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Comb-shaped graft copolymers with cellulose side-chains prepared *via* click chemistry

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ABSTRACT

Comb-shaped copolymers with cellobiose acetate or cellulose triacetate (CTA) side-chains, PPMA-*g*-(CTA2-C15) and PPMA-*g*-(CTA13-C15), were prepared by grafting *N*-(15-azidopentadecanoyl)-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosylamine (CTA2-C15-N_3) and *N*-(15-azidopentadecanoyl)-tri-*O*-acetyl-β-cellulosylamine (CTA13-C15-N_3, number average degree of polymerization (DP_n) = 13) onto poly(2-propyn-1-yl methacrylate) (PPMA, weight average degree of polymerization (DP_w, $X+Y = 5.59 \times 10^2$)) *via* “click chemistry”. The copolymers were characterized by ^1H, ^{13}C and two-dimensional NMR and size exclusion chromatography–multi-angle laser light scattering (SEC–MALS) measurements. The numbers of CTA side-chains (X) of PPMA-*g*-(CTA2-C15) and PPMA-*g*-(CTA13-C15) were calculated as 4.03×10^2 and 2.45×10^2, respectively. Copolymers with cellulosic side-chains, PPMA-*g*-(CELL2-C15) and PPMA-*g*-(CELL13-C15), were successfully obtained after deacetylation of PPMA-*g*-(CTA2-C15) and PPMA-*g*-(CTA13-C15), respectively. X-ray diffraction measurements revealed that PPMA-*g*-(CELL13-C15) showed crystalline pattern of cellulose II, which is believed to have anti-parallel orientation.

1. Introduction

Cellulose is a linear (1→4)-β-glucopyranan having three hydroxyl groups per anhydroglucose unit. There have been several studies on cellulose graft copolymers, and a variety of polymers have been grafted onto the cellulose main-chain through its hydroxyl groups at C2, C3, and C6 positions to alter properties of cellulose or cellulosic materials (Dou & Jiang, 2007; Kang et al., 2006; Nishio, 2006). On the other hand, there have been many reports on glycopolymers with mono-, di-, or oligo-saccharides as pendent groups binding at the reducing-end on a main-chain (Kamitakahara et al., 1998; Ladmiral et al., 2006; Narumi, Miura, et al., 2006; Narumi, Otsuka, et al., 2006; Ohno, Fukuda, & Kitano, 1998; Ohno, Tsujii, & Fukuda, 1998). However, to our knowledge, there has been no report on a graft copolymer having cellulose side-chains. A comb-shaped copolymer with cellulose side-chains could achieve parallel orientation of cellulose molecules, and might have unique unknown properties such as crystallinity.

We have therefore prepared a novel graft copolymer with cellulose side-chains *via* free radical copolymerization of a cellulose macromonomer with methyl methacrylate, based on “graft through” (homo- or co-polymerization of macromonomers) strategy (Enomoto-Rogers, Kamitakahara, Nakayama, Takano, & Nakatsubo, 2009; Enomoto-Rogers, Kamitakahara, Takano, & Nakatsubo, 2009). The number of cellulose graft chains per main-chain was, however, only 3.86, because of low reactivity of a cellulose macromonomer, while the weight average degree of polymerization of poly(methyl methacrylate) main-chain was 4.14×10^2. It has been reported that the semi-flexible or rigid macromonomers such as methacrylate-end capped poly(n-hexyl isocyanate) (Kawaguchi, Mihara, Kikuchi, Lien, & Nagai, 2007; Se & Aoyama, 2004) are hard to polymerize, whereas other flexible macromonomers such as poly(ethylene oxide) (Ito, Tomi, & Kawaguchi, 1992) or poly(dimethylsiloxane) (Shinoda, Miller, & Matyjaszewski, 2001) are not.

On the other hand, “grafting onto” (attachment of side-chains to the backbone) strategy is known as another way to synthesize graft copolymers (Hourdet, L'Alloret, & Audebert, 1997; Kamitakahara et al., 1998; Poe, Jarrett, Scales, & McCormick, 2004). Thus, the “grafting onto” strategy was investigated to introduce more cellulose molecules as side-chains compared to our former study, and to control the orientation of cellulose chains.

Recently, the concept of “click chemistry” invented by Sharpless and coworkers (Kolb, Finn, & Sharpless, 2001; Wu et al., 2004) has been introduced into the synthesis of polymeric materials with well-defined and complex chain architectures. Specifically, Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition between organic azides and terminal alkynes for the transformation of 1,2,3-triazoles has been received much attention as a highly efficient

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and stereoselective reaction coupled with an excellent compatibility for functional groups. Ladmiral et al. (2006) have reported the preparation of glycopolymers by attaching azide containing mono-saccharides onto the alkyne containing polymer main-chain *via* "click chemistry".

In the present study, based on "grafting onto" strategy and "click chemistry", we prepared comb-shaped copolymers with cellulose side-chains, by grafting cellulose derivatives carrying a single azide group onto the alkyne containing polymer main-chain. Chemical structures of the synthesized graft copolymers and their crystalline patterns were investigated.

2. Experimental

2.1. Materials

N-(15-Azidopentadecanoyl)-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosylamine (CTA2-C15-N_3) ($M = 874.97$) and *N*-(15-azidopentadecanoyl)-tri-*O*-acetyl-β-cellulosylamine (CTA13-C15-N_3, $DP_n = 13$) ($M_n = 4.12 \times 10^3$, $M_w = 5.33 \times 10^3$) were prepared as described in our previous articles (Kamitakahara, Enomoto, Hasegawa, & Nakatsubo, 2005; Kamitakahara & Nakatsubo, 2005). The cellulose and cellulose triacetate derivatives were abbreviated as CELL or CTA with the number of the degree of polymerization (DP_n) of the cellulosic chain (2 or 13), followed by the abbreviation of the pentadecanoyl group (C15), and the end group (N_3). MALDI-TOF MS measurements revealed that the molecular weights of CTA13-C15-N_3 with each DP value agreed well with theoretical values, and quantitative substitution of the reducing-end was confirmed. 2,2′-Azobis(isobutyronitrile) (AIBN) was crystallized from ethanol before use. 3-(Trimethyl silyl)-2-propyn-1-ol, methacryloyl chloride, triethylamine (Et_3N), *N,N,N′,N′,N″*-pentamethyldiethylenetriamine (PMDETA), copper(I)bromide (Cu(I)Br), tetrabutyl ammonium fluoride trihydrate (TBAF·$3H_2O$), and all other reagents were commercially obtained and used without further purification.

