Inclusion of isolated α-amino acids along the polylactide chain through organocatalytic ring-opening copolymerization

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ABSTRACT

Degradable polymers based on α-hydroxy acids and α-amino acids constitute a potent class of biomaterials, combining high hydrolyzability with structural features that mimic peptides. Driven by the design criteria to construct isolated α-amino acid units along a main polylactide chain, a copolymer system was developed based on two monomers with distinctly different equilibrium behaviors. This was uncovered by detailed understanding on the kinetic and thermodynamic polymerizability of 3S,6S-dimethylmorpholine-2,5-dione (DMMD) and L-lactide (LLA) at low reaction temperatures. Under Brønsted base-promoted ring-opening copolymerization (ROCOP) conditions, the equilibrium nature of the copolymerization was shown susceptible to changes in the system, such as catalyst basicity, solvent polarity and initial monomer concentrations. Subsequently, high equilibrium conversions of both monomers with control over molecular weight and dispersity could be achieved within short reaction times by modulation of these factors. Thermodynamic elucidations of the copolymerization system revealed that DMMD behaved as an unstrained monomer with a large propagation barrier, favored by an increase in polymerization temperature. Ultimately, the high propagation barrier of DMMD in the system resulted in a kinetically controlled mechanism with the formation of completely isolated units of DMMD along the polylactide backbone. These results extend current ROCOP strategies of morpholine-2,5-diones and cyclic esters to a mild and selective copolymerization platform for the construction of sequence-controlled α-amino acid decorated polyesters for medical applications.

1. Introduction

In order to meet the demands for sequence-controlled biomaterials that are able to integrate with biological systems [1], a fundamental understanding of the synthetic pathway to afford such material with defined microstructure is desirable. Degradable polyesters are among the most well studied polymers for biomedical applications [2-5], and their synthesis via ring-opening polymerization (ROP) [6,7] offers the ability to obtain polymers or copolymers with highly controlled sequence microstructure. This in turn translates to their macromolecular architecture, ultimately controlling their mechanical properties [8,9], processability [10], and degradability [5,11]. Among the degradable polyesters, copolymers based on polylactide have received significant attention owing to its renewable origin, tailorable physical properties and in vivo degradation into non-toxic metabolites [5,12,13]. Polylactide is an ideal polymer in many ways, but its hydrophobic character, lack of functionality for further conjugation and inability to actively interact in a biological milieu remains disadvantageous for more refined medical applications [14].

To increase hydrophilicity, functionality, and cell interaction abilities of polylactide, copolymerization of L-lactide (LLA) with functional cyclic monomers following post-polymerization modifications with bioactive motifs has been a widely used strategy [15]. We have previously demonstrated several strategies for surface-grafting of hydrophilic and electroactive motifs [16], as well as conjugation of peptides [17,18] and other bioactive molecules [19] to functionalized polyesters. While surface-grafted peptides and bioactive molecules have an advantage when it comes to the creation of materials with protein-binding motifs, incorporation of α-amino acids along the polylactide chain could circumvent the inherent hydrophobicity and inertness of the main polymer chain without the need for post-polymerization modifications [14]. Inclusion of isolated α-amino acid sequences would retain the hydrolyzable ester-junctions, provide completely toxic-free degradation products, and ensure low probability of antigenicity [2].
One of the most widely-used methods for preparation of hybrid polypeptide-based polyesters is through ROP of α-amino acid derived N-carboxyanhydrides (NCA) affording polyester-polypeptide block copolymers [20]. The construction of random copolymers from NCAs and LLA is challenging due to the difference in kinetics of LLA and NCAs under ROP conditions. The initiation of NCAs is often promoted by amine initiators, while hydroxyl initiators proceed significantly slower, leading to uncontrolled polymerizations [21,22]. To circumvent these kinetically challenging differences in order to obtain polylactide with random incorporation of peptidic moieties, a monomer able to propagate as an alkoxide, while bearing a latent α-amino acid motif would be a desirable design.

One such type of monomer, morpholine-2,5-diones, was introduced by Helder and Feijen in 1985 for ROP, and since then several homopolymers and copolymers based on morpholine-2,5-dione and cyclic esters have been prepared using metal-based catalysts [23–29]. The ROP of morpholine-2,5-diones has generally been hampered by slower kinetics compared to LLA, attributed to a favorable chelating effect between the metal complex and the amide-moiety, leading to kinetically inert species [30]. Unfavorable equilibrium monomer conversions, racemization and side-reactions have also been observed during the high reaction temperatures required for ring-opening homopolymerization of 3,6-dimethyl-morpholine-2,5-dione (DMMD) under melt conditions [30–32].

