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Ring-opening polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride in ionic liquids

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Abstract

This work deals with ring-opening polymerization of a representative *N*-carboxy-α-amino acid anhydride (NCA) in ionic liquids. The polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) with *n*-butylamine as an initiator in an ionic liquid ([BMI][PF_6]) proceeded as a milky white dispersion with no evidence of macroscopic precipitation. The polymerization with the primary amine under suitable conditions afforded poly(amino acid) having narrow polydispersity, molecular weights close to the theoretical values, and helical secondary structure. The polymerization rate was slightly affected by the nature of the anion and hydrophobicity of the ionic liquids, while poly(BLG)s having low polydispersities were obtained regardless of the kind of the ionic liquids. Several parameters, such as the existence of organic solvent as a co-solvent and monomer concentration, had also clear effects on the polymerization rate and/or the polydispersity of the resulting poly(BLG)s. The possible interactions between the ionic liquid and NCA monomer or the ionic liquid and the initiator were characterized using FT-IR, ^{1}H and ^{13}C NMR measurements. The character of this polymerization process was also studied by performing kinetic investigations. We believe that this represents the first report on amine-initiated ring-opening polymerization of NCA in ionic liquid.

Keywords: Ring-opening polymerization; Amino acid; Ionic liquid

1. Introduction

Ionic liquids are organic salts that are liquid at ambient temperatures, preferably at room temperature. Their interesting properties include high ionic conductivity, high polarity, high density, high heat capacity, high thermal and chemical stability, which can be tuned widely by adjusting the structures of the cation and the anion. Ionic liquids are becoming widely recognized as environmentally friendly reaction media 'green solvents', replacing volatile organic solvents, because of their non-flammability and non-volatility [1,2]. An advantage in using these ionic liquids is their ability to dissolve a wide range of organic and ionic compounds. Recently, several efforts have been also directed at the usage of ionic liquids for various polymerization systems [3], such as coordinative [4,5], radical [6–11], cationic [12], group transfer [13], enzymatic [14], electrochemical [15,16], and step-growth [17,18] polymerizations. The unique and attractive properties of ionic liquids may provide useful methodologies to create new polymeric materials and novel environmental process. In some cases, the use of ionic liquid in place of conventional organic solvent was reported to lead to enhancement in reaction rate [10,11], higher molecular masses [7], and effective separation of polymer from catalyst residues [6]. However, only a few attempts to use ionic liquids in ring-opening polymerizations have appeared in the literature [19]. Ring-opening polymerization of ethylene carbonate with acidic chloroaluminate or chlorostannate ionic liquids was reported, in which the ionic liquids were used as a catalyst [20]. Living ring-opening polymerization of strained cyclic esters, such as lactides and lactones,

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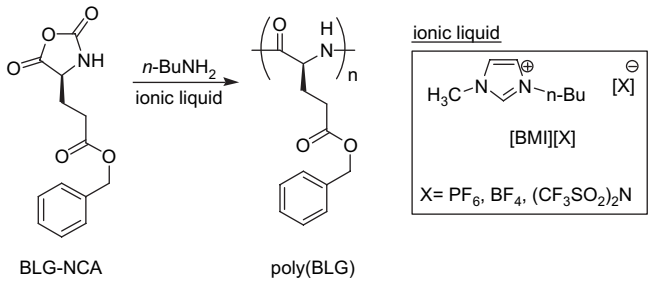
with carbenes generated by in situ activation of ionic liquids was reported [21]. Water-soluble ionic liquids were also used as reaction media to perform cationic ring-opening polymerization of oxazoline derivatives under microwave irradiation [22].

In recent years, much interest has been devoted to synthetic polypeptides, because of their potential application as biodegradable and biomedical polymers [23–25], as well as their feasibility to create secondary structures (α-helix and β-sheet) and higher ordered structures [26–28]. Ring-opening polymerization of α-amino acid anhydrides (NCAs) is the most popular and economic process for the preparation of long polypeptides [29–31]. By using so-called NCA method, high molecular weight polymers can be prepared in both good yields and large quantities without detectable racemization at the chiral centers. Most of polypeptides prepared by the NCA method form strong intrachain (e.g., α-helix) and interchain (e.g., β-sheet) hydrogen bonds, which play an essential role in determining various functions and properties. Such ordered and hierarchical structures due to the noncovalent forces are related to those of natural peptides and proteins. However, some polypeptides are insoluble in almost all organic and inorganic solvents, due to their characteristic noncovalent interactions. The poor solubility and difficulty in handling them led to limited practical applications.

Herein we report first study of the use of room-temperature imidazolium ionic liquids for the preparation of poly(amino acid)s by ring-opening polymerization of NCA (Scheme 1). The main objective of the present study is to replace the traditional volatile polymerization solvents with environmentally friendly ionic liquids and to investigate the potential for the special solvent properties of the ionic liquids to produce amino acid-based polymers with narrower polydispersities. In this study, we selected γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) as a representative NCA, since the polymerization of BLG-NCA has been widely investigated, which forms predominantly helical polypeptide with a stiff rigid-rod structure [32]. Due to their ionic nature, ionic liquids are highly polar but non-coordinating, which may influence a course of polymerization reaction and secondary structures of the resulting polymers. We also investigated the possible interactions between the ionic liquid and NCA monomer, as well as the ionic liquid and the initiator.



Scheme 1. Ring-opening polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) in ionic liquid.

2. Experimental

2.1. Materials

n-Butylamine (Kanto Chemical, >98.0%) was dried with CaH_2 and distilled from CaH_2, and stored under nitrogen. 1-*n*-Butyl-3-methylimidazolium hexafluorophosphate ([BMI][PF_6], Kanto Chemical, >97.5%), 1-*n*-butyl-3-methylimidazolium tetrafluoroborate ([BMI][BF_4], Kanto Chemical, >97.5%), and 1-*n*-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)-imide ([BMI][$(CF_3SO_2)_2N$], Kanto Chemical) were purchased. Dichloromethane (Kanto Chemical, 99%) was dried with CaH_2 and distilled under nitrogen atmosphere prior to use. 1,4-Dioxane (Kanto Chemical, 99%) was dried with sodium and distilled under nitrogen atmosphere prior to use. Triphosgene (Aldrich, 98%) was used as received. γ-Benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) was prepared by the reaction of γ-benzyl-L-glutamate with bis(trichloromethyl)carbonate (triphosgene) according to a literature procedure [33]. The resulting NCA was recrystallized at least two times from CH_2Cl_2/hexane prior to polymerization. Other materials were used without further purification.

2.2. General polymerization procedure

All polymerization procedures were carried out in a dry glass tube capped with a rubber septum and a two-way glass stopper under dry nitrogen. The ionic liquid was placed in the tube, and then treated by stirring at 100 °C under vacuum for more than 1 h in order to remove volatile impurities and moisture before use. For a typical polymerization, BLG-NCA (0.13 g, 0.50 mmol) was dissolved in [BMI][PF_6] (3.1 mL) by stirring with a magnetic bar in a dry glass tube under nitrogen and then the CH_2Cl_2 solution of *n*-butylamine (0.01 mmol, 0.2 M) was added via syringe to the mixture. The resulting mixture was stirred at 30 °C under nitrogen atmosphere for 20 h. Since BLG-NCA was soluble in [BMI][PF_6], while poly(BLG) was essentially insoluble in the ionic liquid, the transparent monomer solution was changed gradually into white turbid dispersion during the polymerization. After polymerization, the reaction mixture was precipitated in a large excess of methanol and isolated by filtration, and finally dried under vacuum at room temperature, to yield poly(BLG) as a white powder. The polymer yield was gravimetrically determined from the methanol-insoluble polymer sample (yield = 47%, 0.052 g). The polymer had $M_n = 7000$ and $M_w/M_n = 1.08$, measured by a GPC in *N*,*N*-dimethylformamide (DMF) with 10 mM LiBr using polystyrene calibration. The resulting polymer was soluble in dichloromethane, THF, dioxane, DMSO, and DMF, while insoluble in diethylether, acetone, hexane, and water.