2.2. 3-(Trimethylsilyl)-2-propyn-1-yl methacrylate (TMS-PMA)

3-(Trimethylsilyl)-2-propyn-1-yl methacrylate (TMS-PMA) was prepared according to the previous article (Ladmiral et al., 2006; Scarpaci et al., 2009). To a solution of 3-(trimethylsilyl)-2-propyn-1-ol (1.0 ml, 1 eq.) and Et_3N (1.5 ml, 1.5 eq.) in dichloromethane (2.0 ml), methacryloyl chloride (1.0 ml, 1.5 eq.) was added. The mixture was stirred at room temperature for 0.5 h under nitrogen. After completion of the reaction, the mixture was extracted with ethyl acetate, washed with water and brine, dried with Na_2SO_4, and concentrated to dryness. Crude product was purified by open preparative column chromatography (eluent:ethyl acetate/*n*-hexane (1:19, v/v)) to give colorless syrup (1.12 g, 81.1% yield). ^1H NMR ($CDCl_3$): δ 0.20 (CH_3 (TMS)), 1.98 (CH_3), 4.78 (–O–CH_2–), 5.64, 6.20 (C=CH_2). ^{13}C NMR ($CDCl_3$): δ −0.35 (CH_3 (TMS)), 18.3 (CH_3), 52.9 (–O–CH_2–), 91.9 (–C≡*C*-TMS), 99.1 (–*C*≡C-TMS), 126.4 (CH_2=C–CH_3), 135.7 (CH_2=*C*–CH_3), 166.5 (*C*=O).

2.3. Poly (3-(trimethylsilyl)-2-propyn-1-yl methacrylate) (TMS-PPMA)

TMS-PMA (0.3 ml, 1 eq.) and AIBN (2.5 mg, 0.01 eq.) in benzene (0.3 ml) were loaded into a glass tube, and degassed by three freeze–pump–thaw cycles. The tube was sealed under vacuum, and placed in an oil bath at 75 °C for 1 day. The tube was cooled to room temperature, and opened. The reaction mixture was poured into methanol, filtered, and dried *in vacuo* to give an amorphous solid (158.6 mg, 58.7% yield). ^1H NMR ($CDCl_3$): δ 0.20 (CH_3 (TMS)), 0.95, 1.20 (CH_3), 1.85, 1.95 (CH_2), 4.60 (–O–CH_2–). ^{13}C NMR ($CDCl_3$): δ −0.24 (CH_3 (TMS)), 17.4, 19.0 (CH_3), 44.8, 45.1 (–CH_2–*C* (CH_3)–), 53.0 (–O–CH_2–), 54.3 (–CH_2–C (CH_3)–), 92.3 (–C≡*C*-TMS), 98.4, 98.7 (–*C*≡C-TMS), 176.1, 176.5 (*C*=O).

2.4. Poly(2-propyn-1-yl methacrylate) (PPMA)

To a solution of TMS-PPMA (128 mg) and acetic acid (50 μl. 1.5 eq. to PMA monomer unit) in tetrahydrofuran (THF) (10 ml), TBAF·$3H_2O$ was added dropwise at −20 °C. The mixture was stirred at room temperature for 3 h under nitrogen. The reaction mixture was poured into methanol. The precipitate was collected by centrifugation at 1000 rpm for 3 min, and dried *in vacuo* to give an amorphous solid (78.1 mg, 96.4% yield). The number and weight average molecular weights ($M_{n,PS}$, $M_{w,PS}$) estimated by polystyrene standards were $M_{n,PS} = 1.92 \times 10^4$ and $M_{w,PS} = 3.54 \times 10^4$, respectively. The absolute molecular weight (M_w) was calculated as 6.94×10^4 by MALS measurements. The weight average degree of polymerization (DP_w) was calculated as 5.59×10^2 from $M_w(PPMA)/M(PMA) = 6.94 \times 10^4/124.21$. The DP_w of PPMA is described as $DP_w = X + Y$: X and Y are the numbers of cellulosic graft chains and PMA units of the graft copolymer (see Fig. 1). ^1H NMR ($CDCl_3$): δ 0.94, 1.09 (CH_3), 1.90, 1.98 (CH_2), 2.51 (–C≡CH), 4.62 (–O–CH_2–). ^{13}C NMR ($CDCl_3$): δ 16.9, 18.9 (CH_3), 44.8, 44.9 (–CH_2–*C* (CH_3)–), 52.2 (–O–CH_2–), 53.9 (–CH_2–C (CH_3)–), 75.5 (–*C*≡CH), 77.2 (–C≡*C*H), 175.5, 176.7 (*C*=O).

2.5. PPMA-g-(CTA2-C15)

CTA2-C15-N_3 (17.6 mg, 1 eq. to PMA monomer), PPMA (2.5 mg), and PMDETA (7 μl, 2 eq. to PMA monomer) in *N,N*-dimethylformamide (DMF) (0.3 ml) were loaded into a glass flask, and degassed by three freeze-pump-thaw cycles. The flask was purged with nitrogen, Cu(I)Br (5.7 mg, 2 eq. to PMA monomer) was added, and the flask was degassed again and sealed. The mixture was stirred at room temperature for 24 h. After completion of the reaction, the mixture was extracted with chloroform, washed with water and brine, dried with Na_2SO_4, and concentrated to dryness. The crude product was analyzed by SEC–MALS measurements. The compound was dried *in vacuo* to give an amorphous solid PPMA-g-(CTA2-C15) (17.8 mg, 88.6% yield). The number and weight average molecular weights ($M_{n,PS}$, $M_{w,PS}$) estimated by polystyrene standards were $M_{n,PS} = 7.58 \times 10^4$ and $M_{w,PS} = 2.24 \times 10^5$, respectively. The absolute molecular weight (M_w) was determined to be 4.22×10^5 by MALS measurements. Number of graft chains (X) was calculated as $X = 4.03 \times 10^2$ from $X = (M_w(\text{PPMA-}g\text{-(CTA2-C15)}) - M_w(\text{PPMA}))/M(\text{CTA2-C15-}N_3) = (4.22 \times 10^5 - 6.94 \times 10^4)/874.97$. Number of PMA units ($Y$) was calculated as $Y = 1.56 \times 10^2$ from $Y = DP_w(\text{PPMA}) - X = 5.59 \times 10^2 - 4.03 \times 10^2$. ^1H NMR ($CDCl_3$): δ 1.24 (br. s, aliphatic-H), 1.99, 2.01, 2.03, 2.04, 2.09, 2.11 (CH_3–CO), 3.72 (C5′-H, C5-H), 3.78 (C4-H), 4.01–4.17 (C6′-H_b, C6-H_b), 4.04, 4.16, 4.40, 4.44, 4.46 (C6′-H_a, C6-H_a), 4.57 (C1′-H), 4.84 (C2-H), 4.93 (C2′-H), 5.09 (C4′-H), 5.17 (C3′-H), 5.23 (C1-H), 5.27 (C3-H), 6.67 (NH), 7.93 (triazole). ^{13}C NMR ($CDCl_3$): δ 18.3 (CH_3), 20.4, 20.6, 20.8 (CH_3–CO–), 25.1 (C1–NH–CO–CH_2–CH_2–), 26.5, 29.2, 29.6, 30.3 (aliphatic-C), 36.4 (C1–NH–CO–CH_2–), 44.7 (CH_2–*C*), 50.4 (CH_2–O), 58.3 (CH_2–C), 61.5 (C6′), 61.9 (C6), 67.8 (C4′), 70.8 (C2), 71.5 (C2′), 71.8 (C5′), 72.5 (C3), 72.9 (C3′), 74.6 (C5), 77.9 (C1), 100.6 (C1′), 124.4 (CH (triazole)), 141.6 (C (triazole)), 169.1, 169.3, 169.5, 170.2, 170.3, 170.5, 171.0 (CH_3–*C*O–), 173.7 (C1–NH–*C*O–), 176.1, 177.1 (CO).

