Synthesis and Property of Novel Amino Acid-Based Polymers by Self-Polyaddition of Monomers Containing both Oxetanyl and Carboxyl Groups

Hiroto Kudo,[1] Toshiki Ohshiro,[1] Hiroyuki Atsumi,[1] Ayako Kamio,[1] Tadatomi Nishikubo,[1] Saeko Murakami[2]

[1]Department of Materials and Life Chemistry, Faculty of Engineering, Kanagawa University, 3-27-1, Rokkakubashi, Kanagawa-ku, Yokohama 221-8686, Japan
[2]Kanagawa Industrial Technology Center, 705-1, Shimoimaizumi, Ebina-Shi, Kanagawa 243-0435, Japan
Correspondence to: T. Nishikubo (E-mail: nishikubot@kanagawa-u.ac.jp)

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ABSTRACT: A new synthetic strategy for polymers containing amino acids in the main chain was developed. Monomers *N*-oxetanylalanine (*N*-Oxe-Ala-COOH), *N*-oxetanylglutamic acid (*N*-Oxe-Glu-COOH), and *N*-oxetanyllysine (*N*-Oxe-Lys-COOH) containing both oxetanyl and carboxyl groups were synthesized, and self-polyaddition and self-copolyaddition of these monomers afforded the corresponding polymers containing amino acids in the main chain [poly($_{Ox}$Ala), poly($_{Ox}$Lys), poly ($_{Ox}$Glu), poly($_{Ox}$Ala-*co*-$_{Ox}$Glu), poly($_{Ox}$Glu-*co*-$_{Ox}$Lys), and poly ($_{Ox}$Ala-*co*-$_{Ox}$Lys)] with molecular weight in the range of 920–6620, in satisfactory yields. The physical properties, such as solubility, glass transition temperature, and thermal stability, were consistent with the amount of carboxyl groups at the chain ends. Biodegradability of the polymers was examined by the biochemical oxygen demand method; the decomposition ratios of poly($_{Ox}$Ala) and poly($_{Ox}$Ala-*co*-$_{Ox}$Glu) were about 60%, whereas that of poly($_{Ox}$Glu) was nearly 100% after 28 days. © 2011 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 50: 458–465, 2012

KEYWORDS: amino acid; biodegradable; hyperbranched; self-polyaddition; synthesis

INTRODUCTION Amino acid-based polymers, that is, poly (amino acid)s, are commonly synthesized by means of anionic ring-opening polymerization of *N*-carboxyanhydrides (NCAs) derived from amino acids.[1] Deming[2] reported new living polymerization of NCA by the transition metal initiators system using cobalt or iron and developed the functional well-defined block copolypeptides.[3] However, this synthetic strategy has some problems. NCAs are usually synthesized via the noncatalytic reaction of amino acids with phosgene in an aprotic solvent, and the reaction proceeds with release of HCl gas. As phosgene (triphosgene) and HCl are both poisonous, great care is needed. Bases such as pyridine and triethylamine cannot be used to trap HCl *in situ* during the synthesis, because NCAs are highly reactive and readily polymerize in the presence of weak bases or H_2O as initiators, that is, NCAs are not easy to handle.

Sanda and coworkers[4] reported various polymers containing amino acids in the side chain and showed that the secondary structure of the obtained polymers could be controlled. Furthermore, they succeeded in developing an alternating ring-opening metathesis copolymerization by using the interaction between acid and base moieties.[5] Ferruti et al.[6] reported the certain polymers containing amino acid in their main chain by the polyaddition of amines and acrylamides and showed that physicochemical and biological properties of the obtained polymers were examined. Endo and coworkers[7] reported the synthesis of various polymers containing amino acid moieties in the main or side chains using a wide variety of polymerization methods and suggested that the products could find application as optically active, biocompatible, or biodegradable materials. Recently, they also reported that NCAs could be synthesized through *N*-phenylcarbamate amino acids as intermediates, that is, NCAs could be synthesized without the use of phosgene.[8] In spite of these attempts at the synthesis of amino acid-based polymers, NCA is still the only general monomer for the synthesis of amino acid-based polymers. This is presumably because direct condensation of amino acids is unsatisfactory to obtain the corresponding polymers, and only a cyclic compound, 2,5-piperazinedione, is formed selectively.

We have examined the synthesis and self-polyaddition of monomers containing both oxetanyl and carboxyl groups.[9] The self-polyaddition proceeded in the presence of onium salts as catalysts to give the corresponding linear hetero-

telechelic polymers with oxetanyl and carboxyl groups at the chain ends, with satisfactory molecular weights and yields. As the reaction of oxetane and carboxylic acid can proceed only in the presence of quaternary onium salts as catalysts, the monomers containing both oxetanyl and carboxyl groups are stable and easy to handle. In the course of our study on the self-polyaddition, we considered that these monomers might be suitable for developing a general synthetic strategy for amino acid-based polymers.

In this article, we examined the synthesis and self-polyaddition reaction of oxetanyl and carboxyl functionalized monomers derived from alanine, glutamic acid, and lysine. Furthermore, the biodegradability of the products was examined.

EXPERIMENTAL

Measurements

Infrared (IR) spectra were measured on a Thermo Electron Model NICOLET 380 spectrometer. The ^{1}H NMR spectra were recorded on JEOL Model JNM α-500 (500 MHz for ^{1}H NMR) instruments in DMSO-d_6 and $CDCl_3$ using Me_4Si (TMS) as an internal standard reagent for ^{1}H NMR. The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) of the polymers were estimated by size-exclusion chromatography (SEC) with a Tosoh model HLC-8120 GPC equipped with refractive index and ultraviolet detectors and TSK gel columns (eluent dimethylformamide (DMF), calibrated with narrow-molecular weight polystyrene standards). Thermal analysis was performed on a Seiko Instruments thermogravimetric analyzer Model EXSTAR6000/TG/DTA6200 and differential scanning calorimetry Model EXSTAR6000/DSC6200 at a heating rate of 10 °C/min under nitrogen.

Materials

1-Methyl-2-pyrrolidinone was dried over CaH_2 and purified in the usual way before use. 3-Ethyl-3-hydroxymethyloxetane (EHO) was a gift from Toa Gosei Company.