2.3. Characterization methods

The ^1H (270 MHz and 400 MHz) and ^{13}C NMR (67.5 MHz and 100 MHz) spectra were recorded with a JEOL EX-270 and a JEOL JNM ECX400. FT-IR spectra were obtained with

a JASCO FT/IR-210 spectrometer. The samples for the evaluation of the interactions were obtained by casting the ionic liquid solution of the initiator or BLG-NCA on NaCl plates. The spectra of the resulting polymers were recorded from KBr pellets, prepared by mixing the sample with KBr. The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) were estimated by size-exclusion chromatography (SEC) using a Tosoh HPLC HLC-8220 system equipped with refractive index and ultraviolet detectors at 40 °C. The column set was as follows: four consecutive hydrophilic vinyl polymer-based gel columns [TSK-GELs (bead size, exclusion limited molecular weight): α-M (13 μm, $>1 \times 10^7$), α-4000 (10 μm, 4×10^5), α-3000 (7 μm, 9×10^4), α-2500 (7 μm, 5×10^3), 30 cm each] and a guard column [TSK-guardcolumn α, 4.0 cm]. The system was operated at a flow rate of 1.0 mL/min, using *N*,*N*-dimethylformamide (DMF) containing 10 mM LiBr as an eluent. Polystyrene standards were employed for calibration.

3. Results and discussion

3.1. Polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) in ionic liquid

The polymerization of BLG-NCA was conducted using *n*-butylamine as an initiator at 30 °C for 20 h at a constant monomer-to-initiator ratio, $[\text{BLG-NCA}]_0:[n\text{-butylamine}]_0 = 50$, under nitrogen atmosphere. The results are summarized in Table 1. We first selected 1-*n*-butyl-3-methylimidazolium hexafluorophosphate ([BMI][PF_6]) as a solvent, because this was the most frequently employed ionic liquid for various polymerization systems [3]. When BLG-NCA was dissolved in [BMI][PF_6], the monomer solution was visually transparent, while a turbid dispersion was formed gradually during the polymerization. The reaction proceeded as a milky white dispersion with no evidence of macroscopic precipitation even at the end of the polymerization. After the reaction, the polymer was easily separated from the ionic liquid, because [BMI][PF_6] was soluble in methanol used for the precipitation. Under these conditions, the moderate polymer yield (47%) was obtained, and the resulting polymer showed sharp symmetrical SEC peak ($M_w/M_n = 1.08$) without shoulder and tailing, as can be seen in Fig. 1. The number-average molecular weight of the poly(BLG), measured by a GPC in DMF with 10 mM LiBr, was $M_n = 7000$, which was fairly comparable to the theoretical value ($M_n = 5100$) calculated from the monomer/initiator molar ratio in the feed and the polymer yield. Similar tendency was reported on atom transfer radical polymerization of butyl acrylate in [BMI][PF_6], in which the polymerization proceeded in a controlled fashion, in spite of partial solubility of the monomer and insolubility of the polymer in the ionic liquid [34]. Hence, it seems reasonable to suggest that good solubility of the resulting polymer is not a necessary requirement for achieving the product with low polydispersity in ionic liquid.

In order to evaluate the effect of the counteranion, we compared the polymerizations of BLG-NCA in three different ionic liquids, [BMI][PF_6], 1-*n*-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide ([BMI][$(CF_3SO_2)_2N$]), and 1-*n*-butyl-3-methylimidazolium tetrafluoroborate ([BMI][BF_4]). Among them, [BMI][PF_6] and [BMI][$(CF_3SO_2)_2N$] are water

Table 1
Polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) with *n*-butylamine at 30 °C for 20 h[a]

Entry	Solvent (weight ratio)	Yield[b] (%)	M_n[c] (theory)	M_n[d] (SEC)	M_w/M_n[d] (SEC)
1[e]	[BMI][PF_6]	47	5100	7000	1.08
2[e]	[BMI][BF_4]	36	3900	6000	1.19
3[e]	[BMI][$(CF_3SO_2)_2N$]	53	5800	6200	1.22
4[e]	[BMI][PF_6]/CH_2Cl_2 (1/3)	60	6500	8600	1.34
5	[BMI][PF_6]/CH_2Cl_2/dioxane (1/3/1)	80	8700	12,200	1.33
6	CH_2Cl_2/dioxane (3/1)	92	10,800	9700	1.34

[a] $[\text{BLG-NCA}]_0/[n\text{-butylamine}]_0 = 50$, [M] = 0.17 M.
[b] MeOH-insoluble part.
[c] The theoretical molecular weight ($M_{n,\ \text{theory}}$) = (MW of BLG) × $[\text{BLG-NCA}]_0/[n\text{-butylamine}]_0$ × polymer yield.
[d] Measured by size-exclusion chromatography (SEC) using polystyrene standards in *N*,*N*-dimethylformamide (DMF, 10 mM LiBr).
[e] Homogeneous transparent solution was changed into white turbid dispersion during the polymerization.

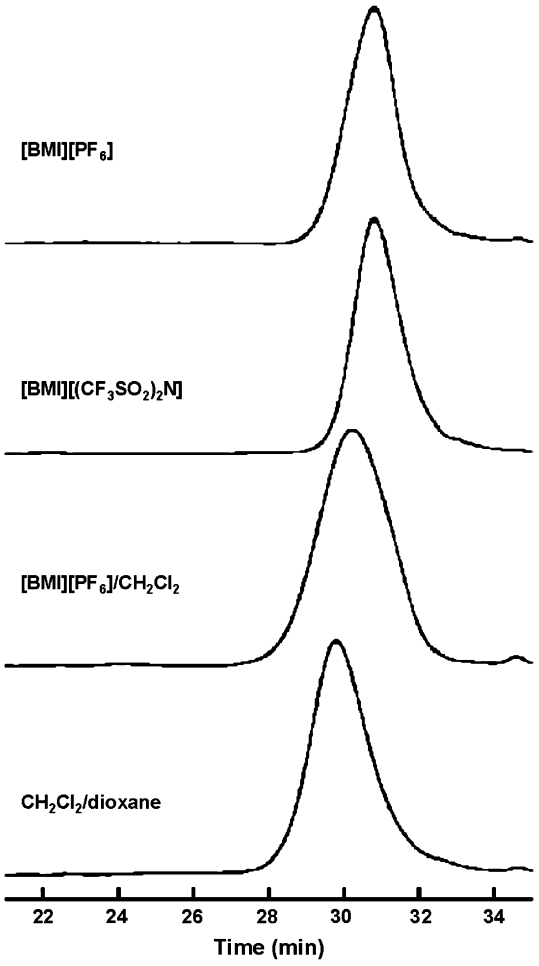
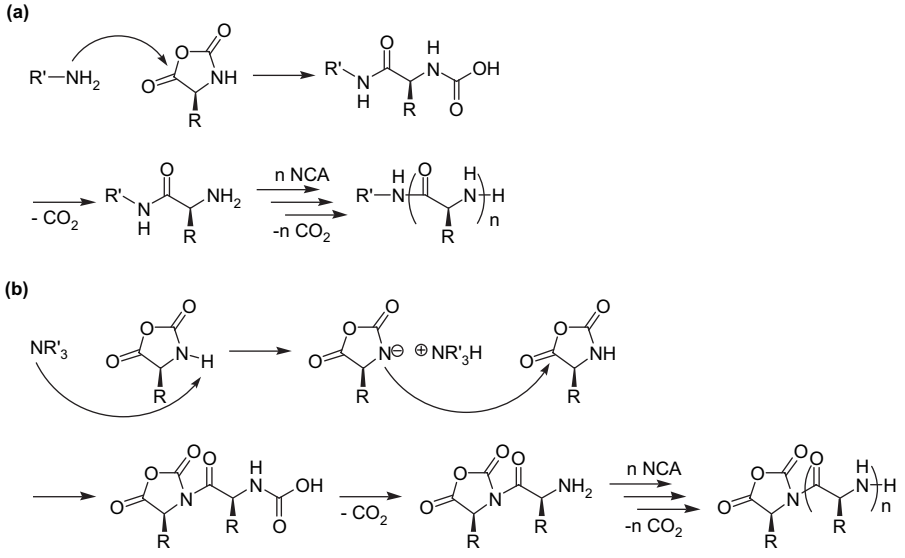


