) and for capillary electrophoresis (6). Recently, Kataoka and his coworkers synthesized a new type of hydrophilic/hydrophobic block polymers composed of PEO semitelechelic as one block segment (7). The block copolymers formed extremely stable polymeric micelle in water and can be used as a vehicle of anti-cancer drugs (8,9).

Therefore, end-reactive PEO becomes very important. Most of the end-reactive PEO, however, were semi-telechelic or homo-telechelic oligomers (10). To expand an opportunity of PEO for the applications as stated above, a synthesis of hetero-telechelic oligomers (11) has been expected. If such heterotelechelics are synthesized easily, these materials can be utilized as modification agents with high performances. There are several reports on the synthesis of

*Corresponding authors

hetelobifunctional PEO using homo-telechelic PEO as starting materials (12). The synthetic methods, however, are complicated because they had to use several reaction steps to derivatize PEO terminus. In addition, the efficiency for derivatizations are not so high, meaning the resulting PEO to be the mixture of starting homo-telechelics and the resulting heterotelechelics in some extent.

Our strategy for hetelotelechelics is to create a novel polymerization route of ethylene oxide (EO) using new initiators with certain functionality. We reported previously that a potassium bis(trimethylsilyl)amide initiated an anionic polymerization of EO to form PEO with a primary amino group in the initiation end and a hydroxyl group at the other end (11). During the polymerization, however, growing chain end attacked trimethylsilyl protecting group at the end of polymer chain to take place a chain transfer reaction. As a result, the oligomers contained secondary and tertiary amino terminuses in some extent. In this paper, we report on synthesis of heterotelechelics with primary amino group at one end and hydroxyl group at the other end without any contamination using new sila-protected potassium amide as an initiator.

Experimental

Materials

Commercial tetrahydrofuran (THF), 1,2-bis(chlorodimethylsilyl)ethane (DSE), and ethylene oxide (EO) were purified using conventional method (13). Butyllithium was used as a hexane solution, the concentration of which was determined using Gilman's double titration method (14). Potassium naphthalene was prepared according to the literature (15). N-methylethylene diamine (MEDA) was used as received from Tokyo Kasei Co.

Synthesis of N-2-(2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentyl)ethylmethyl amine [1a]

A hexane solution of butyllithium (0.1 mol, 63 mL) was added to a solution of N-methyl ethylenediamine (0.1 mol, 7.4 g) in 125 mL of dry THF at room temperature under argon atmosphere. After stirring for 30 min, a solution of DSE (10.7 g, 0.05 mol) in 50 mL of dry THF was added to this mixture at room temperature under argon atmosphere and then stirred for 3h at room temperature. The solution was filtered and then evaporated in vacuo. The crude product was purified by repeated fractional distillation at 70-90 °C (8 mmHg). Finally, 1.8g (7 mmol, 16%) of **1a** was obtained as a colorless liquid. The purity was checked by gas chromatography and found to be more than 94%.

Anionic polymerization of EO using potassium N-2-(2,2,5,5-tetramethyl-1-aza-2,5-di-silacyclopentyl)ethylmethyl amide [1b] as an initiator

All of anionic polymerizations of EO were carried out under argon atmosphere in a 100 mL glass reactors equipped with a 3-way stopcock. One of the representative procedures of anionic polymerizations is described. THF solution of potassium naphthalene (1 mmol, 2.08 mL) was added to a stirred THF solution (15 mL) of sila-protected amine, **1a** (1 mmol, 0.21 g) using a syringe. After stirring for a few minutes to form **1b**, EO (3 mL, 60 mmol) was added to the mixture using a syringe. The color of the mixture was observed to turn brownish red immediately. After the mixture was allowed to react for 2.5 day at room temperature, few drops of acetic acid was added to stop the reaction. The mixture was poured into a 15 fold volume of diethyl ether to precipitate. The polymer sample collected was subjected to freeze drying with benzene to remove the solvents employed. The silyl protecting groups at the polymer ends were completely deprotected during acid treatment and the reprecipitation steps.