Few catalytic systems able to operate at low reaction temperatures have been employed for the ROP of morpholine-2,5-dione derivatives [33–36]. Schubert et al. demonstrated the ROP of alkyl-substituted morpholine-2,5-diones using Brønsted base catalysis for the first time in 2018 [35]. More recently, Li et al. elegantly employed Brønsted base catalysis alone or in the form of binary catalytic systems with a thiourea analogous for well-controlled homopolymerization or block copolymerization of several morpholine-2,5-dione derivatives [36]. Notably, high dependency on the equilibrium monomer conversions of morpholine-2,5-dione derivatives were shown using Brønsted bases even at lower reaction temperatures [35].

To date, no organocatalytic systems nor metal-based systems have successfully enabled the ring-opening homopolymerization of 35,6S-dimethylmorpholine-2,5-dione (DMMD). However, the combination of monomers of high equilibrium concentrations with monomers that are readily polymerizable unlocks a potential synthetic platform to afford copolymers with inclusion of isolated units along a polymer main chain [37–39]. Our hypothesis was that this could be translated into a copolymerization system with DMM and LLA for the selective incorporation of α-amino acids along a degradable polyester backbone (Scheme 1). Since the polymerizability of a monomer is influenced by several parameters in the system such as temperature, concentration, and solvent [40–43], we reasoned that these factors would have a crucial effect on the polymerization-depolymerization equilibriums, ultimately determining the sequence-distribution of the final copolymer. Thus, our aim was to develop a synthetic platform to create isolated α-amino acids along the polylactide chain. To elucidate on this synthetic possibility, we centered our study on the kinetics and thermodynamics of the ring-opening copolymerization (ROCOP) behavior of LLA and DMMD.

2. Results and discussion

The unfavorable equilibrium concentration of DMMD previously described [30,31] makes it an ideal model compound to explore the possibility of isolated inclusion within the polylactide chain. For most cyclic medium-sized rings, where both the change in enthalpy and entropy of polymerization are negative, such as LLA [44], polymerization will only occur if ∆Hp > Tgs, [45,46] suggesting that lower reaction temperatures could promote the copolymerization of LLA and DMMD.

2.1. Organocatalyzed ROCOP of LLA and DMMD

Although low reaction temperatures often promote thermodynamic polymerizability of medium-sized lactones, the kinetics are generally slower at lower temperatures. The kinetic polymerizability is inherently dependent on the catalyst [47,48], requiring high activity catalysts for sufficient monomer conversion in short reaction times. Organocatalysts can operate with high rates under both high and low reaction temperatures [49,50], while displaying high activity and selectivity [51–53]. To open up for the diverse activation modes organocatalysts are known to operate through, both the strong organic acid polyol sulfonic acid (PTSA) and the Brønsted bases 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) were screened [52,54–57]. The total molar ratio was kept at a total monomer to catalyst ratio of 100 ([M]0/[C]0 = 100), and benzyl alcohol (BnOH) was used as the initiator at a catalyst to initiator ratio of [C]/[I] = 1. With the intention of isolated inclusions of the DMMD along the polylactide chain, the feed ratio was kept to [DMMD]=LLA0 = 5:95. As DMMD showed low visual solubility in tetrahydrofuran (THF) and toluene, commonly used solvents for ROP, dichloromethane (DCM) was used at 1.0 M concentration in respect to total initial monomer concentration [M]0 at 30 °C.

A clear trend was shown regarding the basicity and acidity of organocatalysts during the copolymerization. No conversion of DMMD and 10% conversion of LLA after 14 days was observed using the acidic organocatalyst PTSA (Table 1, entry 1), while promising results were shown for the Brønsted bases DBU and TBD. DBU afforded 18% conversion of DMMD and near quantitative conversion of LLA (Table 1, entry 2), while TBD afforded 78% conversion of DMMD and near quantitative conversion of LLA within 2 h reaction time (Table 1, entry 3). This trend is in agreement with previous studies showing high rates of polymerization for LLA using basic catalytic systems such as TBD and DBU but lower rates for acidic catalytic systems [51,58,59].