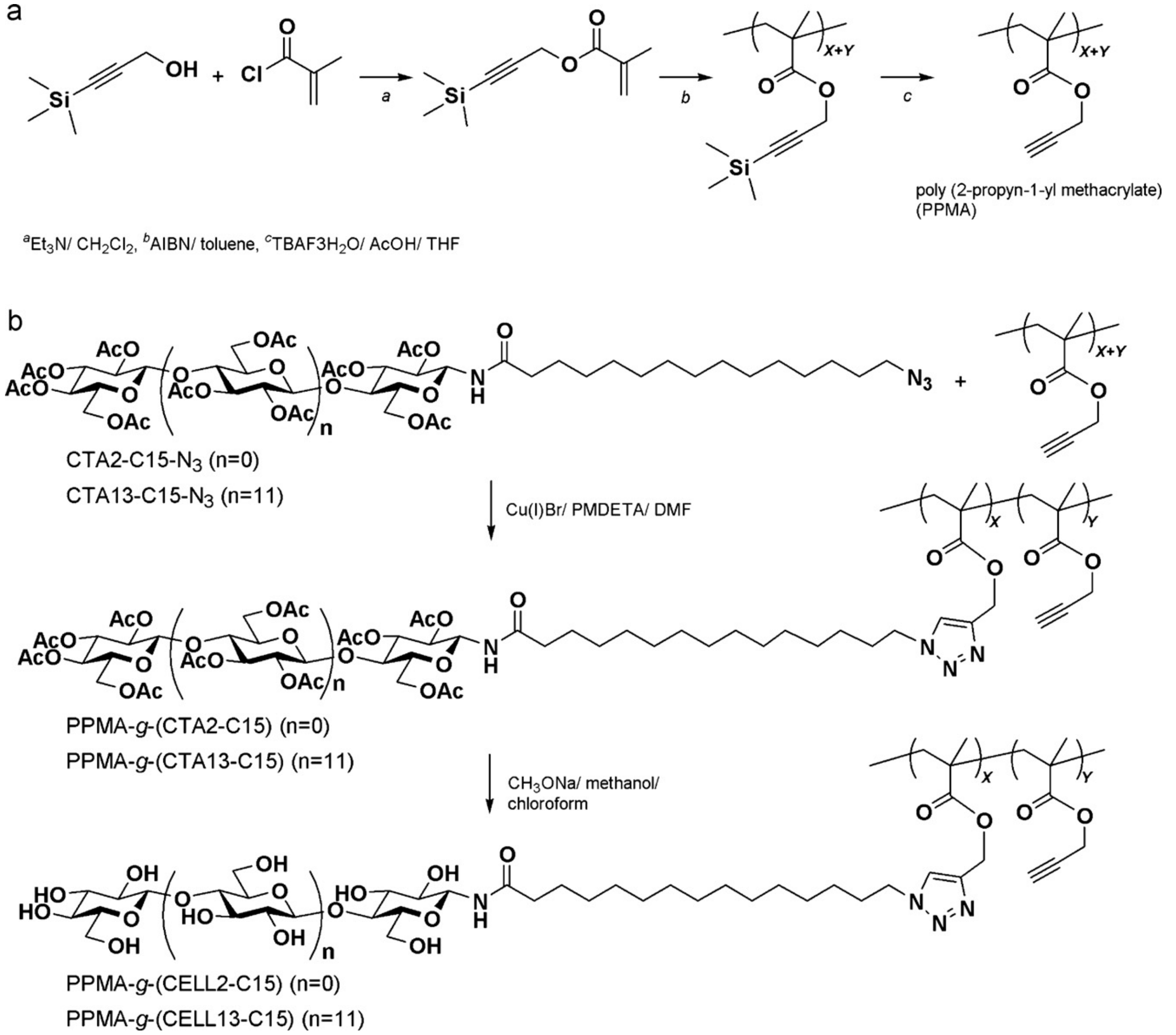

Fig. 1. Preparation of: (a) poly (2-propyn-1-yl methacrylate) (PPMA), (b) PPMA-*g*-(CELL2-C15) and PPMA-*g*-(CELL13-C15).