Jones reagent was prepared from a mixture of CrO_3 (3.80 g) and concentrated H_2SO_4 (3.25 mL) in H_2O (19.7 mL) according to the usual method.[10] 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC HCl), isopropyl alcohol, NEt_3, CH_2Cl_2, L-alanine methyl ester hydrochloride, L-glutamic acid dimethyl ester hydrochloride, L-lysine methyl ester hydrochloride, and tetraphenylphosphonium bromide (TPPB) were commercial-grade products and were used without further purification. *o*-Dichlorobenzene was distilled over P_2O_5 before use.

Synthesis of 3-Carboxy-3-ethyloxetane

A solution of Jones reagent (22.8 mL) and acetone (180 mL) was stirred at 0 °C. After 1 h, a solution of EHO (2.78 g, 24 mmol) in acetone (180 mL) was added slowly and stirring was continued at −5 °C for 2 h, then at 25 °C. After 4 h, isopropyl alcohol (5 mL) was added to the mixture at below 0 °C. The resulting suspension was filtered through Celite to remove insoluble products, and the filtrate was concentrated in a rotary evaporator. Then, NaOH aqueous (6 M) (300 mL) was added to the residue, and the resulting mixture was washed with ethyl acetate (300 mL) thrice. The aqueous phase was separated and acidified to pH = 3.0 with concentrated H_2SO_4. The resulting aqueous mixture was extracted with ethyl acetate (300 mL) five times. The organic phase was dried over $MgSO_4$ and concentrated in a rotary evaporator. The residue was purified by recrystallization from ethyl acetate at −20 °C to obtain 3-carboxy-3-ethyloxetane (CEO) as a colorless solid.

Yield = 2.41 g (86%). Mp = 31.0–31.9 °C. IR (film, cm^{-1}): 3400 (ν COOH), 2886 (ν CH_2), 1724 (ν C=O carbonyl), 970 (ν C—O—C of oxetane). ^{1}H NMR (600 MHz, DMSO-d_6, TMS) δ (ppm): 0.83 (t, J = 7.2 Hz, 3.0H, —CH_3), 1.93 (q, J = 7.2 Hz, 2.0H, —CH_2—), 4.33 and 4.70 (AB quartet, J = 6.0 Hz, 4.0H, —CH_2— of cyclic ether), 12.64 (s, 1.0H, —COOH). ^{13}C NMR (150 MHz, DMSO-d_6, TMS) δ (ppm): 8.76 (CH_3), 28.3 (—CH_2CH_3), 48.4 (>C<), 76.9 (—O—CH_2—), 175.2 (>C=O). Elemental analysis data for CEO. Calcd. for $C_{14}H_{20}O_5$ C, 55.37; H, 7.74. Found: C, 55.32; H, 7.91.

Synthesis of Monomers Containing both Oxetanyl and Carboxyl Groups

Synthesis of N-Oxetanylalanine Methyl Ester (N-Oxe-Ala-COOMe)

EDC HCl (5.75 g, 30 mmol) was added to a solution of CEO (3.52 g, 27 mmol) in CH_2Cl_2 (27 mL) at 0 °C. The resulting solution was stirred for 2 h. NEt_3 (4.17 mL, 30 mmol) and L-alanine methyl ester hydrochloride (4.19 g, 30 mmol) were added, and the mixture was stirred at 25 °C for 24 h, then washed successively with 1 N hydrogen chloride solution, saturated aqueous sodium hydrogen carbonate, and water. The organic phase was dried over $MgSO_4$ and concentrated in a rotary evaporator to obtain *N*-oxetanylalanine methyl ester (*N*-Oxe-Ala-COOMe) as a colorless oil, which was used for the next step without further purification.

Yield = 2.46 g (42%). IR (KRS, cm^{-1}): 3306 (ν NH), 2965 and 2880 (ν CH_2CH_3), 1746 (ν C=O of ester), 1650 (ν C=O of amide), 982 (ν C—O—C of oxetane). ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 0.78 (t, J = 7.5 Hz, 3.0H, —CH_3), 1.29 (d, J = 7.5 Hz, 2.0H, —CH_2—), 1.95 (q, J = 7.5 Hz, 2.0H, —CH_2CH_3), 3.63 (s, 3.0H, —OCH_3), 4.21–4.24 (m, 1.0H, >CH—), 4.27 and 4.66 (AB quartet, 4.0H, —O—CH_2—), 8.20 (s, 1.0H, —NH—).

Synthesis of N-Oxetanylalanine (N-Oxe-Ala-COOH)

A solution of *N*-Oxe-Ala-COOMe (2.46 g, 11.42 mmol) in 6 M NaOH (13 mL) was stirred at 25 °C. After 12 h, the aqueous solution was acidified to pH 3.0 with concentrated HCl. The resulting mixture was extracted with ethyl acetate (100 mL) 10 times. The organic phase was dried over $MgSO_4$ and concentrated in a rotary evaporator. The residue was poured into large amount of ethyl ether to precipitate *N*-Oxe-Ala-COOH as a white solid.

Yield = 1.33 g (54%). Mp = 146.0–146.6 °C. IR (KRS, cm^{-1}): 3299 (ν NH), 1731 (ν C=O), 1616 (ν C=O of amide),1220 and 1186 (ν C—O—C), 968 (ν cyclic ether). ^{1}H NMR (600 MHz, DMSO-d_6, TMS) δ (ppm): 0.77 (t, J = 6.5 Hz, 3.0H, —CH_3), 1.26 (d, J = 6.5 Hz, 3.0H, >CHCH_3), 1.92 (q, J = 6.5

Hz, 2.0H, —CH_2—), 4.25–4.65 (m, 5.0H, —O—CH_2— and >CH—), 8.07 (s, 1.0H, —NH—), 12.04 (s, 1.0H, —COOH). ^{13}C NMR (150 MHz, DMSO-d_6, TMS) δ (ppm): 8.49 (—CH_2CH_3), 16.93 (>CH—CH_3), 29.37 (—CH_2CH_3), 47.57 (quaternary carbon of cyclic ether), 48.96 (quaternary carbon of alanine), 77.27 (—OCH_2—), 174.21 (—COOH), 181.2 (—CONH—). Elemental analysis data for *N*-Oxe-Ala-COOH. Calcd. for $C_9H_{15}NO_4$ C, 53.72; H, 7.51; N, 6.96. Found: C, 53.48; H, 7.43; N, 6.71.

