Synthesis of Poly(ethylene glycol)-*block*-poly(ethylenimine) Possessing an Acetal Group at the PEG End

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ABSTRACT: A new linear block copolymer of poly(ethylene glycol) (PEG) and poly(ethylenimine) (PEI) possessing an acetal group at the PEG chain end was prepared by a heterotelechelic PEG macroinitiator technique. Heterotelechelic PEG with an acetal group at one end and a methanesulfonic group at the other end (acetal-PEG-SO_2CH_3) was synthesized by the anionic ring-opening polymerization of ethylene oxide (EO) initiated with potassium 3,3-diethoxypropanolate (PDP), followed by end-capping with methanesulfonyl chloride. Acetal-PEG-SO_2CH_3 was then used as a macroinitiator for the cationic ring-opening polymerization of 2-methyl-2-oxazoline (Oz) to form the block copolymer, acetal-PEG-POz. The block efficiency attained almost 100%, allowing to control the molecular weight of the POz segment by the initial monomer/initiator ratio. Alkaline hydrolysis of the repeating acetyl groups in the POz segment gave the completely deacylated block copolymer retaining the acetal group at the PEG chain end. The acetal-PEG-poly(ethylenimine) block copolymer thus obtained may have a potential utility as targetable DNA carrier in the field of gene delivery.

Introduction

Since Szwarc[1] pioneered living anionic polymerization, living cationic,[2] radical,[3] and coordination polymerizations[4] have been developed for the preparation of a variety of block and graft copolymers. Along with the development of novel synthetic routes, study on the functionality of these block and graft copolymers has attracted a growing interest in a diverse field of polymer science. Especially, the supramolecular assembly formed from well-defined block copolymers has been investigated extensively from both fundamental and applied viewpoints because of its unique characteristics developed in the mesoscopic size range.[5]

We have recently established the novel synthetic route of amphiphilic block copolymers functionalized at both ends. Ethylene oxide (EO) was anionically polymerized using potassium 3,3-diethoxypropanolate as the initiator,[6] followed by the block copolymerization of lactide (LA) to form the acetal-PEG-PLA block copolymers.[7] Because the acetal-PEG-PLA block copolymer thus prepared is amphiphilic, it forms a core−shell polymeric micelle in aqueous media.[8] The acetal group at the PEG chain end is locating on the surface of the micelle and can be converted into an aldehyde group by acid hydrolysis[9] to prepare polymeric micelles with reactive aldehyde groups on the surface. Reactive polymeric micelles may be useful as a targetable drug carrier by installing biospecific ligands, including sugars and peptides, on their periphery.

Similar core−shell micelles can also be prepared by mixing block ionomers with oppositely charged polyelectrolytes of synthetic and natural origins.[10] These "polyion complex micelles" should be useful as novel vectors for plasmids and antisense DNA for gene therapy,[11] because of the anionic nature of natural DNA compounds to form polyion complex with cationic ionomers. The cationic block ionomer used for this purpose should have terminal functional group at the end of the shell-forming segment to install biospecific ligands. However, an effective and convenient method for such molecular design has not been established yet expect the one based on comb-type graft copolymers of PEI with PEG.[12]

This paper reports a novel synthetic route to prepare an end-functionalized cationic block ionomer that may be useful in the area of DNA delivery. The heterotelechelic PEG macroinitiator method was utilized for cationic polymerization of oxazoline followed by hydrolysis of side chain to obtain end-functionalized PEG−poly(ethylenimine) block copolymer (PEG-PEI) where secondary amino groups of PEI segment should be protonated in aqueous media to become cationic block ionomer (Scheme 1). Note that plasmids complexed with poly(ethylenimine) (PEI) was reported to show an impressive transfection efficiency,[13] probably because PEI acts as a buffer to keep the microenvironmental pH neutral in the endosome where the PEI/plasmid complex should be located after cellular internalization and thus prevents activation of the endosomal nuclease and attack of the plasmid DNA.[14]