2.2. Kinetics of the Brønsted base promoted copolymerization

The high activity of the Brønsted bases in the copolymerization system was further explored by comparing the kinetics using DBU and TBD at [M]0/[C]0 = 50 (Table 1, entry 5 and 8). The kinetic curves displayed a high dependency of equilibrium conversion for DMMD,
Table 1
Organocatalyzed Ring-Opening Copolymerization of LLA and DMMD.

| Entry | Catalyst (C) | [C]:[I]:[DMMD]:[LLA] | Solvent | [M] (b) | t (h) | conv DMMD (%) | conv LLA (%) | % DMMD d | $M_n$(SEC) (kg mol$^{-1}$) | $M_n$(theory) (kg mol$^{-1}$) | Đ (Mw/Mn) |
|-------|--------------|-------------------------|---------|--------|------|----------------|--------------|--------|-----------------------------|-----------------------------|-----------|
| 1     | PTSA·H2O     | 1:0:5:95                | DCM     | 1.0    | 336  | 0               | 10           | 0      | 4.7                         | 1.5                         | 1.11      |
| 2     | DBU          | 1:1:5:95                | DCM     | 1.0    | 2    | 18              | > 98         | 2      | 6.6                         | 13.8                        | –         |
| 3     | TBD          | 1:1:5:95                | DCM     | 1.0    | 2    | 78              | > 98         | 4      | 10.9                        | 14.2                        | –         |
| 4     | TBD          | 1:1:10:40               | DCM     | 1.0    | 0.3  | 72              | > 98         | 11     | 6.9                         | 6.8                         | 1.27      |
| 5     | TBD          | 1:1:10:40               | DCM     | 1.0    | 2    | 90              | > 98         | 13     | 5.7                         | 7.1                         | 1.61      |
| 6     | TBD          | 1:1:10:40               | DCM     | 1.0    | 0.3  | 20              | > 98         | 5      | 7.2                         | 6.0                         | 1.15      |
| 7     | DBU          | 1:1:10:40               | DCM     | 1.0    | 2    | 42              | > 98         | 8      | 7.1                         | 6.4                         | 1.39      |
| 8     | DBU          | 1:1:10:40               | DCM     | 1.0    | 5    | 70              | > 98         | 13     | 4.9                         | 6.7                         | 1.62      |
| 9     | TBD          | 1:1:10:40               | DCM     | 2.0    | 5    | 45              | > 98         | 8      | 9.5                         | 6.3                         | 1.38      |
| 10    | DBU          | 1:1:10:40               | DCM     | 1.0    | 5    | 19              | > 98         | 3      | 7.9                         | 6.1                         | 1.11      |
| 11    | DBU          | 1:1:20:80               | DCM     | 0.5    | 24   | < 10            | 23           | 3      | 1.2                         | 1.5                         | 1.38      |
| 12    | DBU          | 1:1:20:80               | DCM     | 0.25   | 24   | < 10            | 10           | 0.7    | 0.6                         | 1.24                        |
| 13    | TBD          | 1:1:20:80               | DCM     | 1.0    | 27   | < 10            | < 10         | n.d.  | n.d.                        | –                           | n.d.      |
| 14    | TBD          | 1:1:50:50               | DCM     | 1.0    | 96   | < 10            | < 10         | n.d.  | n.d.                        | –                           | n.d.      |
| 15    | TBD          | 1:1:10:40               | NMP     | 1.0    | 96   | < 10            | < 10         | n.d.  | n.d.                        | –                           | n.d.      |
| 16    | TBD          | 1:1:10:40               | DMA     | 1.0    | 144  | < 10            | < 10         | n.d.  | n.d.                        | –                           | n.d.      |

n.d. refers to not determined.

a Copolymerization was carried out by solubilizing the monomers at 30 °C prior to addition of BnOH used as initiator (I) and subsequent catalyst (C). The data are reported from crude aliquots that were terminated by excess (5 equiv. to catalyst) triethylamine (TEA) or acetic acid (AcOH) at the indicated time point unless otherwise stated.
b Total initial monomer concentration.
c Hours (h).
d Percentage of DMMD in poly(LLA-co-DMMD).
e THF-SEC referenced to polystyrene standards.
f $M_n$(theory) calculated from $^1$H NMR (400 MHz, CDCl$_3$).
g Isolated samples.