Fig. 1. SEC traces of poly(BLG)s prepared by the polymerization of BLG-NCA in different solvents. See Table 1 for detailed conditions.

insoluble ionic liquids, and [BMI][$(CF_3SO_2)_2N$] is known to have a strong hydrophobic property. Whereas, [BMI][BF_4] is a water miscible ionic liquid. At a low monomer concentration (0.17 M), BLG-NCA was dissolved completely in all ionic liquids to give transparent solutions at room temperature, which were gradually changed into turbid dispersion during the polymerization. As shown in Table 1, the polymerization in [BMI][BF_4] shows lower polymer yield than that in [BMI][$(CF_3SO_2)_2N$], while no significant influence is observed in the molecular weights and polydispersity. These results indicate that the polymerization rate is slightly affected by the nature of the anion and hydrophobicity of the ionic liquids, while poly(BLG)s having low polydispersities can be obtained regardless of the kind of the ionic liquids.

The most representative models of NCA polymerizations in organic solvent are the so-called ''amine'' and ''activated monomer'' mechanisms, as shown in Scheme 2 [29,31]. The amine mechanism is a simple nucleophilic ring-opening chain growth process, whereas the activated monomer mechanism is initiated by deprotonation of an NCA, which acts as the nucleophile for chain growth. Ideally, NCA polymerizations with primary amine proceed by the amine mechanism, where the polymer grows linearly with monomer conversion if side reactions are absent. However, the activated monomer mechanism takes place, since primary amines can act as both a nucleophile and a base. The polymerization can switch back and forth between the ''amine'' and ''activated monomer'' mechanisms, and a propagation step for one mechanism is a side reaction for the other. With this in mind, we investigated the polymerization of BLG-NCA in organic solvents in the presence of [BMI][PF_6], as comparisons. When the polymerization was conducted in a mixed organic solvent (CH_2Cl_2/dry dioxane = 3/1 wt%), the transparent solution maintained throughout the polymerization and almost full conversion (>90% determined by FT-IR spectroscopy and gravimetrically) was reached at 30 °C after 20 h. The resulting polymer had relatively low polydispersity ($M_w/M_n = 1.34$) and reasonable molecular weights. This is a typical feature of primary amine-initiated polymerizations of α-amino acid NCAs, which are not ''living'', but provide good control of chain length for polypeptides having relatively narrow polydispersity [31]. The polymerization in a mixed organic solvent with ionic liquid (dioxane/CH_2Cl_2/[BMI][PF_6] = 1/3/1 wt%) showed the same tendency in terms of the transparent reaction mixture throughout the polymerization and relatively low polydispersity ($M_w/M_n = 1.33$). In contrast, the transparent solution was changed into white turbid dispersion during the polymerization in CH_2Cl_2/[BMI][PF_6] (3/1 wt%), achieving 60% polymer yield. Nevertheless, the resulting poly(BLG) exhibits relatively low polydispersity ($M_w/M_n = 1.34$, Fig. 1). These results suggest that the usage of ionic liquid as a solvent leads to heterogeneous reaction system with decreasing the polymer yield, which may be due to insufficient solubility of the poly(BLG) formed in the ionic liquid, whereas it has no remarkable effect on the molecular weights and polydispersities of the resulting poly(BLG)s.

The influence of monomer concentration on the polymerization of BLG-NCA was investigated in [BMI][PF_6] (Table 2). Generally, a way to increase the polymerization rate and reach higher conversion is to increase monomer concentration, whereas it often leads to an increase in viscosity, resulting in the loss of the controlled character. At low monomer concentration (0.17 M), the polymerization proceeded as a milky white dispersion, but the viscosity was low enough to ensure an efficient stirring the magnetic bar in the reaction mixture, leading to the controlled synthesis of poly(BLG) with a moderate yield (47%). Higher monomer concentration (0.5 M) resulted in faster polymerization as expected, and 70% polymer yield was reached within 20 h. The resulting poly(BLG) shows a low polydispersity ($M_w/M_n = 1.16$) and reasonable molecular weight close to the calculated value. Further increase in the monomer concentration (1.0 M) and longer



Scheme 2. Mechanism for polymerization of *N*-carboxyanhydrides (NCAs). (a) Amine mechanism and (b) activated monomer mechanism.

Table 2
Polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) in ionic liquids with *n*-butylamine at 30 °C under various conditions[a]

Entry	Ionic liquid	[M]	Time (h)	Yield[b] (%)	M_n[c] (theory)	M_n[d] (SEC)	M_w/M_n[d] (SEC)
1	[BMI][PF_6][e]	0.17	20	47	5100	7000	1.08
2	[BMI][PF_6][f]	0.17	20	53	5800	6900	1.09
3	[BMI][PF_6][e]	0.5	20	70	7700	7800	1.16
4	[BMI][PF_6][f]	0.5	20	71	7800	8100	1.14
5	[BMI][PF_6][e]	0.5	72	72	7900	6900	1.35
5	[BMI][PF_6][e]	1.0	72	78	9200	6600	1.43
7	[BMI][$(CF_3SO_2)_2N$][e]	0.17	20	53	5800	6200	1.22
8	[BMI][$(CF_3SO_2)_2N$][e]	0.5	20	73	8000	7700	1.37

[a] $[BLG\text{-}NCA]_0/[n\text{-butylamine}]_0 = 50$.
[b] MeOH-insoluble part.
[c] The theoretical molecular weight ($M_{n,\ theory}$) = (MW of BLG) × $[BLG\text{-}NCA]_0/[n\text{-butylamine}]_0$ × polymer yield.
[d] Measured by size-exclusion chromatography (SEC) using polystyrene standards in *N*,*N*-dimethylformamide (DMF, 10 mM LiBr).
[e] Polymerization under nitrogen.
[f] Polymerization under continuous vacuum condition.

polymerization time afforded the polymers with broader polydispersities, which may be due to the increase in the viscosity. The same tendency was observed in the polymerizations in [BMI][$(CF_3SO_2)_2N$], in which the polymer yield and the polydispersity increased apparently (53%–73% in yield and M_w/M_n 1.22–1.37), as the monomer concentration increased from 0.17 M to 0.5 M.

One of the benefits in using ionic liquids as reaction solvents can be seen in their negligible volatility and nonflammability. In the cases of ring-opening polymerization of NCAs, the chain growth is accompanied by the release of CO_2 (Scheme 2), and effective elimination of the liberated CO_2 from the reaction system is a crucial point to achieve reasonable control of the polymerization of NCA [35,36]. Effect of CO_2 on the NCA polymerizations was also reported by several groups [37]. With this in mind, we conducted the polymerization of BLG-NCA under continuous vacuum condition, in which the glass vessel was connected directly to vacuum pump and the evacuation was continued during the polymerization. As shown in Table 2 (entries 2 and 4), there is no significant influence on the polymer yield, molecular weights, and molecular weight distribution. It means that the continuous vacuum system in ionic liquid is applicable for the controlled synthesis of poly(amino acid)s. In ring-opening polymerization of NCA monomers, water would certainly act as an initiating source for an undesired activated monomer mechanism. Depending on the concentration, the water can be regarded as a slow initiator of NCA monomers or as a terminator [29,35]. In this study, ionic liquid was treated by stirring at 100 °C under vacuum for more than 1 h in order to remove volatile impurities and moisture before use. To prove that the polymerization of BLG-NCA proceeds from the primary amine in the ionic liquid, a control experiment was conducted. The monomer solution in [BMI][PF_6] was stirred without *n*-butylamine under the same conditions used for the polymerization. No polymer formation was confirmed, suggesting that the ionic liquid had no ability to initiate the polymerization of BLG-NCA at 30 °C under nitrogen atmosphere. This is an indication that the effect of the residual or tightly bound water in the ionic liquid is negligible for the initiation step of the polymerization, even if the complete removal of water seems to be difficult.