Experimental Section

Materials. Tetrahydrofuran (THF) (reagent grade; Godo Yozai Co. Ltd., Tokyo, Japan), 3,3-diethoxy-1-propanol (purity 98%; Aldrich Chemical Co. Ltd., Milwaukee, WI), and ethylene oxide (EO) (purity 100%; Saisan Co. Ltd., Saitama, Japan) were purified by distillation according to the conventional procedure.[15] Methanesulfonyl chloride (purity 99%; Wako Pure Chem. Co. Ltd., Osaka, Japan), nitromethane (purity 95%; Aldrich Chemical Co. Ltd., Milwaukee, WI), and 2-methyl-2-

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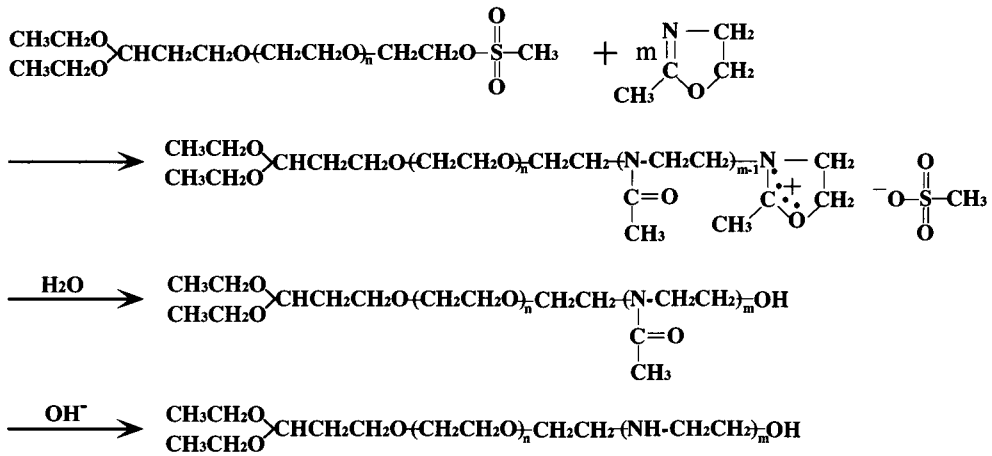
Scheme 1. Synthetic Route of Acetal-PEG-PEI Using Acetal-PEG-SO_2CH_3 as a Macroinitiator



oxazoline (purity 98%; Aldrich Chemical Co. Ltd., Milwaukee, WI) were dried over calcium hydride followed by the distillation under argon atmosphere. After distillation, no detectable impurity was observed for these reagents by gas chromatography. Other reagents were purchased from Aldrich Chemical Co. Ltd. and used as received. A THF solution of potassium naphthalene was prepared by adding 0.16 mol (6.26 g) of freshly cut potassium (purity 99.5%; Aldrich Chemical Co. Ltd., Milwaukee, WI) into 500 mL of ice-cooled THF solution including 0.15 mol (19.23 g) of naphthalene (purity 98%; Wako Pure Chem. Co. Ltd., Osaka, Japan). The concentration of potassium naphthalene thus prepared was determined by titration.[16]

Techniques. Gel permeation chromatography (GPC) measurements were carried out using a TOSOH HLC-8020 equipped with a Shodex-Gel column (KD-806M×2, pore size 1000 Å) and an internal refractive index (RI) detector. DMF containing 10 mM L^{-1} litium bromide was used as the eluent at a flow rate of 1 mL min^{-1} at 40 °C. Molecular weight calibration was done using a series of standard PEGs (Polymer Laboratories, Ltd., U.K.). Gas chromatography (GC) measurement was carried out so as to check the purity of the reagents after the purification using a HP 5890 (Hewlett-Packard, Co. Ltd. USA) equipped with a glass capillary column (DB-1; 0.248 mm × 0.25 μm × 30 m). The ^{1}H and ^{13}C NMR spectra were measured with a JEOL EX400 spectrometer (JEOL, Tokyo, Japan) at 400 MHz.