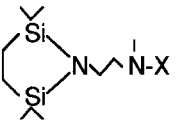
Characterization

IR spectra were recorded on a JASCO IR-Report 100 spectrophotometer. Gas chromatograms were taken with a HP 5890 gas chromatography. Proton NMR spectra were observed on a Varian VXR-500S spectrometer at room temperature, using 5 mm glass tube containing a solution of the sample in chloroform-d_1 and DMSO-d_6 (50 mg/mL) for polymer and benzene-d6(30 mg/mL) for initiator. Chloroform, DMSO and benzene were used as internal references for the measurements. Carbon-13 NMR spectra of polymers were recorded

on a JEOL EX-90 spectrometer at room temperature. The polymers(0.25g) were dissolved in 1 mL chloroform-d1, which was used as internal reference. For GPC measurements, a Shimadzu 6A Liquid Chromatograph and a Toyo Soda HLC-8020 were used (column: TSK-Gel G4000H8+G3000H8+G2500H8). TLC was performed on 250 mm silica gel TLC plates using a fluorescent indicator with chloroform/methanol (80/20 v/v) as the mobile phase. MALDI TOF-MS spectrum was obtained from samples in Gentistic acid as a liquid matrix using a Shimadzu KOMPACT MALDI III with time of flight detector.

Results and Discussion

Trialkylsilyl groups are being widely utilized as protective groups owing to several reasons. One of the reasons is easiness to prepare silyl-protection using several reagents such as silyl halide, silazane, silyl cyanide, silyl isocyanate, etc. (16). Another advantage is easiness to control a strength of silyl protective bond by changing substituents around Si atom. In the case of EO polymerizations initiated with potassium bis(trimethylsilyl) amide, trimethylsilyl protection did not have enough resistivity against nucleophilic attacks by potassium alkoxide at the end of the growing chain as stated above. A five membered disilazane ring is known to show much higher resistivity in nucleophilic attack as compared to bis(trimethylsilyl)amino group in terms of primary amino protection (17). We chose a potassium N-[2-(2,2,5,5-tetramethyl-1-aza-2,5-disila-cyclopentyl)ethyl]methyl amide **[1b]** as an initiator for EO polymerization. **1b** initiated an anionic polymerizations of EO to form polymers, the polymerization conditions and the results of which are summarized in Table 1.



X: H **1a**
K **1b**

Table 1. Results of anionic polymerizations of ethylene oxide (EO) with **1b** as an initiator[a]

Run	$[EO]_0 / [\mathbf{1b}]_0$	Time	yield	$10^{-3} \cdot Mn$		Mw / Mn[b]
		in d	in %	Obsd.[b]	Calcd.	
1	60	3	78	3.7	2.8	1.18
2	60	3	89	2.8	2.8	1.17
3	25	2.5	73	1.6	1.3	1.32
4	60	2.5	92	2.9	2.8	1.32
5	200	2.5	~100	8.3	8.8	1.27

[a] Solv. :THF; Temp.: r. t. [b] Determined from GPC results

The polymerizations proceed smoothly and gave the polymer almost quantitative yields. The molecular weight of the polymers determined from GPC results agreed well with expected values calculated from the monomer / initiator molar ratios. In addition, the molecular weight distri-butions of the polymers formed were relatively narrower (less than 1.32). From these re-sults, it is indicated that the initiation efficiency of

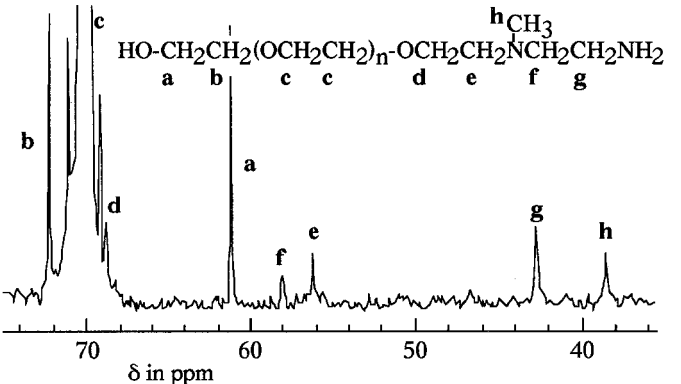


Fig.1. ^{13}C NMR spectrum of PEO prepared with **1b** as an initiator (The same sample as Run 1 in Table 1)

1b was almost one and the polymerization proceeded without any side reactions.

Table 2. ^{13}C NMR Chemical shifts data of PEO initiated with **1b** (ppm)[a]

Carbon	a	b	c	d	e	f	g	h
Observed	61.6	72.2	70.1	69.3	56.4	59.4	42.6	38.9
Calculated	63.7	72.8	70.6	69.3	56.8	60.2	41.4	38.7

[a] $HO\text{-}CH_2CH_2(OCH_2CH_2)_n\text{-}OCH_2CH_2N(CH_3)CH_2CH_2NH_2$ (a b c c d e f g, h)

To make sure the above consideration, an end group analysis of the polymer was carried out using ^1H, ^{13}C NMR, TLC and MALDI TOF-MS spectrometry. Fig. 1 shows ^{13}C NMR spectrum of the polymer formed (Run 1 in Table 1). As can be seen in the figure, there were several signals other than $CDCl_3$. Referring to a literature on hydroxyl terminated PEO (18) and MEDA as reference compound, the assignments of these signals were carried out and are described in the figure. The assignments of these signals were in good accordance with the calculated data (19) as shown in Table 2.