Fig. 2 shows FT-IR spectra of the poly(BLG)s obtained by the ring-opening polymerization of BLG-NCA in an ionic liquid ([BMI][PF_6]) and an organic solvent (dioxane/CH_2Cl_2). Poly(BLG) is known to form α-helix when the degree of polymerization exceeds 7 or 8 [38,39]. Hence, we compared poly(BLG)s having higher molecular weights ($M_n > 4000$), which corresponded to degree of polymerization of more than 18. The amide I and amide II bands observed at 1655 and 1550 cm^{-1} are characteristic for an α-helical secondary structure, whereas the position of the amide I band is shifted to 1630 cm^{-1} for polypeptides possessing a β-sheet conformation [28,40]. The poly(BLG) obtained in [BMI][PF_6] shows the clear peaks at 1655 and 1550 cm^{-1}, while no significant peak is detected at 1630 cm^{-1}, indicating the preferable

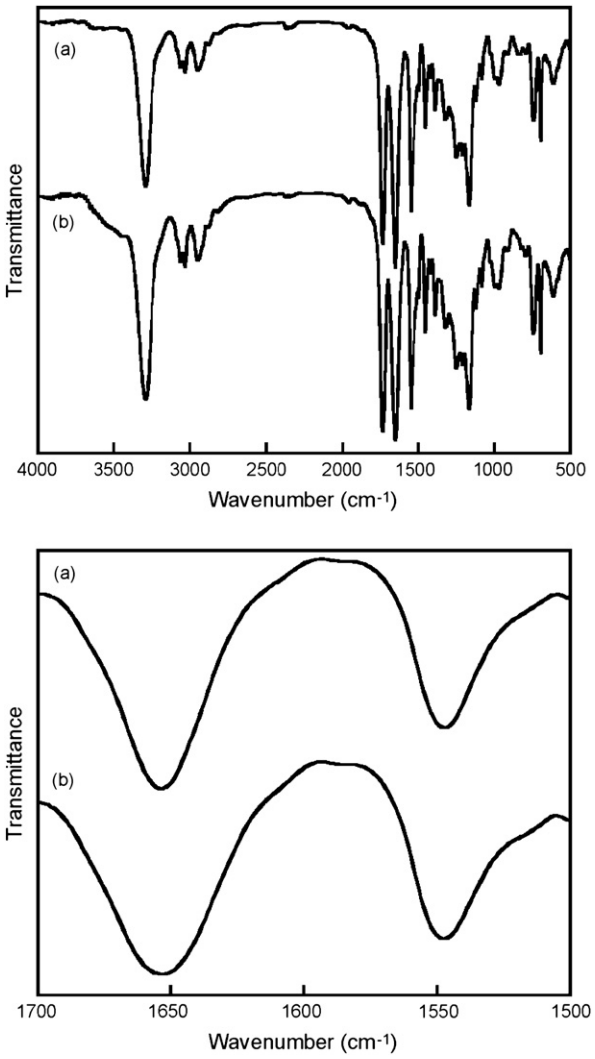


Fig. 2. FT-IR spectra of poly(BLG)s prepared by the polymerization in (a) [BMI][PF_6] and (b) CH_2Cl_2/dioxane (3/1 wt%). Lower figure: magnification in the region of carbonyl bond.

formation of α-helical secondary structure. No significant difference in the characteristic peaks found in the samples prepared in the ionic liquid and the organic solvent suggests that the conformation of the samples is independent of the solvents used during the polymerization.

3.2. Interactions with ionic liquid

To obtain the information on the possible interactions between the ionic liquid and NCA monomer or the ionic liquid and the initiator, we conducted preliminary experiments using FT-IR, ^{1}H and ^{13}C NMR measurements. Fig. 3 shows the FT-IR spectra of *n*-butylamine, a mixture of *n*-butylamine and [BMI][PF_6], and [BMI][PF_6], respectively. The peak associated with the primary amino group of *n*-butylamine shifts slightly to upper field of frequency from 3364 to 3381 cm^{-1}, when 50 wt% of [BMI][PF_6] is added. The FT-IR spectrum of the NCA monomer reveals the stretching vibration (3300 cm^{-1}) corresponding to N–H bond, which shifts significantly to 3416 cm^{-1} in the mixture of the monomer and [BMI][PF_6] (ca. 10 wt% of BLG-NCA), as can be seen in Fig. 4. Furthermore, the C=O stretching bands (1870 and 1783 cm^{-1}) of the anhydride move slightly to 1858 and 1788 cm^{-1}, in addition to the clear shift of the peak attributed to ester carbonyl bond from 1716 to 1734 cm^{-1}, which may be due to the interaction of BLG-NCA with the ionic liquid. These results indicate that the ionic liquid can be interacted with not only the NCA monomer but also the initiator, and these interactions may affect the polymerization behaviors in ionic liquids.

The ^{1}H and ^{13}C NMR analyses were also employed for the evaluation of the interactions between the ionic liquid and the NCA monomer or the ionic liquid and the initiator. Our first attempts using deuterium solvents (DMSO-d_6 and acetone-d_6) were unsuccessful, in which no significant chemical shifts were observed for the NCA monomer and the initiator with the addition of [BMI][PF_6] in the ^{1}H and ^{13}C NMR analyses. Therefore, the NMR measurements were conducted in the absence of the deuterium solvent to avoid the possible influence on the interaction, and the chemical shift was determined on the basis of tetramethylsilane (TMS) added as the internal standard.

As shown in Fig. 5 and Table 3, the lower field chemical shifts of the N–C=O and C=O (152.3 and 170.1 ppm) carbons of the anhydride are clearly visible in ^{13}C NMR spectra of the NCA monomer with the addition of [BMI][PF_6] (153.1 and 171.9 ppm), suggesting that the ionic liquid acts as Lewis

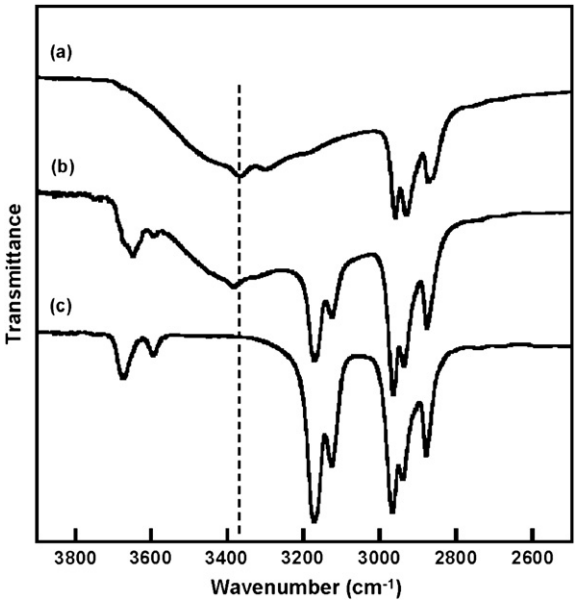


Fig. 3. FT-IR spectra of (a) *n*-butylamine, (b) a mixture of *n*-butylamine and [BMI][PF_6] (ca. 50 wt% of *n*-butylamine), and (c) [BMI][PF_6].