Synthesis of Heterotelechelic α-Acetal-ω-methanesulfonate Poly(ethylene glycol) (Acetal-PEG-SO_2CH_3). 3,3-Diethoxypropanol (2 mmol, 0.315 mL) and potassium naphthalene (2 mmol) in 6.37 mL of THF were added to dry THF (30 mL) in a 100 mL flask with a three-way stopcock under argon atmosphere to form potassium 3,3-diethoxypropanolate (PDP). After stirring for 10 min, liquid EO chilled below 0 °C (140 mmol, 7.0 mL) was added to the solution via a cooled syringe. The mixture was allowed to react for 2 days at room temperature and was then added to dry THF (5 mL) containing 40 mmol of methanesulfonyl chloride (3.10 mL) at room temperature and stirred overnight, followed by a pouring into diethyl ether (500 mL) to precipitate the polymer. The recovered polymer was dried in vacuo and then freeze-dried from benzene. The yield of obtained polymer after purification was 82% (4.92 g); number-averaged molecular weight (M_n) = 3200, molecular weight distribution (MWD) = 1.04. ^{1}H NMR (DMSO): δ 1.12 (t, CH_3CH$_2$O, 3H), 4.58 (t, CHCH$_2$CH$_2$O, 1H), 1.76 (q, CHCH_2CH$_2$O, 2H), 3.56 (s, PEG, 291H), 4.34 (t, CH$_2$CH_2OSO$_2$CH$_3$, 2H), 3.20 (s, CH$_2$CH$_2$OSO$_2$CH_3, 3H). ^{13}C NMR (DMSO): δ 15.4 (*C*H$_3$CH$_2$O), 60.8 (CH$_3$*C*H$_2$O), 100.2 (*C*HCH$_2$CH$_2$O), 33.9 (CH*C*H$_2$CH$_2$O), 60.8 (CHCH$_2$*C*H$_2$O), 70.4 (*C*H$_2$CH$_2$O of PEG backbone), 68.4 (*C*H$_2$CH$_2$OSO$_2$CH$_3$), 66.6 (CH$_2$*C*H$_2$OSO$_2$CH$_3$), 36.9 (SO$_2$*C*H$_3$).

Polymerization of 2-Methyl-2-oxazoline Using Acetal-PEG-SO_2CH_3 as a Macroinitiator. 2-Methyl-2-oxazoline (26.7 mmol, 2.26 mL) was added to a solution of acetal-PEG-SO_2CH_3 (MW = 3200, 1.07 g) in nitromethane (10 mL) and stirred at 60 °C for 70 h under argon. After cooling to room temperature, the product, acetal−poly(ethylene glycol)−poly(2-methyl-2-oxazoline) (acetal-PEG-POz), was isolated in 91% yield by precipitation into diethyl ether and then dialyzed overnight against 1 L of distilled water; M_n = 10 500, MWD = 1.41. ^{1}H NMR (D$_2$O): δ 1.21 (t, CH_3CH$_2$O, 3H), 1.92 (q, CHCH_2CH$_2$O, 2H), 3.59 (s, PEG), 3.40 (s, N(COCH$_3$)CH_2CH_2), 2.10 (s, N(COCH_3)CH$_2$CH$_2$).

Alkaline Hydrolysis of Acetal-PEG-POz. Alkaline hydrolysis of the amide group to a secondary amino group in the main chain of POz was carried out by the addition of NaOH (1.1 g, 27.5 mmol; 3.2 times the residual molar quantity of Oz units in the block copolymer) to acetal-PEG-POz (1.0 g) in an ethylene glycol/ethanol (1:1) cosolvent (10 mL). The mixture was stirred for 6 h at 98 °C and purified by precipitation into cold water. The polymer was collected by filtration. The yield of obtained polymer after purification was 91% (0.58 g) and characterization. ^{1}H NMR (D$_2$O): δ 1.16 (t, CH_3CH$_2$O, 3H), 1.92 (q, CHCH_2CH$_2$O, 2H), 3.59 (s, PEG), 2.60 (s, NCH_2CH_2).