2.6. PPMA-g-(CTA13-C15)

CTA13-C15-N_3 (42.2 mg, 1 eq. to PMA monomer) and PPMA (1.3 mg) were treated in the same manner with that for PPMA-*g*-(CTA2-C15) using PMDETA (18.2 μl, 10 eq. to PMA monomer) and Cu(I)Br (15.0 mg, 10 eq. to PMA monomer) in DMF (0.3 ml). The remaining CTA13-C15-N_3 in the crude product was removed by gel permeation chromatography with chloroform using a Shimadzu SEC system equipped with a fraction collector to give an amorphous solid PPMA-*g*-(CTA13-C15) (11.6 mg, 26.6% yield). The area ratio (A(%)) of the high molecular fraction to the total area including the remaining CTA13-C15-N_3 in chromatogram (RI) of the crude product was A(%) = 43.8%. Number of graft chains (X) was calculated as 2.45×10^2 from $X = \mathrm{DP_w(PPMA)} \times A(\%) = 5.59 \times 10^2 \times 43.8(\%)$, on the assumption that the initial molar ratio of CTA13-C15-N_3 to PMA monomer unit was 1.0. The number of PMA units (Y) in PPMA-*g*-(CTA13-C15) was calculated to be 3.14×10^2 from $Y = \mathrm{DP_w(PPMA)} - X = 5.59 \times 10^2 - 2.45 \times 10^2$. The molecular weight of PPMA-*g*-(CTA13-C15) was estimated to be 1.38×10^6 from $M(\text{PPMA-}g\text{-(CTA13-C15)}) = M_\mathrm{w}(\mathrm{PPMA}) + X \times M_\mathrm{w,PS}(\text{CTA13-C15-}N_3) = 6.94 \times 10^4 + 2.45 \times 10^2 \times 5.33 \times 10^3$. ^{1}H NMR ($CDCl_3$): δ 1.25 (m, aliphatic-H), 1.9–2.1 (CH_3–CO–), 3.56 (C5-H), 3.72 (C4-H), 4.06 (C6-H_b), 4.37–4.43 (C6-H_a, C1-H), 4.81 (C2-H), 5.08 (C3-H). ^{13}C NMR ($CDCl_3$): δ 20.5 (CH_3–CO–), 29.5 (aliphatic-C), 62.0 (C6), 71.7 (C2), 72.4 (C5, C3), 100.5 (C1), 169.3, 169.7, 170.2 (CH_3–*CO*– of C2, C3, C6, respectively).

2.7. PPMA-g-(CELL2-C15)

To a solution of PPMA-*g*-(CTA2-C15) (25 mg) in methanol/chloroform (1/4, v/v, 1 ml), sodium methoxide (50 μl, 0.03 mmol) was added at room temperature, and stirred for 4 h under nitrogen. The precipitated compound was filtered and washed by methanol to give an amorphous solid, PPMA-*g*-(CELL2-C15) (14.9 mg, quantitative). ^{1}H NMR (DMSO-d_6): δ 1.21, 1.46, 1.79, 2.08 (br. s, aliphatic-H), 3.0–3.2, 3.7, 4.2, 4.6, 5.0 (ring-H), 4.74 (C1-H), 8.14 (triazole), 8.32 (NH). ^{13}C NMR (DMSO-d_6): δ 25.9 (C1–NH–CO–CH_2–*CH_2*–), 29.1 (aliphatic-C), 35.4 (C1–NH–CO–*CH_2*–), 44.4 (CH_2–*C*–CH_3), 49.4 (CH_2–O), 57.7 (*CH_2*–C), 60.3 (C6′), 61.1 (C6), 70.0 (C4′), 72.1 (C2), 73.3 (C2′), 75.8 (C5′), 76.4 (C3, C3′), 76.8 (C5), 79.2 (C4), 80.4 (C1), 103.2 (C1′), 125.0 (*C*H (triazole)), 140.8 (C (triazole)), 172.9 (C1–NH–CO–), 176.0 (CO).

2.8. PPMA-g-(CELL13-C15)

To a solution of PPMA-g-(CTA13-C15) (4.8 mg) in methanol/chloroform (1/4, v/v, 0.3 ml), sodium methoxide (50 μl, 0.03 mmol) was added at room temperature, and stirred for 4 h under nitrogen. The precipitated compound was collected by centrifugation at 1000 rpm for 3 min to give an amorphous solid, PPMA-g-(CELL13-C15) (2.4 mg, 88.9% yield).

2.9. General measurements

^{1}H, ^{13}C, and two-dimensional NMR spectra including H–H COSY (correlation spectroscopy), HSQC (heteronuclear single quantum coherence) were recorded with a Varian INOVA300 FT-NMR (300 MHz) or a JEOL JNM-A500 FT-NMR (500 MHz) spectrometer in $CDCl_3$ or DMSO-d_6 with tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) and coupling constants (J) are reported in (ppm) and (Hz), respectively. Fourier transform infrared (FT-IR) spectra were recorded on a FTIR-4000 spectrophotometer equipped with ATR attachment (Durasampl IR II).

2.10. SEC–MALS measurement

Size exclusion chromatography–multi-angle laser light scattering (SEC–MALS) measurements were carried out at 25 °C using a Shimadzu SEC system (CBM-10A, SPD-10A, SIL-10A, LC-10AT, FCV-10AL, CTO-10A, RID-10A, and FRC-10, Shimadzu, Japan) and MALS detector (DAWN EOS, Wyatt Technology Co. Ltd., U.S.A.) ($\lambda = 690$ nm). Chloroform was used as eluent. The flow rate was 1.0 ml/min. The photometer was calibrated with pure toluene. Shodex columns (K802, K802.5, and K805) were used. Number and weight average molecular weights ($M_{n,PS}$, $M_{w,PS}$) and polydispersity index (PDI, $M_{w,PS}/M_{n,PS}$) were estimated using polystyrene standards (Shodex). An absolute molecular weight (M_w) was determined by MALS measurements using Zimm plots. Refractive index (RI) increments (dn/dc) were calculated assuming 100% recovery of injected mass. The dn/dc values were 0.047 for PPMA-g-(CTA2-C15) and 0.064 for poly(2-propyn-1-yl methacrylate) (PPMA).

2.11. X-ray diffraction measurements

X-ray diffraction measurements were carried out with a Rigaku diffractometer Ultima IV. A Nickel-filtered CuKα radiation was used at 40 kV and 30 mA. Cellulose microcrystalline (Avicel, Merck) and low-molecular-weight cellulose (Isogai & Usuda, 1991) was used to obtain reflection pattern of cellulose I and cellulose II, respectively. The peak of X-ray diffraction curves are resolved to four peaks due to (1 −1 0), (1 1 0), (2 0 0) and amorphous reflections. Crystallinity index was calculated from the ratio of the sum of integrated intensities of (1 −1 0), (1 1 0), (2 0 0) reflections to the sum of integrated intensities of the four peaks (Ishikawa, Okano, & Sugiyama, 1997).

3. Results and discussion

3.1. Preparation and characterization of PPMA-g-(CTA2-C15)

Poly(2-propyn-1-yl methacrylate) (PPMA) was obtained *via* free radical polymerization of trimethylsilyl methacrylate monomer using AIBN and subsequent deprotection of trimethylsilyl group using TBAF·$3H_2O$ according to the previous article (Ladmiral et al., 2006), as described in Fig. 1a. We have prepared cellulose derivatives with a ω-azidopentadecanoyl group at the reducing-end in previous work (Enomoto, Kamitakahara, Takano, & Nakatsubo, 2006; Kamitakahara et al., 2005; Kamitakahara & Nakatsubo, 2005). *N*-(15-azidopentadecanoyl)-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosylamine (CTA2-C15-N_3), was grafted onto the PPMA main-chain at the molecular ratio [PMA monomer]/[CTA2-C15-N_3] = 1/1, *via* click chemistry using Cu(I)Br and PMDETA in DMF (Fournier & Du Prez, 2008), as described in Fig. 1b.