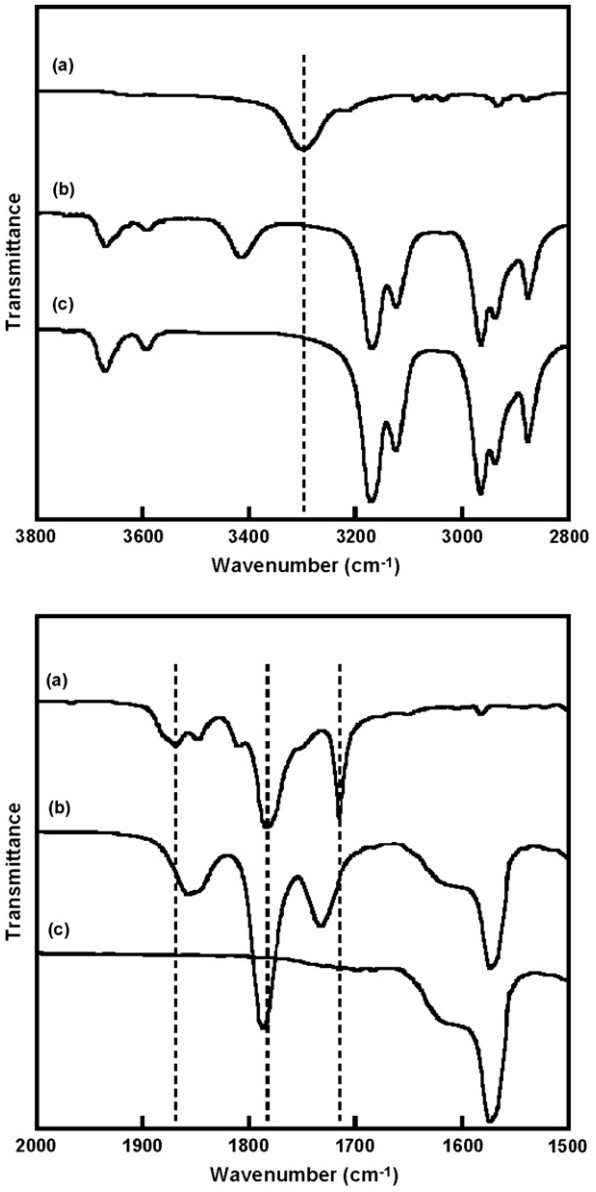


Fig. 4. FT-IR spectra of (a) γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA), (b) a mixture of BLG-NCA and [BMI][PF_6] (ca.10 wt% of BLG-NCA), and (c) [BMI][PF_6]. Upper figure: magnification in the region of amino bond. Lower figure: magnification in the region of carbonyl bond.

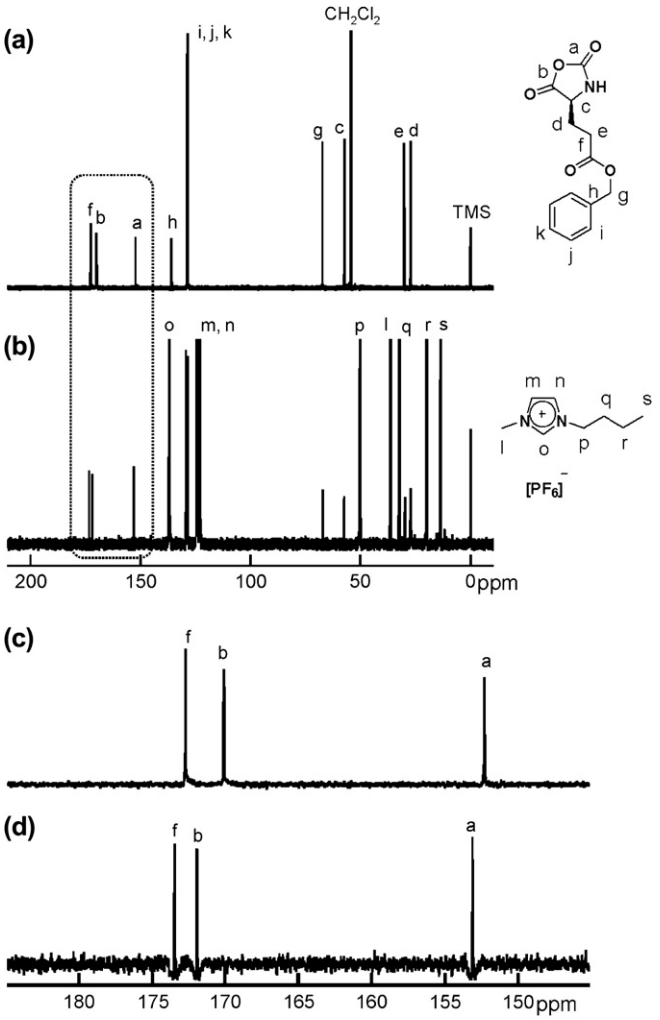


Fig. 5. ^{13}C NMR spectra of (a, c) γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) in CH_2Cl_2 and (b, d) a mixture of BLG-NCA and [BMI][PF_6] (ca. 2.5 mol% of BLG-NCA). Magnification in the range of 145–185 ppm (c, d).

acid under these conditions. It means that the existence of the ionic liquid leads to the increased polarization of the carbonyl group, which is expected to facilitate nucleophilic attack of the primary amine, resulting in the fast initiation of the ring-opening chain growth. This phenomenon may be attributed to the shorter induction period of the polymerization in the ionic liquid than that in the organic solvent, as described in the next section. The change in the chemical shift of the ester carbon from 172.7 to 173.5 ppm is also observed by the addition of the ionic liquid. These results suggest that the ionic liquid has detectable interaction with the ester and anhydride groups of the NCA monomer. Same tendency was also observed in ^1H NMR measurements, as shown in Fig. 6 and Table 4. Judged from the NMR analyses in the presence and absence of deuterium solvents combined with the results of FT-IR measurements mentioned above, it seems reasonable to suggest that the deuterium solvents have some influence on the interactions, which inherently existed between the BLG-NCA and [BMI][PF_6]. This is an indication that the ionic liquid acts simply as a solvent without any interaction with the NCA monomer, when an organic solvent is presented in the system. In contrast, the ionic liquid may affect the polymerization behavior through the interactions in the cases of the polymerization without any organic solvent. Nevertheless, further

Table 3
Changes in the ^{13}C NMR chemical shifts of BLG-NCA with [BMI][PF_6][a]

Group	None (ppm)	[BMI][PF_6] (ppm)	δΔ (ppm)
CH_2–*C*OO	172.72	173.48	+0.75
*C*O–O–CONH	170.07	171.92	+1.84
CO–O–*C*ONH	152.31	153.12	+0.81
O–CH_2–*C*	136.01		
O–CH_2–C_6H_5	128.98, 128.77, 128.61	129.48, 129.11, 128.75	+0.14, +0.34, +0.50
O–*C*H_2–C_6H_5	67.27	67.12	−0.15
NH–*C*H	57.26	57.50	+0.23
CH_2–*C*H_2–COO	30.01	29.81	−0.20
CH_2–CH_2–COO	27.20	27.24	+0.03

[a] Solvent = none; TMS addition; temperature = r.t.; [BLG-NCA]/[ionic liquid] = ca. 0.025.