Synthesis of N-Oxetanylglutamic Acid Methyl Ester (N-Oxe-Glu-COOMe)

N-Oxe-Glu-COOMe was synthesized by the same method as described for the synthesis of *N*-Oxe-Ala-COOMe, but using L-glutamic acid dimethyl ester hydrochloride.

Yield = 47%. IR (KRS, cm^{-1}): 3308 (ν NH), 2,959 and 2,880 (ν CH_2CH_3), 1,741 (ν C=O of ester), 1,653 (ν C=O of amide), 983 (ν C—O—C of oxetane). ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 0.78 (t, J = 7.5 Hz, 3.0H, —CH_3), 1.95–2.03 (m, 4.0H, —CH_2—), 2.40 (q, J = 7.5 Hz, 2.0H, —CH_2CH_3), 3.63 (s, 1.0H, >CH—), 4.28 and 4.67 (AB quartet, 4.0H, —O—CH_2—), 8.40 (s, 1.0H, —NH—).

Synthesis of N-Oxetanylglutamic Acid (N-Oxe-Glu-COOH)

N-Oxe-Glu-COOH was synthesized by the same method as described for the synthesis of *N*-Oxe-Ala-COOH, but using *N*-Oxe-Glu-COOMe.

Yield = 92%. Mp = 144.3–145.6 °C. IR (KRS, cm^{-1}): 3,343 (ν NH), 2,968 (ν C—H), 1,732 (ν C=O of ester), 1,615 (ν C=O of amide), 1,220 and 1,186 (ν C—O—C), 968 (ν cyclic ether). ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 0.78 (t, J = 7.5 Hz, 3.0H, —CH_3), 1.81–1.94 (m, 4.0H, —CH_2CH_2—), 2.40 (q, 2.0H, —CH_2CH_3), 4.21–4.24 (m, 1.0H, >CH—), 4.27 and 4.67 (AB quartet, 4.0H, —O—CH_2—), 8.07 (s, 1.0H, —NH—), 12.40 (s, 2.0H, —COOH). ^{13}C NMR (150 MHz, DMSO-d_6, TMS) δ (ppm): 8.52 (—CH_2CH_3), 29.42 (—CH_2CH_3), 25.98 and 30.27 (—CH_2CH_2—), 49.13 (quaternary carbon of cyclic ether), 51.22 (quaternary carbon of alanine), 77.34 (—OCH_2—), 172.44 (>CHCOOH), 173.82 (—CH_2COOH), 173.85 (—CONH—). Elemental analysis data for *N*-Oxe-Glu-COOH. Calcd. for $C_{11}H_{17}NO_6$ C, 50.96; H, 6.61; N, 5.40. Found: C, 50.94; H, 6.34; N, 5.11.

Synthesis of N-Oxetanyllysine Methyl Ester (N-Oxe-Lys-COOMe)

N-Oxe-Lys-COOMe was synthesized by the same method as described for the synthesis of *N*-Oxe-Ala-COOMe, but using L-lysine methyl ester hydrochloride.

Yield = 28%. IR (KRS, cm^{-1}): 3305 (ν NH), 2969 and 2888 (ν CH_2CH_3), 1724 (ν C=O of ester), 1649 (ν C=O of amide), 982 (ν C—O—C of oxetane). ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 0.76 (t, J = 7.5 Hz, 6.0H, —CH_3), 1.27–1.40 (m, 4.0H, —CH_2CH_2—CH<), 1.61–1.73 (m, 2.0H, —$NHCH_2CH_2$—), 1.94 (q, J = 6.0 Hz, 4.0H, —CH_2CH_3), 3.06 (q, J = 4.0 Hz, 2.0H, —NH—CH_2—), 3.61 (s, 3.0H, —OCH_3), 4.25 and 4.64 (AB quartet, 4.0H, —O—CH_2—), 5.74 (s, 1.0H, >CH—), 7.77 (d, J = 5.5 Hz, 1.0H, —C(O)NH—), 8.11(d, J = 5.5 Hz, 7.5H, —C(O)NH—).

Synthesis of N-Oxetanyllysine (N-Oxe-Lys-COOH)

N-Oxe-Lys-COOH was synthesized by the same method as described for the synthesis of *N*-Oxe-Ala-COOH, but using *N*-Oxe-Lys-COOMe.

Yield = 76% (viscous oil). IR (KRS, cm^{-1}): 3,305 (ν NH), 2,969 and 2,888 (ν C—H), 1,724 (ν C=O of ester), 1649 (ν C=O of amide), 982 (ν cyclic ether). ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ (ppm): 0.76 (t, J = 7.5 Hz, 6.0H, —CH_3), 1.27–1.42 (m, 4.0H, —CH_2CH_2—CH<), 1.54–1.76 (m, 2.0H, —$NHCH_2CH_2$—), 1.96 (q, J = 7.5 Hz, 4.0H, —CH_2CH_3), 3.06 (t, J = 4.0 Hz, 2.0H, —NH—CH_2—), 4.18 (q, J = 5.0 Hz, 1.0H, >CH—), 4.25 and 4.64 (AB quartet, 8.0H, —O—CH_2—), 5.74 (s, 1.0H, >CH—), 7.77 (t, J = 4.0 Hz, 1.0H, —C(O)NH—), 8.11(d, J = 5.0 Hz, 1.0H, —C(O)NH—), 12.4 (s, 1.0H, —COOH). ^{13}C NMR (150 MHz, DMSO-d_6, TMS) δ (ppm): 8.48 and 8.63 (CH_3—), 23.13 (CH_3CH_2—), 28.66, 29.40, and 38.37 (—C_4H_8—), 49.1 (quaternary carbon), 51.80 (>CH—), 77.30 and 79.20 (—CH_2O—), 173.24 (—COOH), and 173.73 and 173.81 (—CONH—). matrix assisted Laser desorption and ionization time of flight mass spectroscopy (MALDI-TOF MS) m/z $(M + Na)^+$ Calcd. for ($C_{18}H_{30}N_2O_6$ + Na): 393.54. Found: 393.43.