Fig. 1. Conversion of DMMD (▲) and LLA (●) as a function over time using (a) DBU, and (b) TBD, as a catalyst; Evolution of DMMD (▲) and LLA (●) as a function over time using (c) DBU, and (d) TBD, as a catalyst. Observed rate constants $k_{obs}$ were derived from the linear slope for LLA ($R^2 = 0.83$ for DBU and 0.99 for TBD) and DMMD ($R^2 = 0.96$ for DBU and 0.97 for TBD). Conditions: [DMMD]$_0$:[LLA]$_0$:[C]:[BnOH]$_0$ = 10:40:1:1; [DMMD]$_0$ + [LLA]$_0$ = 1.0 M in DCM at 30 °C. Monomer conversion was determined by $^1$H NMR (400 MHz, CDCl$_3$).
which for TBD reached a plateau after 90 min at ~90% conversion, while ~70% conversion was reached in the DBU-promoted reaction after 300 min (Fig. 1a and b). With both catalyst systems, LLA reached quantitative conversion within 5 min reaction time. The difference in equilibrium conversion of DMMMD employing either TBD or DBU correlates well to their difference in basicity. TBD having a pKa of 24 as compared to the pKa of 26 for DBU (pKa refers to conjugate acids in acetonitrile) [60]. Differences in equilibrium conversion between these two catalysts have been described previously, such as in the ring-opening polymerization of cyclic carbonates [61].

2.3. Monomer evolution in the copolymerization systems

Looking closer at the monomer evolution in both catalyst systems allowed a more detailed comparison between the monomers. The linear relationship of \( \ln([M]/[M]_0) \) versus time \((t)\) for both catalysts is consistent with pseudo-first order kinetics with respect to monomer (Fig. 1c and d). Half-lives \((t_{1/2})\) were derived from the slope using the relation \(t_{1/2} = \frac{\ln(2)}{k} \). During the DBU-catalyzed copolymerization the observed rate constant \((k_a)\) for LLA was 21.732 sec \(^{-1}\), while for DMMMD the \(k_a\) was 0.558 sec \(^{-1}\). Instead, during the TBD-catalyzed copolymerization the observed rate constant for LLA was 20.904 sec \(^{-1}\), while for DMMMD the \(k_a\) was 3.144 sec \(^{-1}\). The large difference in rate between LLA and DMMMD clearly indicates that the propagation rates, \(k_p\), and/or depolymeration rates, \(k_d\), differs between both monomers.

2.4. Total initial monomer concentration dependency on DMMMD equilibrium

Having established both TBD and DBU as prominent catalysts, albeit with lower equilibrium conversions of DMMMD using DBU as a catalyst, the impact of monomer concentration \([M]\) was explored. Monomer concentration has a direct effect on the thermodynamics of a polymerization system as well as to maintain control over a polymerization reaction \([40,46,62,63]\), suggesting that a change in total initial monomer concentration \([M]_0\) could increase the DMMMD equilibrium conversion and subsequent incorporation into the copolymer chain. Using the same polymerization conditions as in the kinetic runs, and a DBU loading of \([M]/[C] = 100\), the kinetics at \([M]_0 = \) 0.25, 0.5, 1.0, or 2.0 M (Fig. 2) were compared. Increasing the total initial monomer concentration from 1.0 M to 2.0 M led to more than a two-fold increase in DMMMD equilibrium conversion, however at the expense of a longer reaction time required for DMMMD to reach its equilibrium. During 2.0 M conditions, 45% conversion of DMMMD was observed after 300 min (Table 1, entry 10; Fig. 2a and c), as compared to the 150 min required to reach the equilibrium conversion of 18% during 1.0 M conditions (Table 1, entry 11; Fig. 2a and c). This illustrates a thermodynamic effect where increased \([M]_0\) led to higher equilibrium conversion of DMMMD, while kinetically the higher equilibrium monomer conversion also led to longer reaction time required to reach the equilibrium. Comparatively, LLA reached equilibrium conversion (near quantitative conversion in both cases) at 2.0 M conditions within 10 min reaction time, while at 1.0 M conditions 30 min was required (Fig. 2b and d). This instead suggests a kinetic dependency on effective concentration of the catalyst, monomer, and polymer, with higher effective concentration leading to an increase in rate of monomer conversion for LLA.