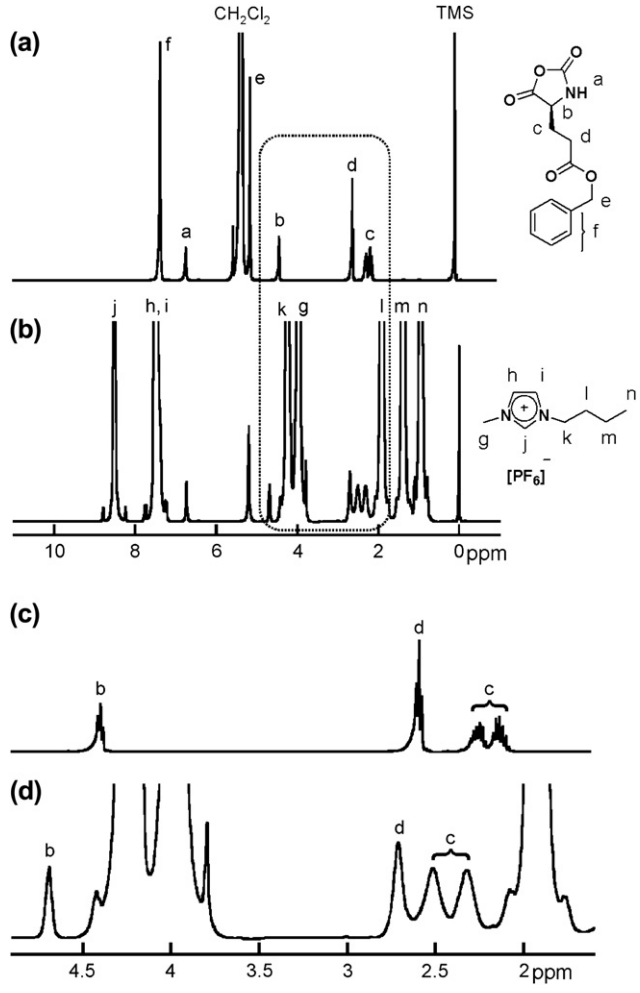


Fig. 6. ^1H NMR spectra of (a, c) γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) in CH_2Cl_2 and (b, d) a mixture of BLG-NCA and [BMI][PF_6] (ca. 2.5 mol% of BLG-NCA). Magnification in the range of 1.5–5.0 ppm (c, d).

Table 4
Changes in the ^{1}H NMR chemical shifts of BLG-NCA with [BMI][PF_6][a]

Group	None (ppm)	[BMI][PF_6] (ppm)	$\delta\Delta$ (ppm)
C_6H_5–CH_2	7.35		
N*H*	6.71	6.73	+0.02
C_6H_5–CH_2–O	5.11	5.20	+0.08
NH–*CH*	4.39	4.68	+0.29
CH_2–COO	2.57	2.71	+0.13
CH_2–CH_2–COO	2.22, 2.11	2.51, 2.31	+0.28, +0.20

[a] Solvent = none; TMS addition; temperature = r.t.; [BLG-NCA]/[ionic liquid] = ca. 0.025.

parameters, such as interaction of the growing polymer chains with the ionic liquid, effects of the concentration, molar ratio of the reagents, and the nature of ionic liquids have to be taken into account for the comprehensive understanding of the interactions in real polymerization system.

3.3. Polymerization kinetics

The character of this polymerization process was studied by performing kinetic investigations. Fig. 7a shows the first-order kinetic plots for the polymerization of BLG-NCA in an ionic liquid ([BMI][PF_6]) and an organic solvent (dioxane/CH_2Cl_2). Approximately linear first-order kinetic plot is seen at the initial stage of the polymerization in the ionic liquid. The first-order kinetic plot is considered to be linear only if the kinetics is first-order with respect to the monomer and the concentration of active species remains constant. Since there is no reason to suspect a higher order dependence of the polymerization rate on monomer conversion, the concentration of the active species is constant at the initial stage of the polymerization in the ionic liquid. The calculated propagation rate constant at $[M]_0 = 0.17\ mol\ L^{-1}$ and $[I]_0 = 0.0034\ mol\ L^{-1}$ is $8.3 \times 10^{-3}\ L\ mol^{-1}\ s^{-1}$, which is comparable to the reported values: $1-8 \times 10^{-3}\ L\ mol^{-1}\ s^{-1}$ for the first slow stage and $1-5 \times 10^{-2}\ L\ mol^{-1}\ s^{-1}$ for the second fast stage [37]. In contrast, an induction period of less than 4 h is seen in the kinetic plot for the polymerization in the mixed organic solvent, followed by fast polymerization. Such two successive stages during the primary amine-initiated polymerization of BLG-NCA in an organic solution were also reported in previous papers, in which the first slow stage was ascribed to the growth of random coils of relatively low molecular weight, whereas the second fast polymerization was attributed to helical growth [37,41,42]. The poly(BLG) having relatively low molecular weight and random coil structure is soluble in MeOH, which was used for the precipitation in our system. Hence, the induction period observed in this study may correspond to the initial stage of the polymerization with virtually no activity for the production of MeOH-insoluble poly(BLG) having sufficient molecular weight and/or helical structure. The induction period roughly estimated simply by extrapolating the linear part of each curve to the time axis is about 3 h in the organic solvent, while it apparently decreases to less than 1.5 h in the ionic liquid. The difference in the induction

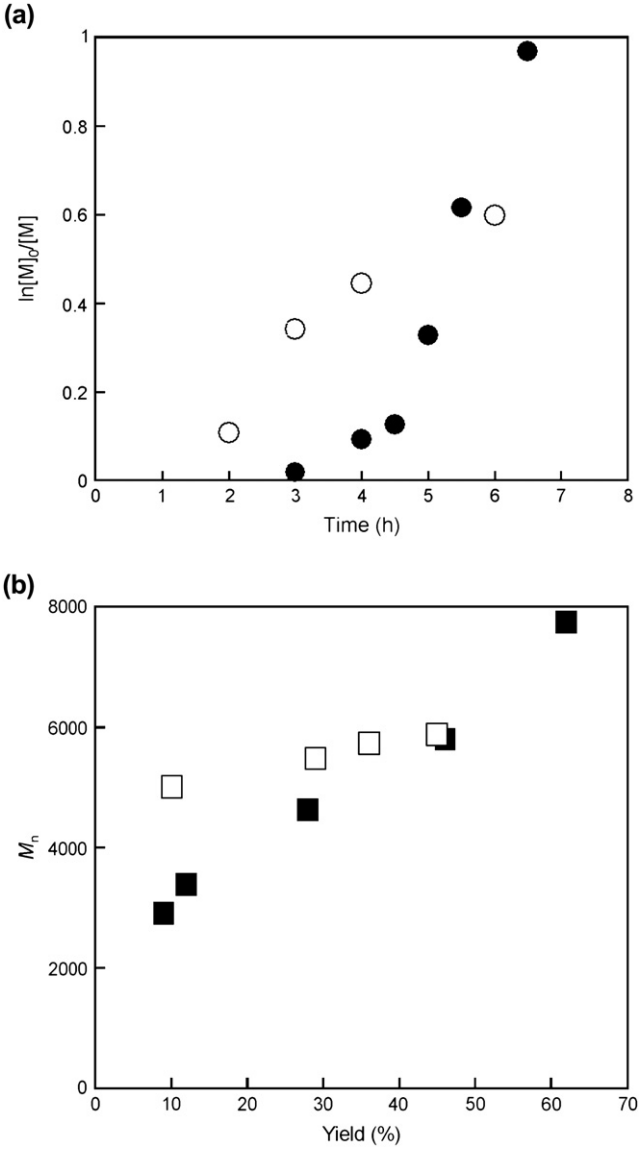


Fig. 7. (a) The first-order kinetic plots and (b) number-average molecular weights as a function of the polymer yield for the polymerizations of BLG-NCA at 30 °C in [BMI][PF_6] (open circles and squares) and CH_2Cl_2/dioxane (3/1 wt%, closed symbols). $[BLG\text{-}NCA]_0/[n\text{-butylamine}]_0 = 50$, and [M] = 0.17 M.

period, depending on the polymerization solvent (the ionic liquid or the organic solvent), may be related to the interaction between the ionic liquid and the NCA monomer, as described in the previous section. The Lewis acid nature of the ionic liquid may lead to the increased polarization of the carbonyl group, ultimately resulting in fast chain growth. Another possible explanation is that the interaction between the ionic liquid and the NCA monomer leads to the formation of poly(BLG) having higher molecular weights and/or preferable helical structure even at the initial stage of the polymerization. Note that the induction period in the polymerization of BLG-NCA in the ionic liquid corresponds roughly to the time when the transparent monomer solution was changed into a turbid dispersion.