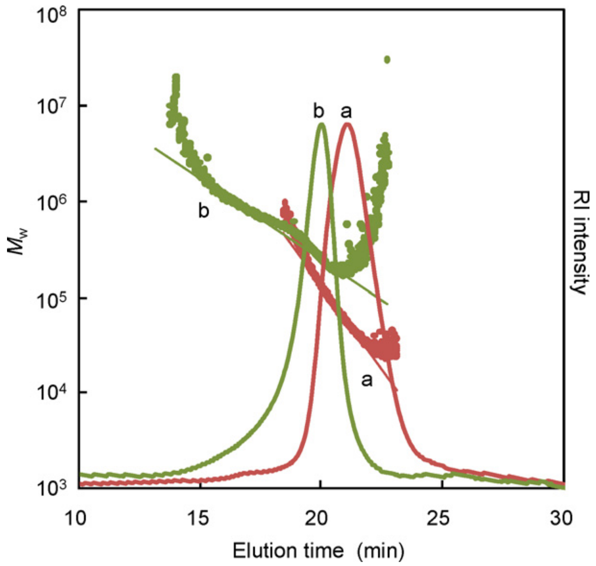

Fig. 2. The chromatograms (RI) and the molecular weight (M_w) plots of: (a) PPMA and (b) PPMA-g-(CTA2-C15).

The chromatograms (RI) and plots of the molecular weight determined by MALS (M_w) of PPMA and PPMA-g-(CTA2-C15) are shown in Fig. 2. No peak corresponding to a free CTA2-C15-N_3 was observed in its chromatogram as shown in Fig. 2b. The characteristics of the copolymers are summarized in Table 1. In ^{1}H NMR spectrum of PPMA-g-(CTA2-C15) in Fig. 3a, the resonances assigned to the triazole appeared at 7.93 ppm and that assigned to the alkyne proton of the PPMA main-chain disappeared. In Fig. 3b, ^{13}C resonances assigned to the triazole were observed at 124.4 and 141.6 ppm. The heteronuclear connectivity (CH) of the triazole was confirmed by its HSQC spectrum (data not shown). The relative integral area of the triazole (1H) to the ring-H (14H) was 1.12–14, indicating a quantitative formation of the triazole. In the FT-IR spectrum of PPMA-g-(CTA2-C15) in Fig. 4c, disappearance of the N_3 absorbance at 2120 cm^{-1} after click reaction supports a quantitative formation of the triazole.

3.2. Structural characterization of PPMA-g-(CTA2-C15)

Conformational structure of PPMA-g-(CTA2-C15) in chloroform was investigated by means of SEC–MALS measurements. The absolute molecular weight (M_w) of PPMA chain was determined to be 6.94×10^4 by MALS measurements. The weight average degree of polymerization of the PPMA (DP_w, $X+Y$) was calculated as 5.59×10^2 from M_w(PPMA)/M(PMA), where the molecular weight of PMA monomer is 124.21. The molecular weight ($M_{w,PS}$) (7.58×10^4) of PPMA-g-(CTA2-C15) was underestimated by PS standards, compared to the absolute molecular weight (M_w) (42.2×10^4) determined by MALS measurements. It is well known that SEC, when calibrated only with linear standard polymers such as polystyrene (PS), severely underestimates the molecular weight of a branched polymer which has a more compact molecular volume in solution than that of a corresponding linear polymer with the same molecular weight (Ito et al., 1992). This fact supports successful grafting of CTA2-C15-N_3 onto PPMA and the branched structures of the copolymers with CTA side-chains as