The difference in rate of conversion for LLA and DMMMD during the 2.0 M conditions (LLA \(_{eq,conv}\) 10 min; DMMMD \(_{eq,conv}\) 300 min) led to higher dispersity \((D = 1.38)\) compared to the dispersity of 1.11 during the 1.0 M conditions (LLA \(_{eq,conv}\) 30 min; DMMMD \(_{eq,conv}\) 150 min), demonstrating the importance of tuning the relative rates of the comonomer conversion profiles to maintain a controlled copolymerization system (Fig. 2c and d). The higher dispersity at increased initial monomer concentration is likely a consequence from the statistical increase in chain-end entanglement at higher concentrations, leading to higher probability of intra-molecular associations. Lower total initial monomer concentrations substantially decreased the conversion of both LLA and DMMMD, by less than 10% and 5% respectively (Table 1, entry 12 and 13).

The observed linear relationship between the equilibrium concentration \([DMMMD]_0\) and total initial monomer concentration \([M]_0 = [DMMMD]_0 + [LLA]_0\) (Fig. 2c), was used to calculate the theoretical \([M]_0\) required to reach ~98% conversion of DMMMD. Using the linear equation, the concentration required to reach ~98% conversion of DMMMD would in theory be 4.2 M (equivalent to \([DMMMD]_0 = 0.8\) M; \([LLA]_0 = 3.0\) M) (Fig. S11). This assumes that DMMMD would be fully solubilized at this total initial monomer concentration, however the solubility of DMMMD was greatly reduced above \([DMMMD]_0 = 0.4\) M \(([M]_0 = 2.0\) M). In fact, when the copolymerization was carried out with \([DMMMD]_0 = 0.5\) M and \([LLA]_0 = 0.5\) M, the polymerization system remained insoluble and, consequently low conversion of both DMMMD and LLA was observed (Table 1, entry 14). Despite the trend observed of lower \([DMMMD]_0\) at higher \([M]_0\), the threshold of solubility of DMMMD in DCM was observed far below what was practically feasible.

2.5. Solvent polarity effects on the copolymerization equilibrium

While the total initial monomer concentration had a large effect on the equilibrium behavior of DMMMD, the polarity of the solvent demonstrated an even higher dependency on the polymerizability in the system. Despite high solubility of DMMMD being achieved in the more polar solvents 1-methyl-2-pyrrolidinone (NMP) and N,N-dimethylacetamide (DMA), the copolymerization in these solvents resulted in low monomer conversion in respective to both DMMMD and LLA. In 1.0 M NMP and DMA (in respect of total initial monomer concentration), with a feed ratio of \([DMMMD]_0/[LLA]_0 = 5.95\) using TBD as catalyst and a total molar ratio \([M]/[C] = 50\) at 30 °C, less than 10% conversion of both DMMMD and LLA was observed over a period of 4 days (Table 1, entry 15 and 16). Intriguingly, the monomers remained intact throughout this time and no side-reactions were observed by 1H NMR. In an anionic mechanism, the rate would be expected to be higher in a polar aprotic solvent system due to the increase in nucleophilicity by facilitating ionization of the initiator/chain-end [64]. However, this was not observed. Such phenomenon could be explained by catalyst deactivation due to coordination/complexation of solvent molecules and the catalyst active sites. Mechanistic elucidations have shown that TBD operates through a pseudoanionic mechanism, implicating that polar solvents could inhibit the ability to abstract the hydroxyl proton of the propagating chain or prevent H-bonding activation of the monomer carbonyl compound [55]. Similar trends have for example been seen in the phosphazene-catalyzed polymerization of ε-caprolactone [65]. Another explanation could be changes in the polymerization equilibrium in highly polar media. Different type of solvent media can affect the free energies of monomer as well as polymer during the polymerization, ultimately affecting the equilibrium concentration [63]. For example, similar observations in equilibrium behavior in polar solvents have been shown during the DBU-promoted polymerization of cyclic carbonates [41] and the cationic ROP of THF [66].