The usage of the ionic liquid as a solvent has clear effect on the polymerization rate. Except for the initial stage of the polymerization (until 5 h), the polymerization rate is slower in the ionic liquid than in the organic solvent, as shown in Fig. 7a. The retardation is likely due to the interaction of the ionic liquid with butylamine initiator (or the primary amine existed at the growing polymer chain end). The interaction of the ionic liquid with the primary amine may be also related to the unfavorable activated monomer mechanism. Since the induction period during the polymerization in the ionic liquid is shorter than that in the organic solvent, it seems reasonable to expect that the ionic liquid promotes the addition of the amine to the NCA through the predominant interaction between the ionic liquid and NCA monomer at the initial stage of the polymerization. In the course of the polymerization, the ionic liquid can act as a retardant agent through the interaction between the ionic liquid and the primary amine existed at the growing polymer chain end. The behaviors may be related to the assumption that the ionic liquid affects the exchange process between the ''amine'' and ''activated monomer'' mechanisms occurred during the course of the monomer consumption. The formation of a turbid dispersion is another important factor to affect the behavior of the NCA polymerization in the ionic liquid. Such tendency is different from those of previous studies of free radical polymerizations, in which polymerization of methyl methacrylate occurred at a faster rate when performed in ionic liquids [7,10]. In the cases of the radical polymerization, the increase in propagation rate and decrease in the termination rate were observed, which may be due to the increased polarity of the medium and increased viscosity, respectively. In other words, the results obtained in this study suggest that the polarity and viscosity have no significant influence on the BLG-NCA polymerization in the ionic liquid.

Fig. 8 shows the SEC traces of the poly(BLG)s obtained at different reaction times for the polymerization of BLG-NCA

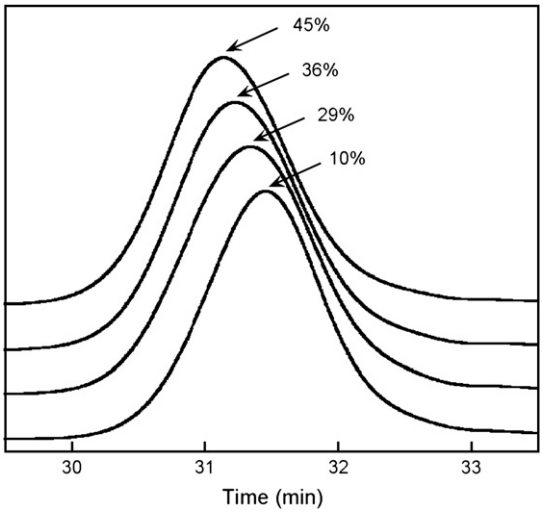


Fig. 8. Evolution of size-exclusion chromatography (SEC) traces with yield for the polymerization of BLG-NCA in [BMI][PF_6].

with *n*-butylamine in the ionic liquid ([BMI][PF_6]). A progressive increase in the molar mass with the polymer yield with the narrow unimodal peaks is clearly seen ($M_w/M_n < 1.2$). Actually, the polymer yields are 10, 29, 36, and 45%, corresponding to the number-average molecular weights, M_n, of 5100 ($M_w/M_n = 1.13$), 5400 ($M_w/M_n = 1.13$), 5600 ($M_w/M_n = 1.14$), and 5900 ($M_w/M_n = 1.13$). As shown in Fig. 7b, M_n increases slightly with the polymer yield, and positive intercept of the M_n vs yield plot is observed in the polymerization in the ionic liquid. Note that the molecular weights obtained by conventional GPC are just the apparent ones due to the use of polystyrene calibration. To clarify this point, NMR technique was used to determine the molecular weight of the polymers, by comparing the integrals of peaks for the chain-end protons to those of the main-chain protons. The ^{1}H NMR spectra of the poly(BLG)s synthesized using *n*-butylamine as an initiator in an ionic liquid and a mixed organic solvent are presented in Fig. 9. The characteristic peaks at 1.8–3.3 (CH_2CH_2COO), 3.8–4.2 (CH), 4.8–5.3 (OCH_2Ph), 7.1–7.6 (aromatic), and 8.1–8.4 ppm (NH) are clearly observed. In addition to these peaks attributed to the poly(BLG), the peaks corresponding to the initiator moiety are also visible at 0.7–1.0 and 1.2–1.5 ppm, which are assigned to methyl and methylene protons of the *n*-butylamino group, respectively. The molecular weight calculated by comparison of the signals at 4.8–5.3 ppm corresponding to the methylene protons in the main chain to the signal at 0.7–1.0 ppm corresponding to three protons of the end group is $M_{n,\ NMR} = 5100$, which is comparable to the theoretical value ($M_{n,\ calcd} = 4900$) and the observed value ($M_{n,\ GPC\ (DMF)} = 5900$). The signals attributed to the end group are clearly seen in poly(BLG) obtained in the mixed organic solvent (Fig. 9b), indicating that the polymer chain end is capped with the *n*-butylamino group as expected according to the amine mechanism (Scheme 2a), regardless of the polymerization solvent. The polymers obtained in the mixed organic solvent at the yield of 28 and 62% had $M_{n,\ NMR} = 4600$, compared to $M_{n,\ calcd} = 3000$, $M_{n,\ GPC\ (DMF)} = 4600$ ($M_w/M_n = 1.51$), and $M_{n,\ NMR} = 7900$, compared to $M_{n,\ calcd} = 6700$, $M_{n,\ GPC\ (DMF)} = 7700$ ($M_w/M_n = 1.39$). At the initial stage of the polymerization, the M_n determined by SEC increases linearly with the polymer yield (Fig. 7b) with slightly broad molecular weight distributions ($M_w/M_n = 1.39$–1.51). In contrast, the molecular weight reached a plateau value in the ionic liquid, which may be due to the formation of a turbid dispersion and/or unfavorable side reactions occurring in the course of the polymerization. These results suggest that the primary amine-initiated polymerizations of BLG-NCA in the ionic liquids used in this study are not perfect ''living'' system, but provide moderate control of the ring-opening polymerization to give poly(BLG)s having relatively narrow polydispersity. Further detailed kinetic investigations, such as the effects of the monomer concentration, the monomer-to-initiator ratio, the nature of the ionic liquids, will be reported separately. An attempt to find suitable ionic liquids to solve poly(amino acid)s is now in progress, which will be required to achieve homogeneous polymerizations of various NCAs.