Results and Discussion

Synthesis of Acetal-PEG-SO_2CH_3. We have previously reported that potassium 3,3-diethoxypropanolate (PDP) initiates the anionic polymerization of EO to form PEG with an acetal group at the α-chain end and a hydroxyl group at the α-chain end (acetal-PEG-OH).[6] To utilize acetal-PEG-OH as a macroinitiator for oxazoline polymerization, the hydroxyl group must be converted to a functional group that can initiate the polymerization of oxazoline without affecting the acetal group at the other end. Sulfonate derivatives can initiate several kinds of oxazoline polymerization.[17] If acetal-PEG-OH can be functionalized with a sulfonate derivative, it should act as a macroinitiator for oxazoline polymerization. After the polymerization of EO initiated with PDP in THF, the reaction mixture was added to a 20-fold excess of methanesulfonyl chloride. Figure 1 shows the GPC trace of the resulting polymer; its number-averaged molecular weight (M_n) and molecular weight distribution (MWD) were 3200 and 1.04, respectively. The M_n is close to the calculated value (3000) based on the monomer/initiator ratio. This and the very narrow MWD demonstrate that the polymerization and subsequent ω-end functionalization were successful without any remarkable side reaction. Parts a and b of Figure 2 show the ^{13}C NMR spectra of the starting acetal-PEG-OH and the obtained acetal-PEG-SO_2CH_3, respectively. After the methanesulfonylation, the signals of the α and β methylene carbons adjacent to the hydroxyl end group of the acetal-PEG-OH appearing at

Figure 1. Gel permeation chromatogram of acetal-PEG-SO_2CH_3.

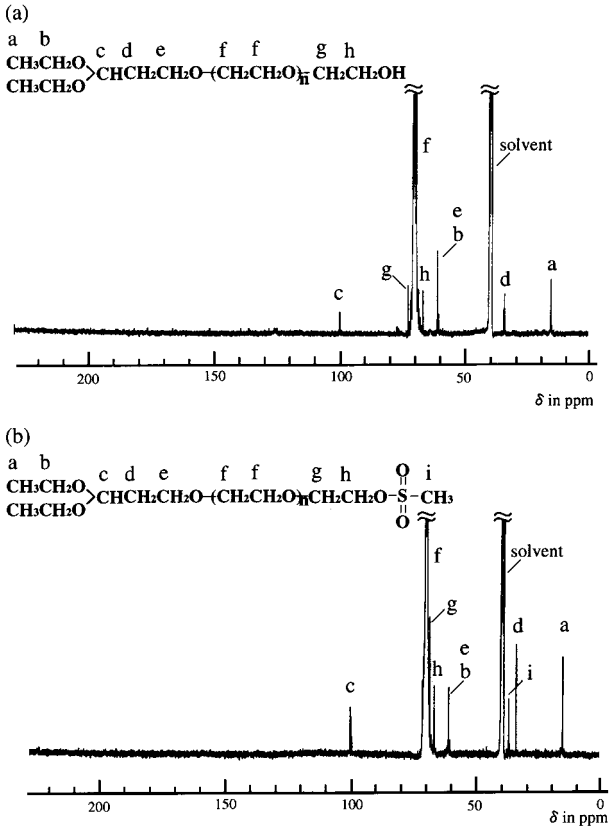


Figure 2. ^{13}C NMR spectra in DMSO-d_6 at room temperature of (a) acetal-PEG-OH and (b) acetal-PEG-SO_2CH_3.