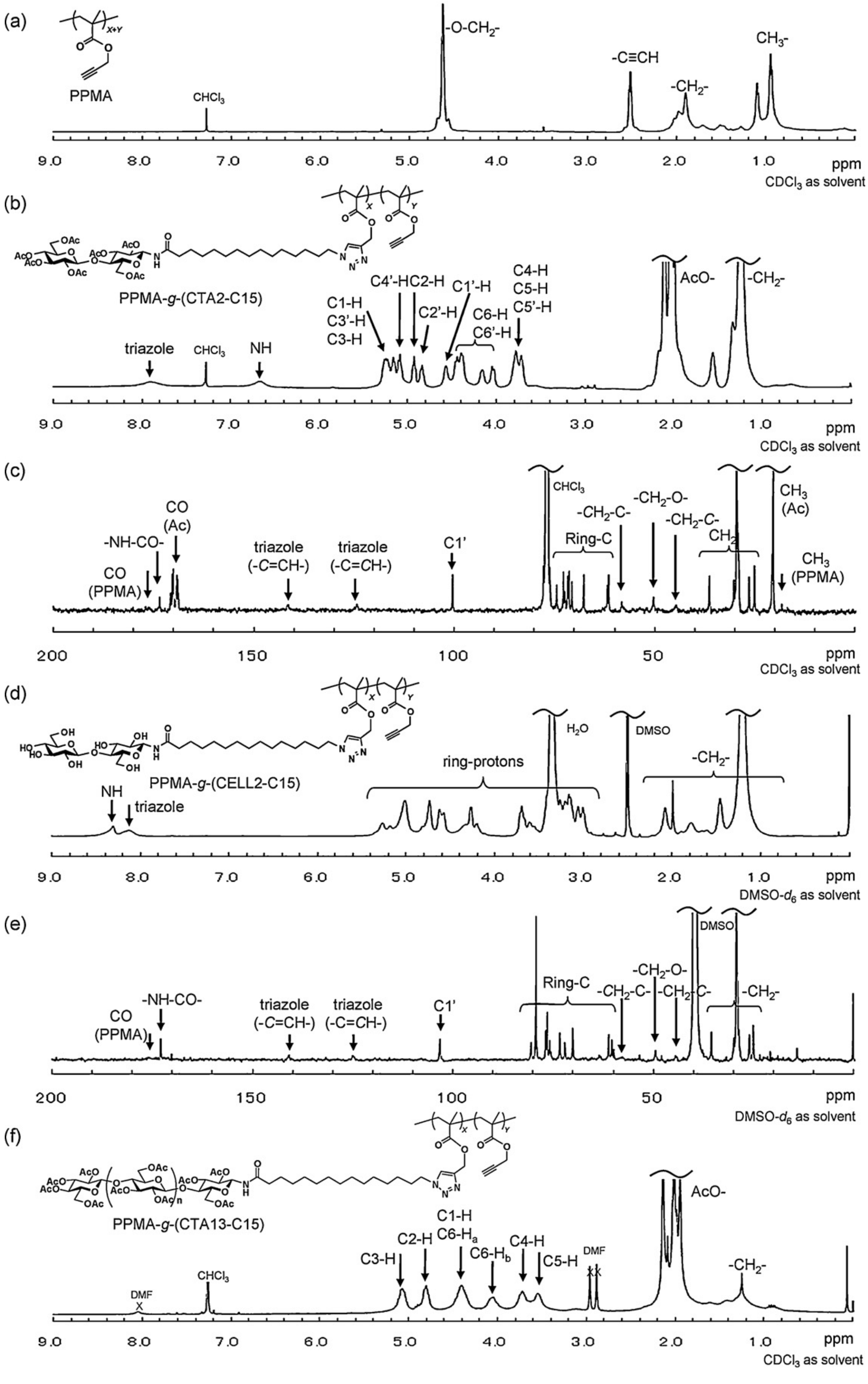

Fig. 3. (a) ^{1}H NMR spectrum of PPMA, (b) ^{1}H and (c) ^{13}C NMR spectra of PPMA-*g*-(CTA2-C15), (d) ^{1}H and (e) ^{13}C NMR spectra of PPMA-*g*-(CELL2-C15) (f) ^{1}H NMR spectrum of PPMA-*g*-(CTA13-C15).

Table 1
Characteristics of PPMA, PPMA-*g*-(CTA2-C15) and PPMA-*g*-(CTA13-C15).

Polymers	A(%)[a]	Yield (%)[c]	$M_{n,PS}$ (10^{-4})[d]	$M_{w,PS}$ (10^{-4})[d]	$M_{w,PS}/M_{n,PS}$[d]	M_w (10^{-4})	$X+Y$ (10^{-2})[f]	X (10^{-2})	Y (10^{-2})
PPMA			1.92	3.54	1.84	6.94[e]	5.59		5.59
PPMA-*g*-(CTA2-C15)	[b]	88.6	7.58	22.4	2.95	42.2[e]	5.59	4.03[g]	1.56[h]
PPMA-*g*-(CTA13-C15)	43.8	26.6	22.9	87.5	3.81	137.57[j]	5.59	2.45[i]	3.14[h]

[a] Peak area ratio (%) of the graft copolymer to total peak area of the graft copolymer and remaining CTA-C15-N_3 calculated from SEC(RI) elution curves.

[b] Quantitative.

[c] Yield of the graft copolymers.

[d] Estimated by polystyrene standards.

[e] Determined by MALS measurements.

[f] DPw of PPMA main-chain calculated as $X+Y=M_w(\text{PPMA})/M(\text{PMA})$.

[g] Number of CTA2-C15 chains calculated from $X=(M_w(\text{PPMA-}g\text{-(CTA2-C15)})-M_w(\text{PPMA}))/M_{w,PS}(\text{CTA2-C15-N}_3)$. $M(\text{PMA})=124.21$. $M(\text{CTA2-C15-N}_3)=874.97$.

[h] Number of PMA units calculated from $Y=(X+Y)-X$.

[i] Number of CTA13-C15 graft chains calculated from $X=\text{DP}_w(\text{PPMA})\times A(\%)$.

[j] Molecular weight calculated from $M_w=M_w(\text{PPMA})+X\times M_{w,PS}$ (CTA13-C15-N_3). $M_{n,PS}$ and $M_{w,PS}$ of CTA13-C15-N_3 = 4.12×10^3 and 5.33×10^3.

discussed in our previous articles (Enomoto-Rogers, Kamitakahara, Nakayama, et al., 2009; Enomoto-Rogers, Kamitakahara, Takano, et al., 2009). The PPMA-*g*-(CTA2-C15) has the same PPMA main-chain with the same DP_w value. The number of CTA chains (X) of PPMA-*g*-(CTA2-C15) was calculated as 4.03×10^2 from $X=(M_w(\text{PPMA-}g\text{-(CTA2-C15)})-M_w(\text{PPMA}))/M(\text{CTA2-C15-N}_3)$. The number of PMA monomers (Y) was calculated as 1.56×10^2 from $Y=(X+Y)-X$.

3.3. Preparation and characterization of PPMA-g-(CTA13-C15)

Under conditions the same as those for CTA2-C15-N_3, *N*-(15-azidopentadecanoyl)-tri-*O*-acetyl-β-cellulosylamine (CTA13-C15-N_3, DP_n = 13) was grafted onto the PPMA main-chain with the molecular ratio [PMA monomer]/[CTA13-C15-N_3] = 100% to obtain PPMA-*g*-(CTA13-C15), as described in Fig. 1b. In chromatogram of PPMA-*g*-(CTA13-C15) before purification, the two