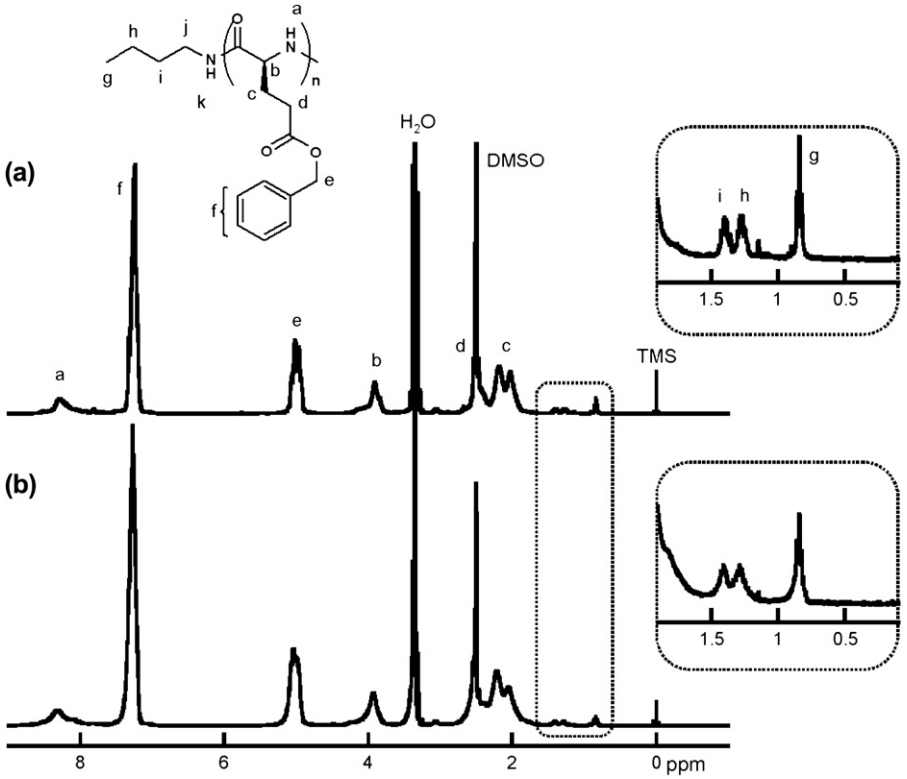


Fig. 9. ^{1}H NMR spectra (DMSO-d_6) of poly(BLG)s obtained by the polymerization of BLG-NCA with *n*-butylamine in (a) [BMI][PF_6] and (b) CH_2Cl_2/dioxane (3/1 wt%).

4. Conclusions

In conclusion, we have shown for the first time that room-temperature imidazolium ionic liquids could be used as a reaction medium for ring-opening polymerization of BLG-NCA. The polymerization with a primary amine in ionic liquids under suitable conditions afforded poly(amino acid)s with low polydispersity and helical secondary structure. The usage of ionic liquid as a solvent led to heterogeneous reaction system by decreasing the polymer yield, whereas it had no remarkable effect on the molecular weights and polydispersities of the resulting poly(BLG)s. The kinetic investigation demonstrated that the polymerization rate was slower in the ionic liquid than in the organic solvent, while the polymerization in the ionic liquid showed shorter induction period than that in the organic solvent. The FT-IR, ^{1}H and ^{13}C NMR analyses indicated the existence of the interactions between the ionic liquid and NCA monomer, as well as ionic liquid and the initiator, which should be related to the polymerization behaviors. Further investigations to clarify the influence of ionic liquid on the polymer properties, such as thermal and mechanical properties, and extend this methodology to other NCAs are now in progress.

References

[1] Welton T. Chemical Reviews 1999;99:2071–83.
[2] Dupont J, de Souza RF, Suarez PAZ. Chemical Reviews 2002;102:3667–91.
[3] Kubisa P. Progress in Polymer Science 2004;29:3–12.
[4] Pinheiro MF, Mauler RS, de Souza RF. Macromolecular Rapid Communications 2001;22:425–8.
[5] Wasserscheid P, Hilgers C, Gordon CM, Muldoon MJ, Dunkin IR. Chemical Communications (Cambridge) 2001;1186–7.
[6] Carmichael AJ, Haddleton DM, Bon SAF, Seddon KR. Chemical Communications (Cambridge) 2000;1237–8.
[7] Hong K, Zhang H, Mays JW, Visser AE, Brazel CS, Holbrey JD, et al. Chemical Communications (Cambridge) 2002;1368–9.
[8] Perrier S, Davis TP, Carmichael AJ, Haddleton DM. Chemical Communications (Cambridge) 2002;2226–7.
[9] Strehmel V, Laschewsky A, Wetzel H, Gornitz E. Macromolecules 2006;39:923–30.
[10] Harrisson S, Mackenzie SR, Haddleton DM. Chemical Communications (Cambridge) 2002;2850–1.
[11] Harrisson S, Mackenzie SR, Haddleton DM. Macromolecules 2003;36:5072–5.
[12] Vijayaraghavan R, MacFarlane DR. Chemical Communications (Cambridge) 2004;700–1.
[13] Vijayaraghavan R, MacFarlane DR. Chemical Communications (Cambridge) 2005;1149–51.
[14] Nara SJ, Harjani JR, Salunkhe MM, Mane AT, Wadgaonkar PP. Tetrahedron Letters 2003;44:1371–3.
[15] Sekiguchi K, Atobe M, Fuchigami T. Journal of Electroanalytical Chemistry 2003;557:1–7.
[16] Pringle JM, Forsyth M, MacFarlane DR, Wagner K, Hall SB, Officer DL. Polymer 2005;46:2047–58.
[17] Vygodskii YS, Lozinskaya EL, Shaplov AS. Macromolecular Rapid Communications 2002;23:676–80.
[18] Vygodskii YS, Lozinskaya EI, Shaplov AS, Lyssenko KA, Antipin MY, Urman YG. Polymer 2004;45:5031–45.
[19] Biedron T, Bednarek M, Kubisa P. Macromolecular Rapid Communications 2004;25:878–81.

[20] Kadokawa J, Iwasaki Y, Tagaya H. Macromolecular Rapid Communications 2002;23:757–60.
[21] Nyce GW, Glauser T, Connor EF, Möck A, Waymouth RM, Hedrick JL. Journal of the American Chemical Society 2003;125:3046–56.
[22] Guerrero-Sanchez C, Lobert M, Hoogenboom R, Schubert US. Macromolecular Rapid Communications 2007;28:456–64.
[23] Bellomo EG, Wyrsta MD, Pakstis L, Pochan DJ, Deming TJ. Nature Materials 2004;3:244–8.
[24] Yoshida T, Aoyagi T, Kokufuta E, Okano T. Journal of Polymer Science: Part A: Polymer Chemistry 2003;41:779–87.
[25] Fukushima S, Miyata K, Nishiyama N, Kanayama N, Yamasaki Y, Kataoka K. Journal of the American Chemical Society 2005;127: 2810–1.
[26] Klok H-A, Langenwalter JF, Lecommandoux S. Macromolecules 2000;33:7819–26.
[27] Schlaad H, Kukula H, Smarsly B, Antonietti M, Pakula T. Polymer 2002;43:5321–8.
[28] Floudas G, Papadopoulos P, Klok H-A, Vandermeulen GWM, Rodriguez-Hernandez J. Macromolecules 2003;36:3673–83.
[29] Kricheldorf HR. Model of biopolymers by ring-opening polymerization. Boca Raton, FL: CRS Press; 1990.
[30] Deming TJ. Advanced Materials 1997;9:299–311.
[31] Deming TJ. Journal of Polymer Science: Part A: Polymer Chemistry 2000;38:3011–8.
[32] Yu SM, Conticello VP, Zhang G, Kayser C, Fournier MJ, Mason TL, et al. Nature 1997;389:167–70.
[33] Daly WH, Poché D. Tetrahedron Letters 1988;29:5859–62.
[34] Biedron T, Kubisa P. Macromolecular Rapid Communications 2001;22: 1237–42.
[35] Aliferis T, Iatrou H, Hadjichristidis N. Biomacromolecules 2004;5: 1653–6.
[36] Kricheldorf HR, von Lossow C, Schwarz G. Macromolecules 2005;38: 5513–8.
[37] Thunig D, Semen J, Elias H-G. Makromolekulare Chemie 1977;178: 603–7.
[38] Rahman SAE, Anzinger H, Mutter M. Biopolymers 1980;19:173–87.
[39] Kricheldorf HR, Müller D. Makromolekulare Chemie 1983;184:1407–21.
[40] Kubelka J, Keiderling TA. Journal of the American Chemical Society 2001;123:6142–50.
[41] Doty P, Lundberg RD. Journal of the American Chemical Society 1956;78:4810–2.
[42] Lundberg RD, Doty P. Journal of the American Chemical Society 1957;79:3961–72.