For a quantitative analysis of the terminal MEDA moiety, ^1H NMR spectra of the PEO sample were measured. Using MEDA as reference compound, the singlet signal and three triplet signals appearing 1.1 ppm and 2.3 - 2.7 ppm, respectively, are assignable to N-methyl and N-methylene protons shown in the Fig. 2(a). NH_2 signal was appearing around 2.1 ppm as broad peak together with hydroxy protons at the another end and water protons. In DMSO-d_6 as solvent for ^1H NMR, however, hydroxy protons were appearing clearly at 4.5 ppm separated from water protons signals shown in Fig. 2(b). The peak intensity ratio of OH signal vs. NCH_2 (at 2.3 ppm) were 1 / 2, indicating the polymer thus obtained to have one hydroxyl and one MEDA terminuses stoichiometrically.

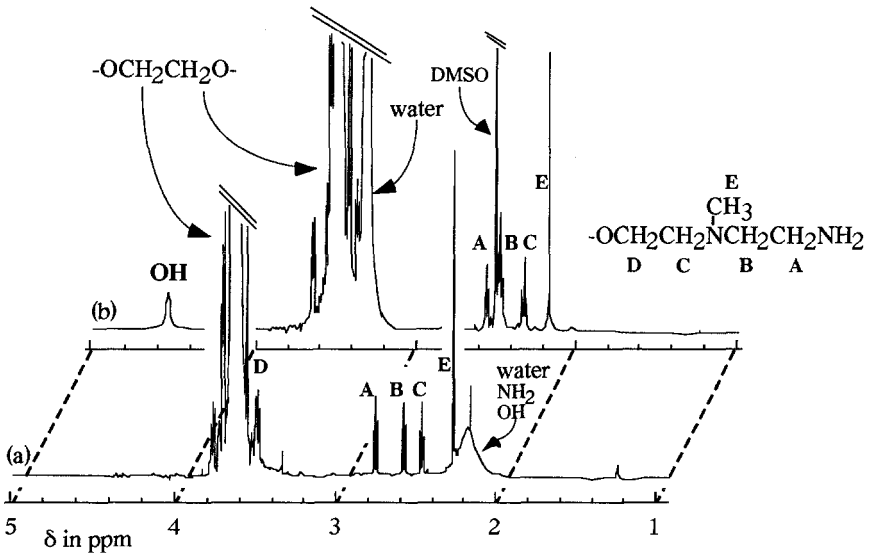


Fig.2 ^1H NMR spectra of PEO (a) in $CDCl_3$ and (b) in DMSO (The same sample as Run 1 in Table 1)

To get further information if the PEO thus obtained really has primary-amino group, several analyses were carried out. Silica gel TLC separation of the PEO (Run 1 in Table 1)

using chloroform / methanol (80 / 20 v/v) as a mobile phase showed clear one spot (Rf value = 0.38). A ninhydrin test of the spot was positive, which strongly indicated again that the PEO had the primary amino group at the end of the polymer chain. One of the absolute methods to analyze the end group of the each polymer chain is to determine a molecular mass of each polymer molecules. MALDI TOF-MS spectrum is one of the methods to determine the molecular mass of the each polymer molecule (20). Fig. 3 shows the MALDI TOF-MS spectrum of the PEO (Run 1 in Table 1).

In the case of MALDI MS, it is known that molecular ions of the polymers are generally detected as cation adduct (usually Na^+ as an impurity). From the spectrum shown in Fig. 3, the molecular mass of the each molecule can be determined as follows:

$$MW_{mass} = 44.053n + 118.18 + 23 \quad [1]$$

where, 44.053 and 23 means molecular weights of EO and sodium cation, respectively. For example, the highest peak in the figure showed 3269 mass, in which n is 71. The third signal toward higher mass range from the highest peak (n = 71) showed 3401 mass which agreed well with n = 74 from the calculation using Eq. [1]. The number, 118.18 in Eq. [1] agreed exactly with a summation of molecular mass of both terminal moieties, *viz.* $HO\text{-}CH_2\text{-}CH_2$ (45.06) + $N(CH_3)CH_2\text{-}CH_2\text{-}NH_2$ (73.12) = 118.18.