Self-Polyaddition of N-Oxe-Ala-COOH

A typical procedure for the self-polyaddition of *N*-Oxe-Ala-COOH was as follows: *N*-Oxe-Ala-COOH (0.1 g, 0.5 mmol), TPPB (0.021 g, 0.05 mmol), and *o*-dichlorobenzene (0.5 mL) were charged into a glass tube, which was evacuated and sealed using a gas torch. The reaction was performed at 130 °C for 24 h in the sealed tube with stirring. The polymer solution was then poured into a large amount of ethyl ether to precipitate poly($_{ox}$Ala), which was dried *in vacuo* at room temperature for 24 h. The yield of poly($_{ox}$Ala) was 0.089 g (89%). The number-average molecular weight (M_n) of the polymer estimated by SEC was 5100 (M_w/M_n = 1.70).

IR (KRS, cm^{-1}): 3,374 (ν O—H), 3,340 (ν —NH—), 1740 (ν C=O ester), 1,226 (ν C—O—C ester). ^{1}H NMR (600 MHz, DMSO-d_6, TMS) δ (ppm): 0.75 (s, —CH_2CH_3), 1.25 (s, >$CHCH_3$), 1.47–1.53 (m, —CH_2CH_3), 3.49–3.60 (m, >CH—), 4.03–4.26 (m, —CH_2OH and —$COOCH_2$—), 4.65 (s, —OH), 7.81–8.16 (m, —NH—).

Self-Polyaddition of N-Oxe-Glu-COOH

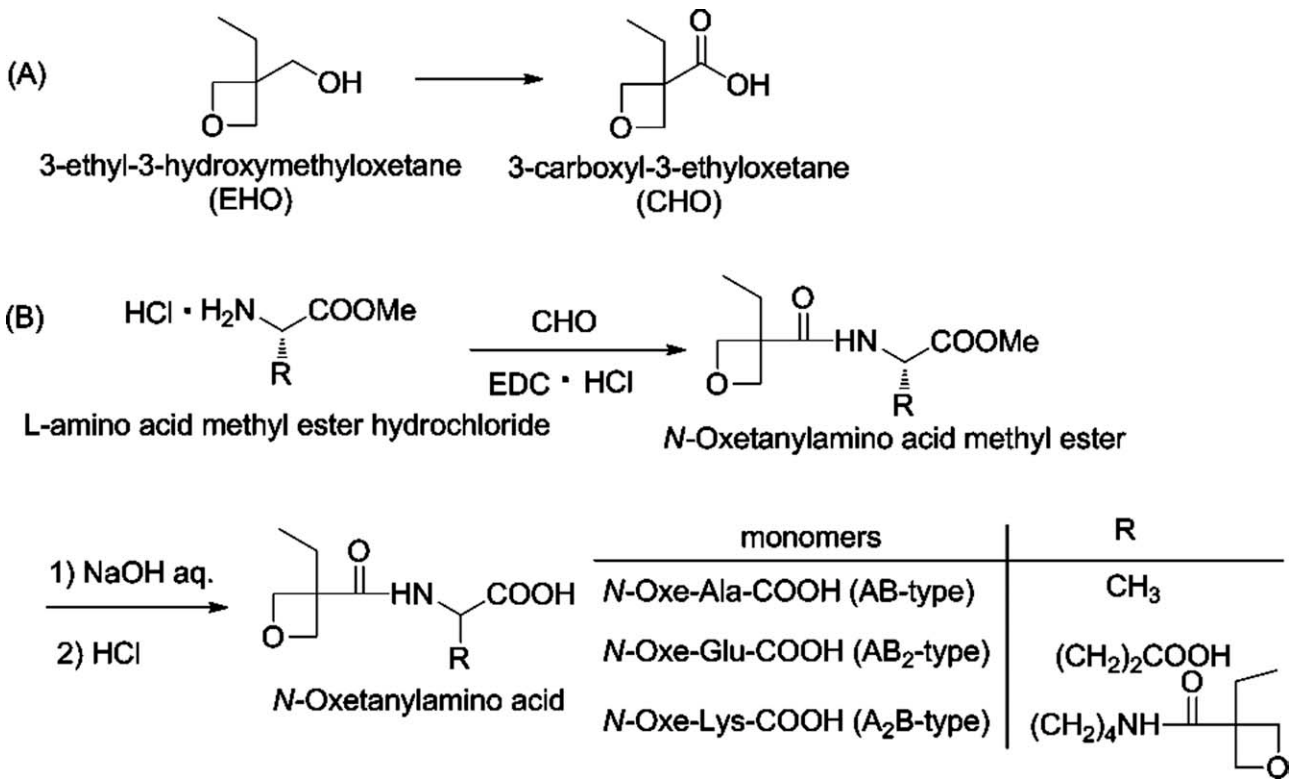
The self-polyaddition of *N*-Oxe-Glu-COOH was performed by the same method as described for *N*-Oxe-Ala-COOH. Yield of poly($_{ox}$Glu) was 85%. Poly($_{ox}$Glu) was insoluble in common organic solvents, and SEC and NMR measurements could not be done.

IR (KBr, cm^{-1}): 3420 (ν O—H), 2,974 (ν —CH—), 1,742 (ν C=O ester), 1,651 (ν C=O amide), 1,206 (ν C—O—C ester).

Self-Polyaddition of N-Oxe-Lys-COOH

The self-polyaddition of *N*-Oxe-Lys-COOH was performed by the same method as described for *N*-Oxe-Ala-COOH. The yield of poly($_{ox}$Lys) was 89%. M_n = 6620, M_w/M_n = 3.81.

IR (KBr, cm^{-1}): 3,345 (ν O—H), 2,965 and 2,937 (ν —CH—), 1,740 (ν C=O ester), 1,650 (ν C=O amide), 1,267 (ν C—O—C ester), 980 (ν C—O—C oxetane). ^{1}H NMR (600 MHz, DMSO-d_6,



SCHEME 1 Synthesis of *N*-oxetanylamino acids.