2.6. Thermodynamics of DMMMD in the copolymerization system

The high dependency on the equilibrium concentration of DMMMD, led to further elucidation on the thermodynamics of the copolymerization system. For LLA, both \(\Delta H^\circ\) and \(\Delta S^\circ\) remains below zero [44], and an increase in temperature during polymerization leads to an increase in \([M]_q\) according to equation (55) [46]. At \([M]_q = [M]_0\), the temperature is equal to the ceiling-temperature \((T = T_f)\) and above this temperature, depolymerization occurs. Conversely, for monomers of which \(\Delta H^\circ > 0\) and \(\Delta S^\circ > 0\), a decrease in temperature leads to an increase in \([M]_q\) and at \([M]_q = [M]_0\), the temperature is defined as the
floor-temperature (T = T_f). Below this temperature, polymerization is thermodynamically forbidden.

While absolute values on the thermodynamic polymerizability of DMMD would not be obtained from a copolymer system, the temperature-dependency was used to elucidate on the equilibrium behavior of DMMD in this copolymerization system. The polymerization reaction was performed at different temperatures ranging from −40 °C, −20 °C, 10 °C, to 20 °C (homogenous conditions at all temperatures), using a feed ratio of [DMMD]_0:[LLA]_0:[BnOH]_0:[TBD]_0 = 20:80:1:1 in DCM (1.0 M in respect to total initial monomer concentration). The kinetics of DMMD and LLA were monitored by 1H NMR until the equilibrium DMMD conversion was reached. Surprisingly, for the temperature range from −40 °C to 20 °C, [DMMD]_eq changed from 0.93 mol L⁻¹ to 0.13 mol L⁻¹ illustrating a decrease in equilibrium concentration with an increase in temperature (Fig. 3). This is in contrast to the equilibrium behavior of most medium-sized cyclic monomers, where [M]_eq increases with increasing temperature [40]. It should be noted that previous works on thermodynamics of equilibrium polymerization behavior have dealt with single monomer systems, whereas in this case the system includes two monomers.

As for a single monomer system, the thermodynamic polymerizability of a monomer during a copolymerization system is dictated by the free energy change (∆G_{eq}) in the system, dependent on the change in enthalpy (∆H_{eq}) and entropy (∆S_{eq}) during the reaction progress at the absolute temperature T. Only when ∆G_{eq} < 0, copolymerization is thermodynamically feasible. However, in a copolymerization system there’s a higher degree of freedom because of the multiple number of reaction possibilities. This opens the possibility to copolymerize monomers that are otherwise non homo-polymerizable even above its T_c, as previously described [40,67,38].

The intriguing interplay between equilibrium conversion of DMMD...
...and temperature during copolymerization with LLA prompted a more detailed evaluation of the polymerization thermodynamics. By plotting the natural logarithm of [DMMD]eq over the inverse temperature (Fig. 3), access to the enthalpy and entropy change of the polymerization is given. In this system, both the obtained change in entropy and the obtained change in enthalpy of DMMD in the copolymerization system were above zero (ΔH_{DMMD,copol} = 4.1 kJ mol\(^{-1}\); ΔS_{DMMD,copol} = 18.2 J K\(^{-1}\) mol\(^{-1}\)). In a homopolymerization system, this would indicate a floor temperature (T\(_f\)) of DMMD at ~46 °C (227 K). However, being in fact a copolymerization system with LLA this value rather emphasizes a propagation barrier for DMMD. Thus, DMMD in this system behaved as an unrestricted monomer, requiring a temperature increase for the polymerization to take place (T\(_f\), ΔS\(_p\) > ΔH\(_p\) for ΔG\(_p\) < 0) [40].

This behavior contrasts with many homopolymerization systems of different morpholine-2,5-dione derivatives that display the inverse dependency; increased monomer concentration with increased temperature (displaying a T\(_f\) dependency rather than a T\(_g\) dependency). Feijen et al. observed that the homopolymerization of DMMD at 165 °C failed completely, attributed to a favorable depolymerization rate alongside side-reactions [31]. Wiggerhorn et al. observed similar trends during homopolymerization of DMMD. At 150 °C, only oligomers were produced and below a polymerization temperature of 80 °C, no conversion of DMMD was observed at all [30]. Contrary, this copolymerization platform shows the possibility to copolymerize DMMD with LLA even as low as at 20-30 °C with up to 13% incorporation of DMMD, equivalent to approximately 65% incorporation of the DMMD feed. This illustrates the ability to completely change the thermodynamic polymerizability of DMMD by including a comonomer in the system, in this case LLA.