67.2 and 72.6 ppm completely disappeared. New peaks appeared at 36.9, 68.4, and 66.6 ppm, which are assignable to SO_2CH_3, $CH_2CH_2OSO_2CH_3$, and $CH_2CH_2OSO_2CH_3$, respectively. The signals based on the acetal end group remain intact. Complete disappearance of the signals due to the hydroxyl group after the ω-end modification reaction demonstrates that the conversion of the ω-end group to a methanesulfonic group is quantitative, which was further confirmed by the ^1H NMR analysis shown in Figure 3. The signals appearing at 3.2 ppm (s, 3H) and 4.3 ppm (t, 2H) are assignable to the methyl and methylene groups adjacent to the sulfonate group, respectively, while the clear triplet signals based on the acetal methine proton appears at

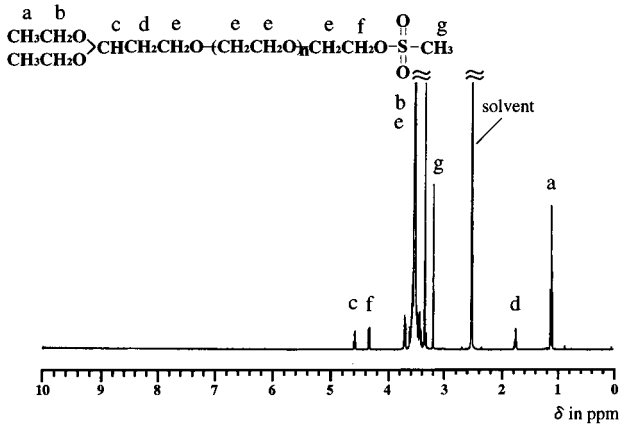


Figure 3. ^1H NMR spectrum of acetal-PEG-SO_2CH_3 in DMSO-d_6 at room temperature.

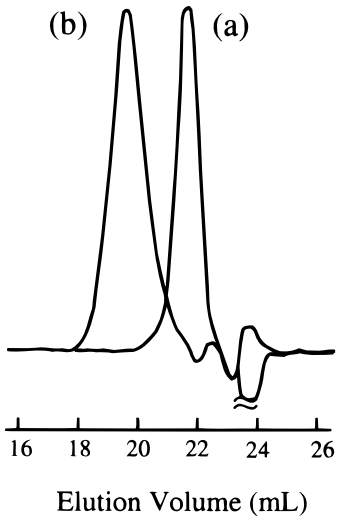


Figure 4. Gel permeation chromatograms of (a) acetal-PEG-SO_2CH_3 and (b) acetal-PEG-POz.

4.6 ppm. The molecular weight (3160) of acetal-PEG-SO_2CH_3 calculated from the relative intensity of the oxymethylene peak of PEG (3.6 ppm) and methyl peak of the acetal-moiety (1.1 ppm), assuming quantitative introduction of an acetal group to the chain end of each polymer molecule, was in good accordance with the molecular weight determined by GPC analysis (M_n 3200). The conversion of the ω-end to a methanesulfonic group was 97% based on the relative area of the signals from the methyl protons of the methanesulfonyl group at 3.2 ppm and methyl protons of the acetal moiety at 1.1 ppm in the ^1H NMR spectrum in DMSO at room temperature.

Synthesis of Acetal-PEG-POz Using Acetal-PEG-SO_2CH_3 as a Macroinitiator. Figure 4 shows the GPC profiles of the acetal-PEG-SO_2CH_3 and the block copolymer, acetal-PEG-POz, obtained by ring-opening polymerization of 2-methyl-2-oxazoline (Oz) from the methanesulfonyl end of the acetal-PEG-SO_2CH_3. After the block copolymerization of Oz ((b) in Figure 4), the peak shifted to smaller elution volume due to an increase in the molecular weight. No peak of the prepolymer remained, indicating the high efficiency of the block copolymerization. The M_n and the MWD determined from the GPC data were 10 500 and 1.41, respectively. The molecular weight of the POz segment calculated by subtracting the molecular weight of the PEG segment (M_n = 3200) from the total molecular