TMS) δ (ppm): 0.76 (broad s, —CH_2CH_3), 1.27–1.98 (m, >$CHCH_2$—CH_2—CH_2—CH_2—NH—, and —CH_2CH_3), 3.06 (broad s, >$CHCH_2$—), 3.43–3.58 (m, >CH—), 4.15–4.28 (C(=O)OCH_2— and —CH_2OH), 4.64 (s, —CH_2OH), 7.71–8.07 (m, —NH—).

Biodegradability of the Polymers

The biodegradability of the synthesized polymers was examined by the biochemical oxygen demand (BOD) method using activated sludge according to the Japanese Industrial Standards method, as follows. A mixture of polymer (100 mg) and activated sludge (30 mg) in a culture medium was stirred slowly at 25 °C for 28 days. The value of biodegradability (%) was calculated as the ratio of the values of BOD and theoretical oxygen demand.

RESULTS AND DISCUSSION

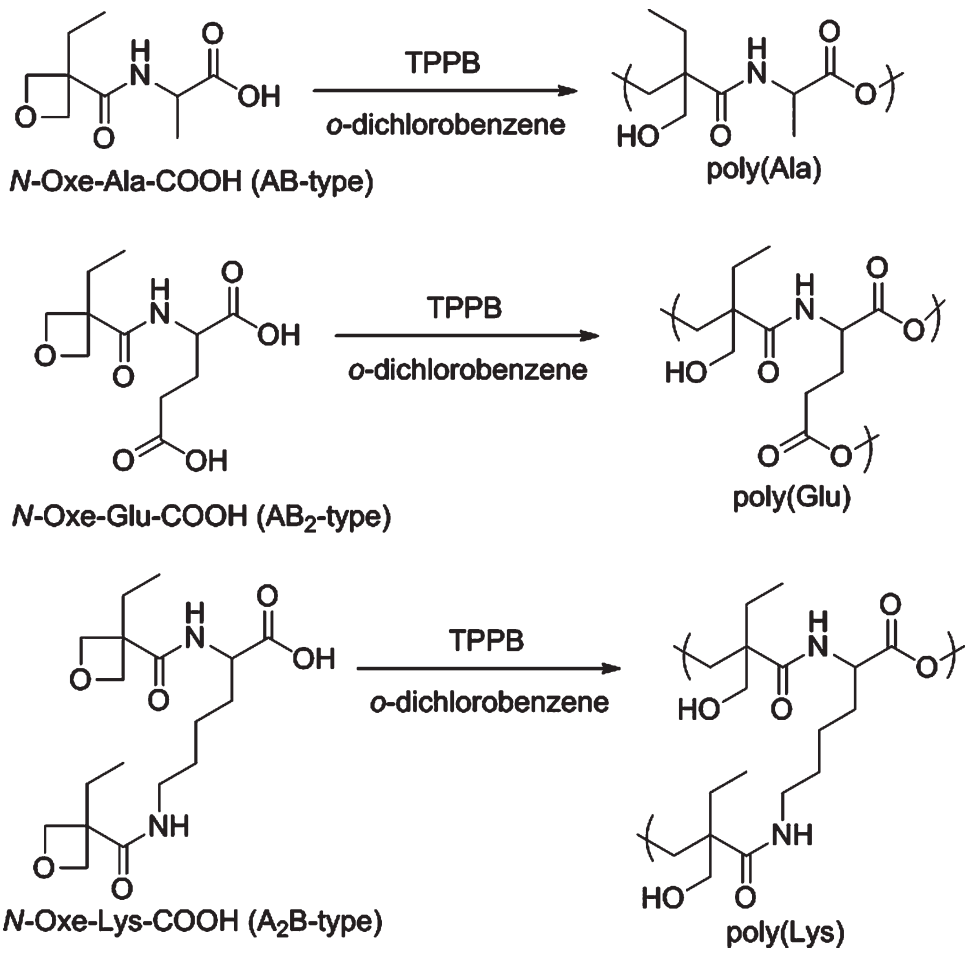
Monomer Synthesis

Careful design is needed to synthesize monomers containing two different reactive groups, which can undergo self-polyaddition. Here, we employed *N*-oxetanylamino acids as monomers, because the reaction of oxetane and carboxylic acid to afford the corresponding polyester can only proceed in the presence of an appropriate catalyst.[11] We carried out the oxidation reaction of EHO using Jones reagent. When this reaction was carried out using H_2SO_4, only hydroxyl groups of EHO were converted to carboxyl groups, and the oxetane moiety remained intact, that is, the corresponding 3-carboxyl-3-ethyloxetane (CEO) could be synthesized. Next, the reaction of CEO and amino acid methyl ester hydrochloride was carried out using EDC HCl as a condensation agent, affording the *N*-oxetanylamino acid methyl ester. The corresponding products were treated with alkali aqueous solution to give the amino acid derivatives containing both oxetanyl and carboxyl groups, as shown in Scheme 1. The structures of the synthesized amino acid derivatives were confirmed by IR, ^{1}H NMR, and ^{13}C NMR spectroscopy, as well as elemental analysis or MALDI-TOF mass spectrometry.

In the case of L-alanine methyl ester hydrochloride, the corresponding *N*-oxetanylalanine (*N*-Oxe-Ala-COOH) containing an oxetanyl group and a carboxyl group could be synthesized. *N*-Oxe-Glu-COOH, containing an oxetanyl group and two carboxyl groups, and *N*-Oxe-Lys-COOH, containing two oxetanyl groups and a carboxyl group, were similarly synthesized from glutamic acid and lysine, respectively. These amino acid derivatives can be regarded as AB, ABB′, and A_2B type monomers for self-polyaddition.