2.7. Sequence distribution and copolymer microstructure

To demonstrate the ability to afford copolymers with inclusion of DMMD into the polylactide chain, while maintaining low dispersity and similar Mn to the theoretical value, the reaction was quenched prior to DMMD reaching equilibrium. After 20 min reaction time, DMMD was expected to reach approximately 70% conversion based on the monomer evolution profile (Fig. 1d). Isolating the copolymer after 20 min reaction time afforded poly(LA-co-DMMD) copolymer of Mn, 6.9 kg mol\(^{-1}\) and D of 1.27 (Table 1, entry 5; Fig. S13). The amount of DMMD incorporation was calculated based on \(^{1}H\) NMR, with 11% incorporation into the polymer chain. Conversely, when DBU was employed as the catalyst, the copolymer reached Mn, 7.3 kg mol\(^{-1}\) and D of 1.15 with 5% incorporation of DMMD into the polymer backbone (Table 1, entry 7; Fig. S13).

\(^{1}H\) NMR, \(^{13}C\) NMR, and 2D NMR techniques were used to determine the copolymer composition and sequence distribution (Fig. 4), with the following nomenclature used to refer to the sequences; lactidyl unit (LL), lactic unit (L), morpholine-dione lactic unit (M\(_e\), e for ester), morpholine-dione amide unit (M\(_a\), a for amide). The microstructural arrangements are illustrated from the TBD-catalyzed copolymerization (Table 1, entry 5).

From the \(^{1}H\) NMR spectrum, a downfield shift could be observed for the methine (CH) proton of M\(_a\), resulting in a resonance shift at 5.23 ppm as compared to the monomer shift at 4.92 ppm (Fig. 4a). The methine (CH) proton of M\(_e\) also shifted downfield from 4.27 ppm to that of 4.62 ppm. This proton showed a 1H–1H cross-coupling (\(\gamma_{J,HH}\)) to the amide-nitrogen (NH) at 6.58 ppm (Fig. S3). The 1H assignment is in agreement with previous published data [31].

The monomer arrangements were identified from the signals pattern in the carbonyl and methine region of \(^{13}C\) NMR together with 1H–1\(^{13}C\) HMBC (Fig. 4c and d). The M\(_a\) carbonyl appeared at 172.1 ppm, compared to the M\(_e\) carbonyl at 169.5 ppm, which was confirmed by the cross-coupling between the M\(_e\) carbonyl to the methine-proton of M\(_e\) at 4.62 ppm and the cross-coupling between the M\(_a\) carbonyl at 169.5 ppm with the amide-NH proton at 6.58 ppm. The presence of one singlet resonance from the DMMD carbonyls in \(^{13}C\) NMR (Fig. 4c, insert) implicates complete isolation of the DMMD monomer along the polymer backbone while a gradual sequence distribution of DMMD along the polylactide chain is suggested from the relative monomer conversions.

The carbonyl carbon at 170.1 ppm cross-couples by 3J to the methine proton of M\(_a\), representing the triad sequence LLM\(_g\). This demonstrates the connection-point between LL and M\(_a\), and suggests that the ring-opening occurs at least on the ester-side of DMMD. The ester carbonyl carbon of M\(_a\) at 172.1 ppm also cross-couples by 3J to lactidyl-methine (LL), confirming that a minimum two lactide-acid-units are connected to the M\(_a\)-group of DMMD. Assuming that the ring-opening occurs on the ester-moiety of the DMMD [30], the subsequent propagation step would be limited to a hetero-propagation step and not a homopropagation step due to the known iterative sequence of DMMD-cot-LL. Compared to the composition derived from the \(^{1}H\) NMR this demonstrates ≈8 lactidyl units per 1 DMMD unit, emphasizing the utility of this reaction system for isolated chain inclusion of α-amino acid moieties into the polylactide chain.

2.8. Mechanistic implications on the equilibrium nature of the copolymerization

The difference in kinetics between LLA and DMMD suggested that the copolymerization favored formation of longer polylactide blocks with a gradual inclusion of DMMD in the polymer chain. However, no blocks of DMMD sequences were observed in \(^{13}C\) NMR at any time point during the kinetic runs, indicating that the homopropagation of DMMD active chain ends was highly unfavorable. Since DMMD did not homopropagate, it is reasonable that once the active DMMD chain end forms, it is rapidly end-capped by LLA, emphasizing a kinetically controlled mechanism (Fig. 5; Step 1–4). However, for a purely kinetically controlled incorporation, the inclusion of DMMD would be dependent on the presence of LLA, which contrasts with what was observed. In this system, the conversion of DMMD and the incorporation of isolated DMMD units along the chain increased even after near quantitative conversion of LLA had been achieved, despite the reluctance of DMMD to homopropagate. While a kinetic inclusion of DMMD monomer into the polylactide chain is reasonable, other mechanistic aspects were evidently playing a key role in the microstructural arrangements.