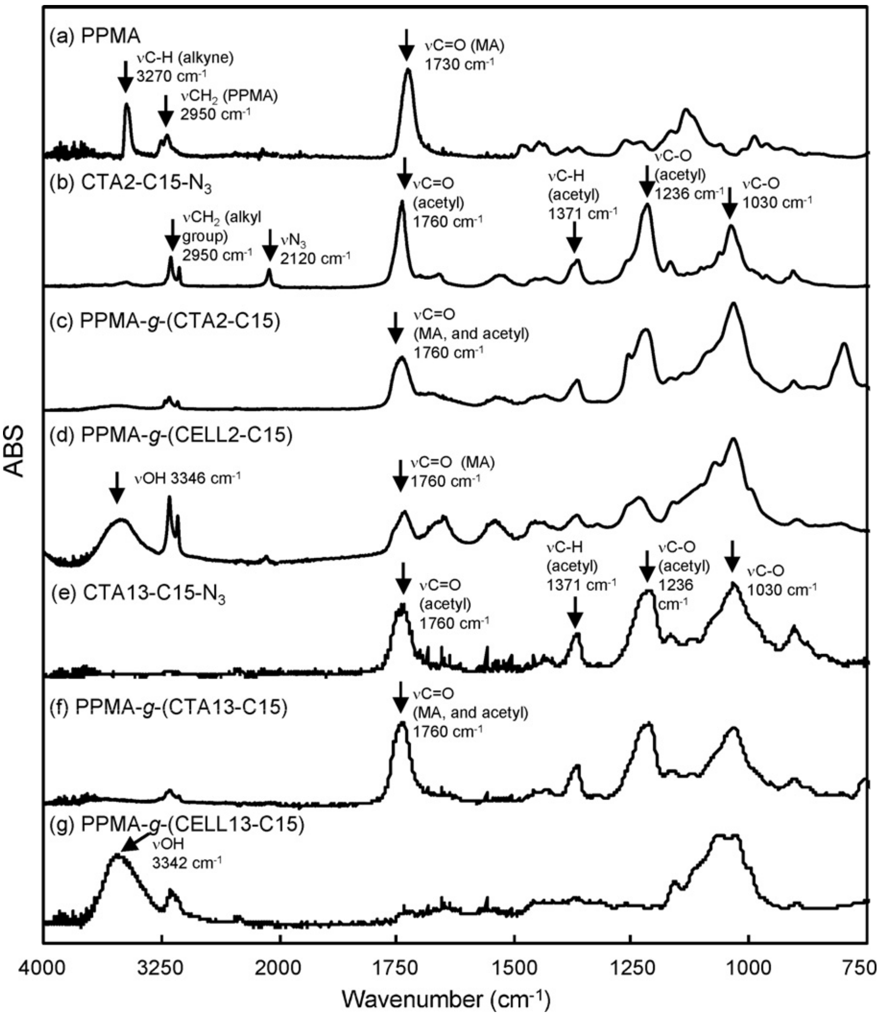

Fig. 4. FT-IR spectra of: (a) PPMA, (b) CTA2-C15-N_3, (c) PPMA-*g*-(CTA2-C15), (d) PPMA-*g*-(CELL2-C15), (e) CTA13-C15-N_3, (f) PPMA-*g*-(CTA13-C15) and (g) PPMA-*g*-(CELL13-C15).

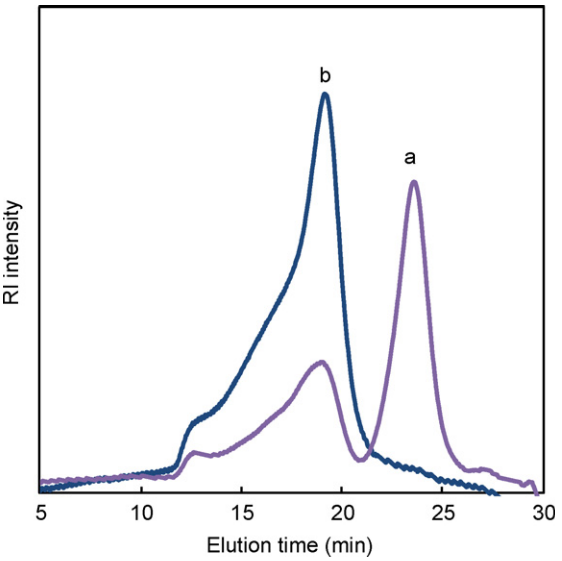

Fig. 5. Chromatograms (RI) of PPMA-*g*-(CTA13-C15) (a) before and (b) after purification.

peaks consisting of a remaining CTA13-C15-N_3 and the fraction with higher molecular weight were observed, as shown in Fig. 5a, indicating a formation of graft copolymer with CTA side-chains. The high-molecular-weight fraction was collected by SEC system equipped with a fraction collector, and PPMA-*g*-(CTA13-C15) was obtained in 26.6% yield as shown in Fig. 5b. In both FT-IR and ^{1}H NMR spectra of purified PPMA-*g*-(CTA13-C15), peaks assigned to CTA chains were observed in Figs. 3f and 4f, indicating that the compounds are composed of CTA chains. The resonances of the triazole and PPMA of PPMA-*g*-(CTA13-C15) were however not identified in both NMR and FT-IR spectra, because of the low PPMA content of the copolymer.

The $M_{n,PS}$ and $M_{w,PS}$ of PPMA-*g*-(CTA13-C15) were estimated to be 2.29×10^5 and 8.75×10^5, respectively by SEC analysis using PS standards. The molecular weight of the branched copolymer should be underestimated by PS standards compared to the absolute molecular weight determined by MALS analysis, as discussed in the previous section. It was, however, impossible to measure an absolute molecular weight of PPMA-*g*-(CTA13-C15) by MALS analysis because of high scattering intensity of the compounds (see supplemental data). This high intensity is due to small amount of insoluble fraction with a large radius, which could not be removed and not dispersed at low concentration or after long-term storage in chloroform. The molecular weight was calculated as 1.2×10^7, and overestimated compared to an possible maximum value (3.03×10^6), which was calculated by initial molar ratio [CTA13-C15-N_3]/[PMA] = 1.0 and 100% grafting rate.

According to the chromatogram of refractive index (RI), the area ratio of the high molecular fraction to the total area including the remaining CTA13-C15-N_3 was $A(\%) = 43.8\%$. Therefore, X value of PPMA-*g*-(CTA13-C15) was calculated as 2.45×10^2 from $X = \mathrm{DP_w(PPMA)} \times A(\%) = 5.59 \times 10^2 \times 43.8(\%)$, on the assumption that the initial molar ratio of CTA13-C15-N_3 to PMA monomer unit was 1.0. The number of PMA units (Y) in PPMA-*g*-(CTA13-C15) was calculated to be 3.14×10^2 from $Y = \mathrm{DP_w(PPMA)} - X$. The molecular weight of PPMA-*g*-(CTA13-C15) was estimated to be 1.38×10^6 from $M(\text{PPMA-}g\text{-(CTA13-C15)}) = M_w(\mathrm{PPMA}) + X \times M_{w,PS}(\text{CTA13-C15-}N_3) = 6.94 \times 10^4 + 2.45 \times 10^2 \times 5.33 \times 10^3$. The density of cellulose side-chains per PPMA main-chain increased compared to that of the copolymer PMMA-*g*-(CTA13-C15) (3.84 CTA side-chains per PMMA main-chain with $\mathrm{DP_w}$ of 4.14×10^2) obtained *via* radical copolymerization of a cellulose macromonomer with methyl methacrylate studied previously (Enomoto-Rogers, Kamitakahara, Takano, et al., 2009). Thus, "graft onto" strategy and "click reaction" were efficient methods to obtain a copolymer with cellulosic side-chains.