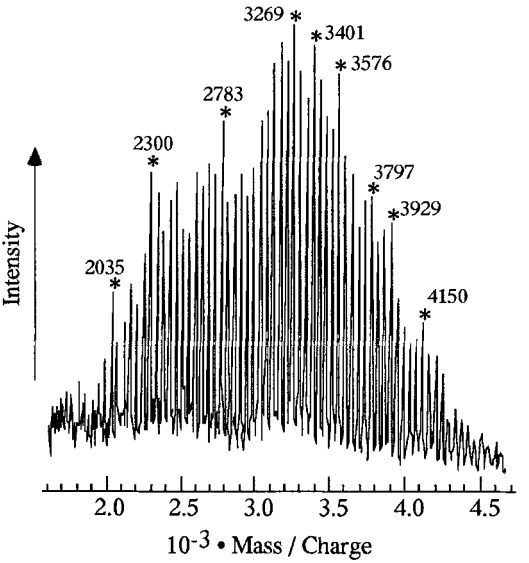


Fig.3 MALDI-TOF-MS spectrum of PEO prepared with **1b** (The same sample as Run 1 in Table 1)

On the basis of all above results, it is concluded that the PEO obtained with **1b** as the initiator has one primary-amino group at one end and one hydroxyl group at the another end quantitatively and can be regarded as heterotelechelic poly(ethylene oxide).

The authors would like to express their sincere appreciation to Mr. Koichi Tanaka, Shimadzu Corporation, for carrying out of MALDI-TOF-MS measurements.

References

1 For review, see: (a)(1991)"Alkylene Oxides and Their Polymers", (ed) Bailey F E Jr., . Koleske J V, Marcel Dekker, New York, ; (b) (1993)"Poly(ethylene glycol) Chemistry", (ed) Harris J M, Plenum Press, New York,
2 Kohler G, Milstein C (1975) Nature 256:495
3 Ohno H, Ito K, (1993) Polymer 34:4171
4 Abuchowski A, van Es T, Palczuk N-C, Davis F-F (1977) J. Biol. Chem. 252:3582
5 Amiji M, Park K (1993) J. Biomater. Sci., Polymer Edn. 4:217
6 Herren B-J, Shafter S-G, Alstine J-V, Harris J-M, Snyder R-S (1987) J. Coll. Int. Sci. 115:46
7 Kataoka K, Kwon G S, Yokoyama M, Okano T, Sakurai Y, (1993) J. Controlled Release, 24:119

8 Yokoyama M, Okano T, Sakurai Y, Ekimoto H, Shibazaki C, Kataoka K (1991) Cancer Res. 51:3229
9 Yokoyama M, Miyauchi M, Yamada N, Okano T, Sakurai Y, Kataoka K, Inoue S (1990) Cancer Res. 50:1693
10 Homo-telechelic oligomers mean telechelic oligomers with same functional groups at both ends.
11 The term of "heterotelechelics" was defined in our previous paper, Yokoyama M, Okano T, Sakurai Y, Kikuchi A, Ohsako N, Nagasaki Y, Kataoka K (1992) Bioconjugate Chem. 3:275
12 For review see: (a) Harris, J M , Yalpani, M, (1985) "Polymer-Ligands Used in Affinity Partitioning and Their Synthesis" in "Partitioning in Aqueous Tow-Phase Systems", (ed) Walter H, Brooks D E, Fisher D, Academic Press, New York, 589; (b) Zalipsky S, (1993) Bioconjugate Chem., 4:296
13 Perrin D-D, Armarego W-L-F, Perrin D-R (1980) Purification of Laboratory Chemicals. Pergamon Press Oxford
14 a) Gilman H, Langham W, Moor F-W (1940) J. Am. Chem. Soc. 66:2327; b) Gilman H, Haubein A-H (1944) J. Am. Chem. Soc. 66:1515
15 Scott N-D, Walker J-F, Hansley V-L (1936) J. Am. Chem. Soc. 58:2442
16 Greene T W G, Wuts G M, (1991)"Protective Groups in Organic Synthesis", 2nd Ed., John Wiley & Sons, Inc., New York
17 Djuric S, Venit J, Magnus P (1981) Tetrahedron Lett. 22:1787; (b) Guggenheim T-L, (1985) Tetrahedron Lett. 25:1253
18 Kinugasa S, Takatsu A, Nakanishi H, Nakahara H, Hattori S (1992) Macromolecules 25:4848
19 Clerc P, Simmon S, (1983) "Tables of Spectral Data for Structure Determination of Organic Compounds", Springer-Verlag
20 For review, see: Creel H-S (1993) Trends Polym. Sci. 1:336

Accepted April 12, 1994 Shi