Self-Polyaddition

In our previous report,[9] the self-polyaddition of monomers containing both oxetanyl and carboxyl groups proceeded using onium salts as catalysts, affording hetero telechelic polyesters with pendant primary hydroxyl groups in the side chains, and oxetanyl and carboxyl groups at the chain ends. In this system, TPPB showed the highest catalytic activity among the quaternary onium salts in *o*-dichlorobenzene as a reaction solvent, and the reaction did not occur in the absence of quaternary onium salt. This means that the monomers containing both oxetanyl and carboxyl groups are stable at room temperature, so they are easy to handle and should be useful for self-polyaddition. This time, we examined the self-polyaddition and



SCHEME 2 Self-polyaddition of *N*-oxetanylamino acids.

self-copolyaddition of *N*-Oxe-Ala-COOH, *N*-Oxe-Glu-COOH, and *N*-Oxe-Lys-COOH using TPPB as a catalyst in *o*-dichlorobenzene at 130 °C for 48 h (Scheme 2). Table 1 summarizes the reaction conditions and results.

In the case of *N*-Oxe-Ala-COOH, polymer with $M_n = 5100$ ($M_w/M_n = 1.70$) was obtained. Its structure was confirmed by IR and ^{1}H NMR spectroscopy. In the IR spectrum, a peak at 982 cm^{-1} assignable to the oxetane moiety disappeared, and new peaks at 3374 cm^{-1} and 1740 cm^{-1} assignable to hydroxyl group and carbonyl group of an ester moiety appeared after the reaction. Figure 1 depicts the ^{1}H NMR spectra before and after the self-polyaddition of *N*-Oxe-Ala-COOH: the peaks at 4.28 and 4.68 ppm assignable to the oxetane moiety disappeared, and we observed new peaks at 4.03–4.26 ppm and 4.65 ppm, which are assignable to methylene and hydroxyl protons of the polymer formed by addition reaction of the oxetanyl and carboxyl groups. These findings indicate that the self-polyaddition of *N*-Oxe-Ala-COOH proceeded to afford poly($_{Ox}$Ala) with pendant hydroxyl groups and alanine moieties in the main chain, as shown in Scheme 2. The yield was 89%.

TABLE 1 Self-Polyaddition of *N*-Oxe-Amino Acids[a]

Run	Monomer	Yield[b] (%)	M_n[c]	M_w/M_n[c]	T_g[d] (°C)	T_d[e] (°C)
1	*N*-Oxe-Ala-COOH (AB type)	89	5,100	1.70	75	289
2	*N*-Oxe-Glu-COOH (ABB′ type)	85	–[f]	–[f]	–[f]	227
3	*N*-Oxe-Lys-COOH (A_2B type)	89	6,620	3.81	63	289

[a] Reaction conditions; monomer: 0.5 mmol, *o*-dichlorobenzene (0.5 mL) at 130 °C for 24 h.
[b] Ethyl ether-insoluble part.
[c] Estimated by SEC (eluent; DMF).
[d] Glass transition temperature (T_g) was determined by differential scanning calorimetry.
[e] 5 wt % loss of thermal decomposition temperature was determined by thermogravimetric analyzer.
[f] Poly(Glu) was insoluble in common organic solvents.

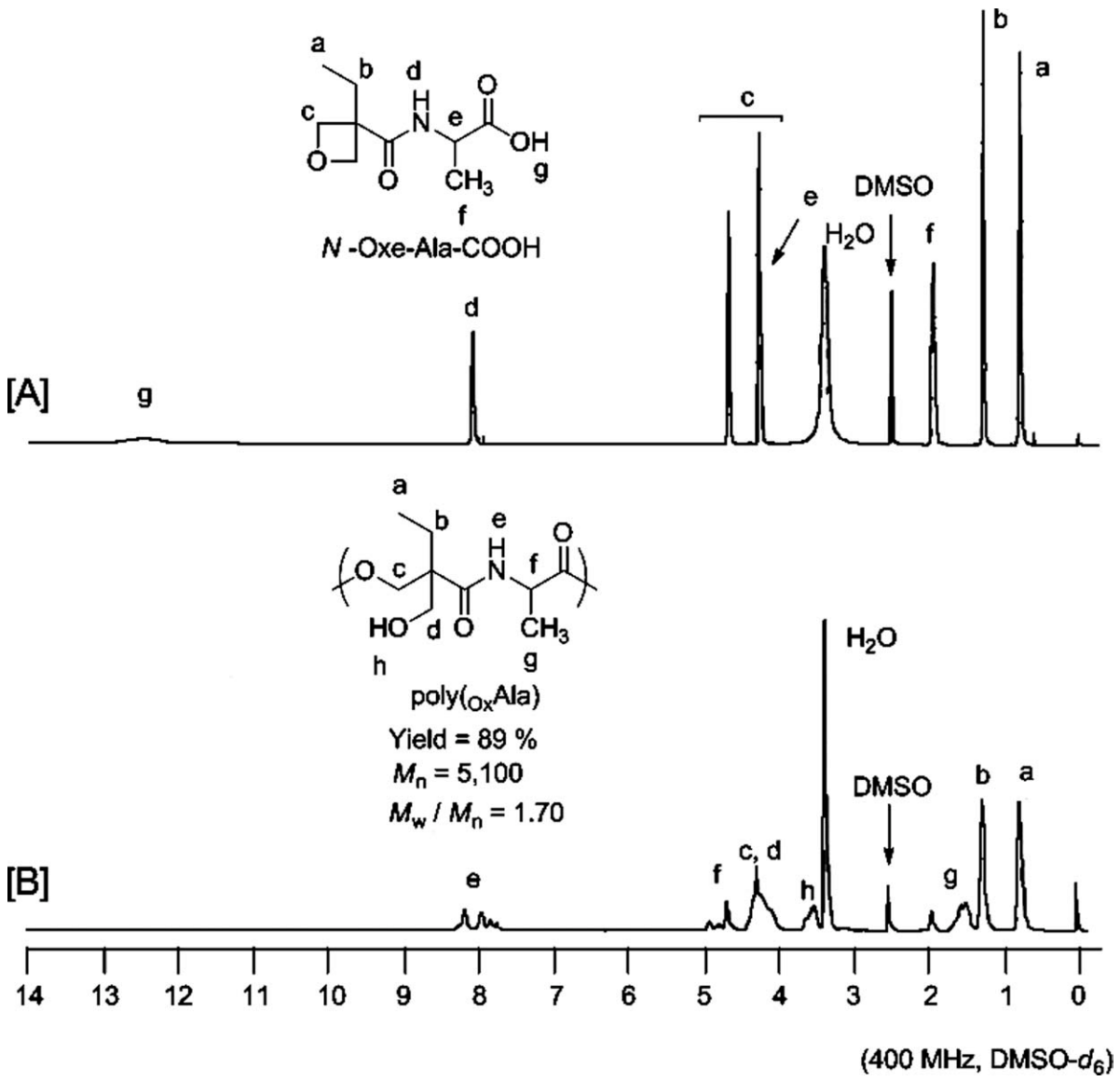


FIGURE 1 ^{1}H NMR spectra of *N*-oxe-Ala-COOH (A) and poly($_{Ox}$Ala) (B).