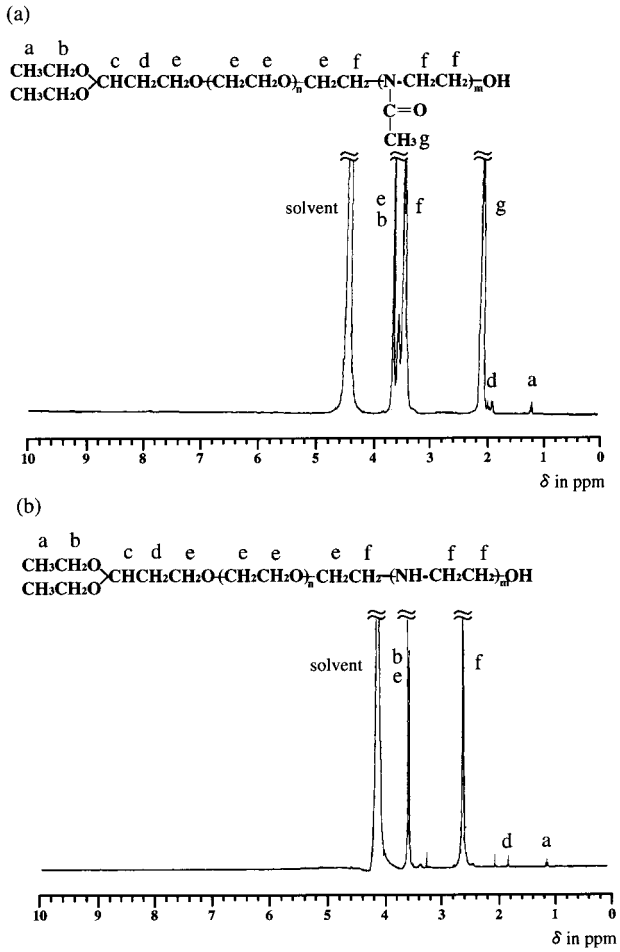


Figure 5. ^{1}H NMR spectra of (a) acetal-PEG-POz at room temperature (solvent, D_2O) and (b) acetal-PEG-PEI at 80 °C (solvent, D_2O).

Table 1. Characterization of Acetal-PEG-POz Prepared at Various Temperatures

run	temp (°C)	M_n[b]	MWD[b]	yield (%)
1	60	10500	1.41	91
2	65	8900	1.43	84
3	70	8560	1.56	86
4	90	4950	1.35	48

[a] All the samples were prepared using acetal-PEG-SO_2CH_3 with M_n of 3200 as an initiator in nitomethane ([monomer]/[initiator] = 80). [b] Estimated by GPC.

weight was 7300, which agrees with that calculated from the initial monomer/macromonomer ratio (80/1). The ^{1}H NMR is shown in Figure 5a. Along with the peaks corresponding to both the PEG and POz segments, the end acetal signals (1.1 and 1.9 ppm) are clear. The characteristics of the acetal-PEG-POz prepared at different temperatures are summarized in Table 1. The block copolymerization initiated by the acetal-PEG-SO_2CH_3 proceeded smoothly except at a temperature of 90 °C. Some side reaction may take place at elevated temperature.

Synthesis of Acetal-PEG-PEI by Alkaline Hydrolysis of Acetal-PEG-POz. Acetal-PEG-PEI was prepared by alkaline hydrolysis of the acetyl group of each repeating unit in acetal-PEG-POz using NaOH in ethylene glycol/ethanol (1:1). Figure 5 shows the ^{1}H NMR spectra of the block copolymer before (Figure 5a) and after (Figure 5b) hydrolysis. The methyl protons of the acetyl group appearing at 2.1 ppm completely disappeared after hydrolysis, and methylene protons adjacent to the nitrogen shifted from 3.4 to 2.6 ppm. The acetal signals (1.1 and 1.8 ppm) are still clearly observed even after the treatment with a strong base, because acetal group is stable to alkaline conditions.

Micellization of acetal-PEG-PEI with plasmid DNA and subsequent ligand conjugation on the periphery of the obtained micelles are now underway in our laboratory, and results will be reported elsewhere.

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