3.4. Preparation and characterization of PPMA-g-(CELL2-C15) and PPMA-g-(CELL13-C15)

PPMA-*g*-(CTA2-C15) and PPMA-*g*-(CTA13-C15) were treated with sodium methoxide to remove acetyl groups, as described in Fig. 1b, and the deprotected copolymers, PPMA-*g*-(CELL2-C15) and PPMA-*g*-(CELL13-C15), were obtained. Completion of deacetylation was supported by a strong OH absorbance appeared at $\nu = 3446\,\mathrm{cm^{-1}}$ in FT-IR spectrum as shown in Fig. 4d and g. The PPMA-*g*-(CELL2-C15) was soluble in DMSO, and insoluble in water. The PPMA-*g*-(CELL13-C15) was insoluble in water and other common solvents, such as DMSO and DMF. In ^{1}H NMR spectrum of PPMA-*g*-(CELL2-C15) in DMSO-d_6 (Fig. 3d), the resonances of acetyl protons disappeared, and those of ring-protons of cellobiose moieties remained. In addition, the resonances at 8.14 and 8.32 ppm were assigned to the triazole and amide protons (NH), respectively. In the ^{13}C NMR spectrum (Fig. 3e), the resonances assigned to two carbons of the triazole were observed at 124.4 and 141.6 ppm. The carbonyl carbons of the ester linkage (O–*C*O) of PPMA and the amide linkage (C1-NH-*C*O) remained at 176.0 and 172.9 ppm, respectively. These data indicate that the acetyl groups of the copolymers were selectively removed without cleavage of the triazole, ester and amide linkages. The comb-shaped copolymers having cellulose side-chains with "head-to-tail" orientation were successfully obtained.

3.5. X-ray diffraction measurements

In general, cellulose I, the native form, is believed to have parallel orientation (Gardner & Blackwel, 1974; Sugiyama, Vuong, & Chanzy, 1991). On the other hand, cellulose II, the regenerated or mercellized form, is believed to have anti-parallel orientation (Kolpak & Blackwell, 1976; Langan, Nishiyama, & Chanzy, 1999). X-ray diffraction measurements were carried out to analyze crystalline pattern of PPMA-*g*-(CELL2-C15) and PPMA-*g*-(CELL13-C15). The cellobiose chains of PPMA-*g*-(CELL2-C15) showed no crystalline pattern as shown in Fig. 6c. The cellulose chains of PPMA-*g*-(CELL13-C15) had crystalline structure with crystallinity index = 42.2%, as shown in Fig. 6e, but exhibited crystalline pattern of cellulose II with three (1 −1 1), (1 1 0), and (2 0 0) reflections located at $d = 0.739$, 0.451, and 0.407 nm, respectively (Isogai, Usuda, Kato, Uryu, & Atalla, 1989). The reason for amorphous pattern of cellulose is likely that crystallization of cellulose chains *via* hydrogen bonding was inhibited by their covalent grafting on PPMA chain at the longer interchain distances than those suitable for the crystallization, as discussed in our previous work (Enomoto-Rogers, Kamitakahara, Yoshinaga, & Takano, 2011b). Regarding the crystalline structure, there are some possibilities for the structures of cellulose chains of PPMA-*g*-(CELL13-C15). Parallel cellulose chains might show diffraction pattern of cellulose II, or anti-parallel cellulose chains might be formed by interdigitation of cellulose chains on the PPMA chain and give diffraction pattern of cellulose II. In our previous study, nanoparticles consisting with cellulose chains having radial "head-to-tail" orientation showed a crystalline pattern of cellulose II (Enomoto-Rogers, Kamitakahara, Yoshinaga, & Takano, 2011a). Thus, the XRD data of PPMA-*g*-(CELL13-C15), which has cellulose side-chains with "head-to-tail" orientation, suggested the possibility that not only anti-parallel but also "parallel" orientations of cellulose chains might give a crystal structure of cellulose II.

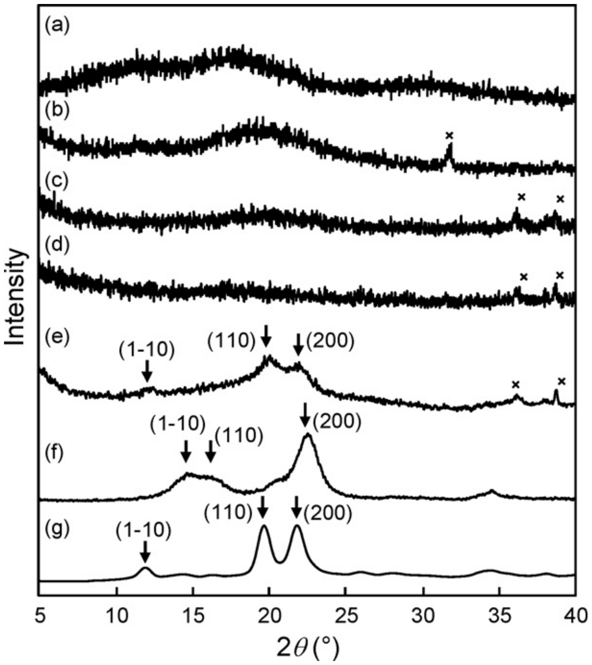

Fig. 6. Wide angle X-ray diffractgrams of: (a) PPMA, (b) PPMA-*g*-(CTA2-C15), (c) PPMA-*g*-(CELL2-C15), (d) PPMA-*g*-(CTA13-C15), (e) PPMA-*g*-(CELL13-C15), (f) microcrystalline cellulose (cellulose I) and (g) regenerated cellulose (cellulose II).

4. Conclusions

Comb-shaped graft copolymers with cellulose triacetate (CTA) side-chains, PPMA-*g*-(CTA2-C15) and PPMA-*g*-(CTA13-C15), were prepared by grafting CTA2-C15-N_3 and CTA13-C15-N_3 ($DP_n = 13$) onto PPMA ($DP_w = 5.59 \times 10^2$), respectively, *via* "click chemistry". The numbers of CTA side-chains (X) of PPMA-*g*-(CTA2-C15) and PPMA-*g*-(CTA13-C15) were calculated as 4.03×10^2 and 2.45×10^2, respectively. The density of cellulose side-chains per main-chain increased compared to that of the copolymers obtained *via* radical copolymerization of a cellulose macromonomer. PPMA-*g*-(CELL2-C15) and PPMA-*g*-(CELL13-C15) were successfully obtained by deacetylation of PPMA-*g*-(CTA2-C15) and PPMA-*g*-(CTA13-C15), respectively. PPMA-*g*-(CELL13-C15) exhibited reflection pattern of cellulose II, suggesting the possibility that not only anti-parallel but also parallel orientations of cellulose chains might give a crystal structure of cellulose II.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.10.055.

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