Self-polyaddition of *N*-Oxe-Glu-COOH and *N*-Oxe-Lys-COOH afforded the corresponding polymers poly($_{Ox}$Glu) and poly($_{Ox}$Lys) in 85% and 89% yields, respectively (runs 2 and 3 in Table 1). The obtained poly($_{Ox}$Glu) was insoluble in common organic solvents, presumably because poly($_{Ox}$Glu) has branched structures and many carboxyl groups at the chain ends which would form strong intermolecular hydrogen bonds. The structure of poly($_{Ox}$Glu) was confirmed only by

TABLE 2 Self-Copolyaddition of *N*-Oxe-Ala-COOH/*N*-Oxe-Glu-COOH[a]

Run	Monomer Composition	Yield[b] (%)	M_n[c]	M_w/M_n[c]	T_g[d] (°C)	T_d[e] (°C)
1	*N*-Oxe-Ala-COOH/*N*-Oxe-Glu-COOH (AB/ABB′ = 1/1)	91	–[f]	–[f]	130	236
2	*N*-Oxe-Ala-COOH/*N*-Oxe-Lys-COOH (AB/A_2B = 1/1)	91	4,070	2.36	47	289
3	*N*-Oxe-Glu-COOH/*N*-Oxe-Lys-COOH (AB_2/A_2B = 1/1)	90	–[f]	–[f]	119	273

[a] Reaction conditions; total monomer: 0.5 mmol, *o*-dichlorobenzene (0.5 mL) at 130 °C for 24 h.
[b] Ethyl ether-insoluble part.
[c] Estimated by SEC (eluent; DMF).
[d] Glass transition temperature (T_g) was determined by differential scanning calorimetry.
[e] 5 wt % loss of thermal decomposition temperature was determined by thermogravimetric analyzer.
[f] Polymer was insoluble in common organic solvents.

TABLE 3 Self-Copolyaddition of *N*-Oxe-Ala-COOH/*N*-Oxe-Glu-COOH[a]

Run	*N*-Oxe-Ala-COOH/*N*-Oxe-Glu-COOH (AB/ABB′)	Yield[b] (%)	M_n[c]	M_w/M_n[c]	T_g[d] (°C)	T_d[e] (°C)
1	1/1	91	–[f]	–[f]	130	236
2	2/1	95	–[f]	–[f]	89	235
3	5/1	75	–[f]	–[f]	88	280
4	6/1	85	1,600	3.91	78	281
5	7/1	90	920	1.81	83	281
6	8/1	88	1,120	1.79	84	281
7	9/1	91	2,870	12.8	84	282
8	10/1	90	1,480	21.6	75	284

[a] Reaction conditions; total monomer: 1.0 mmol, *o*-dichlorobenzene (0.5 mL) at 130 °C for 24 h.
[b] Ethyl ether-insoluble part.
[c] Estimated by SEC (eluent, DMF).
[d] Glass transition temperature (T_g) was determined by differential scanning calorimetry.
[e] 5 wt % loss of thermal decomposition temperature was determined by thermogravimetric analyzer.
[f] The obtained polymer was insoluble in common organic solvents.

IR spectroscopy. Poly($_{Ox}$Lys) also has branched structure, but is soluble in common organic solvents, and its M_n and M_w/M_n were 6620 and 3.81, respectively. The degrees of branching of poly($_{Ox}$Glu) and poly($_{Ox}$Lys) could not be evaluated from the IR and NMR results. Furthermore, the optical activity of the monomers was not examined, because racemization is expected to occur readily both during synthesis of the monomers and during polymerization (aqueous alkaline degradation reaction was used during the synthesis of monomers and self-polyaddition was performed at the high reaction temperature of 130 °C).

Self-Copolyaddition of *N*-Oxe-Ala-COOH (AB Type), *N*-Oxe-Glu-COOH (ABB′ Type), and *N*-Oxe-Glu-COOH (A_2B Type)

Self-copolyaddition of *N*-Oxe-Ala (AB type), *N*-Oxe-Glu (ABB′ type), and *N*-Oxe-Lys (A_2B type) was carried out at the same molar feed ratios, affording the corresponding polymers in satisfactory yields. The conditions and results are summarized in Table 2. Self-copolyaddition of AB + ABB′ and ABB′ + A_2B gave polymers poly($_{Ox}$Ala-*co*-$_{Ox}$Glu) and poly($_{Ox}$Glu-*co*-$_{Ox}$Lys) that were insoluble in common organic solvents (runs 1 and 3 in Table 2). However, these polymers showed higher T_g's compared with those of poly($_{Ox}$Ala) (T_g = 75 °C) and poly($_{Ox}$Lys) (T_g = 63 °C). This is presumably because poly($_{Ox}$Ala-*co*-$_{Ox}$Glu) and poly($_{Ox}$Glu-*co*-$_{Ox}$Lys) contain branched structures and have so many terminal carboxyl groups that form intermolecular and intramolecular hydrogen bonds. In the case of self-copolyaddition using AB and A_2B, the corresponding polymer poly($_{Ox}$Ala-*co*-$_{Ox}$Lys) could be obtained with M_n = 4070 and M_w/M_n = 2.36 in 91% yield (run 2 in Table 2). The unit ratio of alanine and lysine moieties of poly($_{Ox}$Ala-*co*-$_{Ox}$Lys) was almost agree with that of feed ratios, that is, alanine:lysine = 1:1, which was confirmed by ^{1}H NMR spectroscopy. Its value of T_g (47 °C) was the lowest among the copolymers obtained. Furthermore, the values of 5% weight-loss temperature ($T_d^{5\%}$) of the synthesized polymers were in the range between 227 and 289 °C, and polymers containing ABB′ type monomer tended to have lower $T_d^{5\%}$. Thus, the thermal properties of the polymers were consistent with the structures of the monomers.

Next, self-copolyaddition was carried out at feed ratios of *N*-Oxe-Ala-COOH/*N*-Oxe-Glu-COOH = 1/1 to 10/1 under the same conditions as used for the self-polyaddition described above. The results and conditions are also summarized in Table 3. At monomer feed ratios of *N*-Oxe-Ala-COOH/*N*-Oxe-Glu-COOH = 1/1 to 5/1, the obtained polymers were insoluble in common organic solvents, presumably due to strong hydrogen bonding of the many carboxyl groups at the chain ends (runs 1–3 in Table 3). Self-copolyaddition at feed ratios of *N*-Oxe-Ala-COOH/*N*-Oxe-Glu-COOH = 6/1 to 10/1 gave poly($_{Ox}$Ala-*co*-$_{Ox}$Glu) with M_n's = 920–2870 and M_w/M_n's = 1.81–21.6 in 85–91% yields. The large values of M_w/M_n were observed presumably due to adhesive effect on polystyrene gel column caused from many carboxyl groups at the ends and side chain of the obtained polymers. The values of T_g and $T_d^{5\%}$ were in the ranges of 75–130 °C and 236–284 °C, respectively. Decrease of T_g and increase of $T_d^{5\%}$ were observed with increase of the feed ratio of AB type monomer. These results indicate that the amount of terminal carboxyl groups can be controlled by adjusting the feed ratio in the self-copolyaddition. Consequently, it is expected that physical properties such as solubility, glass transition temperature, and thermal stability of the copolymers can also be controlled by the adjusting the amount of terminal carboxyl groups.

Biodegradability of Polymers

Biodegradable polymers such as poly(amino acid)s, poly(lactic acid),[12] and polycaprolactone[13] can be decomposed by aerobic or anaerobic bacteria, affording H_2O and CO_2. We examined the biodegradability of the synthesized polymers poly($_{Ox}$Ala), poly($_{Ox}$Glu), and poly($_{Ox}$Ala-*co*-$_{Ox}$Glu) (*N*-Oxe-Ala-COOH/*N*-Oxe-Glu-COOH = 1/1) by the BOD method using activated sludge. Figure 2 shows the biodegradability of the

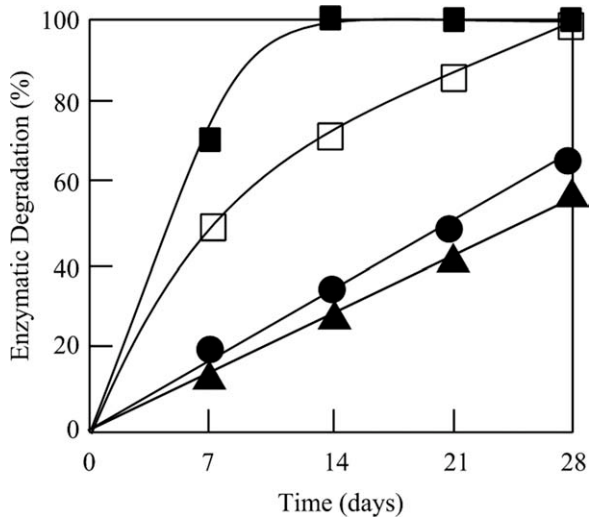


FIGURE 2 Biodegradation ratios of poly($_{Ox}$Ala), poly($_{Ox}$Glu), and poly($_{Ox}$Ala-*co*-$_{Ox}$Glu) and alanine as a reference. □: Alanine (reference), ▲: poly($_{Ox}$Ala), ■: poly($_{Ox}$Glu).●: poly($_{Ox}$Ala-*co*-$_{Ox}$Glu) (*N*-Oxe-Ala-COOH/*N*-Oxe-Glu-COOH = 1/1).

polymers and alanine as a reference. Alanine was quantitatively degraded after 28 days. This confirms that the activated sludge is appropriate for the examination of biodegradability. The degradation ratios of poly($_{Ox}$Ala) and poly($_{Ox}$Ala-*co*-$_{Ox}$Glu) reached 57.8% and 65.3%, respectively. Furthermore, poly($_{Ox}$Glu) could show excellent biodegradability, amounting to >99% after only 14 days. Thus, all these polymers had good biodegradability, and this might be further improved by increasing the composition ratio of glutamic acid moieties in the main chain.

CONCLUSIONS

A novel general method was developed for the synthesis of polymers containing amino acids in the main chain, via self-polyaddition of amino acid-based monomers containing oxetanyl and carboxyl groups. *N*-Oxetanylalanine (*N*-Oxe-Ala-COOH), *N*-oxetanylglutamic acid (*N*-Oxe-Glu-COOH), and *N*-oxetanyllysine (*N*-Oxe-Lys-COOH) were synthesized from alanine, glutamic acid, and lysine, respectively. These synthesized monomers were stable and easy to handle. Self-polyaddition reactions of *N*-Oxe-Ala-COOH, *N*-Oxe-Glu-COOH, and *N*-Oxe-Lys-COOH were carried out using TPPB as a catalyst in *o*-dichlorobenzene at 130 °C for 48 h, to afford the corresponding polymers poly($_{Ox}$Ala), poly($_{Ox}$Glu), and poly($_{Ox}$Lys) with pendant hydroxyl groups in satisfactory yields. Furthermore, self-copolyaddition reactions of these monomers were also performed to give the corresponding polymers, and it was found that the physical properties, such as solubility, glass transition temperatures, and thermal stability, of the polymers reflected the amount of the terminal carboxyl groups. The biodegradability of the polymers was examined by the BOD method, and that the decomposition ratios of poly($_{Ox}$Ala), poly($_{Ox}$Glu), and poly($_{Ox}$Ala-*co*-$_{Ox}$Glu) were 57.8%, >99%, and 65.3%, respectively. The synthesis and properties of other amino acid-based polymers are under investigation.

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