Our reasoning for this center on the reluctance of DMMD to homopropagate in combination with the high degrees of freedom existing in a copolymerization system. In theory, the copolymerization system can undergo an infinite number of reactions leading to various copolymers of different microstructures, driven by the entropy increase in the system [40,68]. This is especially important for monomers exhibiting T\(_f\); polymerization is favored if entropy increases (T\(_f\), ΔS\(_p\) > ΔH\(_p\) for ΔG\(_p\) < 0). In this copolymerization system, such behavior was observed by the close relationship between an increase in DMMD equilibrium conversion in conjunction with a broadening in molecular weight distribution. The Mn increased linearly with conversion of LLA until the theoretical Mn of 7.3 kg mol\(^{-1}\) (Mn, TBD 6.9 kg mol\(^{-1}\); Mn, DBU 7.2 kg mol\(^{-1}\)), while a deviation in Mn and broadening in molecular weight distribution followed once DMMD reached approximately 50% of its equilibrium conversions (Fig. S12; after 10 min using TBD and 30 min using DBU). The broadening in molecular weight distribution over time indicates on either intra-/inter-molecular chain-transfer processes and/or a redistribution between the polymer chains by the polymerization-depolymerization equilibrium of the active chain end. We reasoned that at the time when most of the LLA had been consumed and approximately 50% of DMMD monomer, the reluctance of DMMD to homopropagate would lead to an accumulation of paralyzed chain ends (Fig. 5; Step 5). Such accumulation would increase the dispersity, and likely result in cross-propagation of the active DMMD chain-ends at prolonged reaction times (Fig. 5; Step 6). These findings reveal that the copolymerization is indeed driven by a kinetically controlled mechanism preventing the formation of DMMD-
Fig. 4. (a) $^1$H NMR (400 MHz, CDCl$_3$) of poly(LLA-co-DMMD); (b) Representation of the sequence-determination of poly(LLA-co-DMMD); (c) $^{13}$C NMR (100 MHz, CDCl$_3$) of poly(LLA-co-DMMD); (d) Selected area of $^1$H-13C HMBC spectrum of poly(LLA-co-DMMD). Conditions: [DMMD]$_0$:[LLA]$_0$:[TBD]$_0$:[BnOH]$_0$ = 10:40:1:1; [DMMD]$_0$ + [LLA]$_0$ = 1.0 M DCM at 30 °C.

Fig. 5. Plausible mechanism of the TBD-catalyzed ROCOP of LLA and DMMD. Step 1–2: initiation and ROP of LLA occurs faster than the ROP of DMMD. Step 3–4: once the ring-opening of DMMD occurs, it is rapidly end-capped by another LLA monomer, suggesting a kinetically controlled mechanism. Step 5–6: The slower kinetics of DMMD and reluctance to homopropagate leads to paralyzed chain ends at prolonged reaction time, eventually leading to transesterification and/or redistribution between polymer chain ends, ultimately increasing the randomness in the copolymer.
3. Conclusion

This work describes a thorough platform for the selective incorporation of α-amino acids along a degradable polymer backbone. It highlights how to overcome the kinetic and thermodynamic barriers of a monomer with disadvantageous equilibrium behavior to construct sequence-controlled copolymer microstructures. This was uncovered through detailed understanding on how the copolymer system configures and how the monomer structure relates to the copolymerization behavior. In this system, LLA exhibited higher ring-strain compared to DMMD and rapidly homopropagated at a higher rate than corresponding heteropropagation, resulting in longer block formations of LLA-units. DMMD was reluctant to homopropagation and instead heteropropagation with LLA was favored. The driving force for an increase in entropy during copolymerization and the reluctance of DMMD to homopropagate led to chain shuffling once LLA had reached its equilibrium conversion, resulting in higher degrees of randomness in the polymer chain. Despite this entropic driving force, control over Mn and D could be achieved with high conversions of both monomers within 20 min reaction time. Polymers combining high hydrolytic degradability with structural features that mimic peptides is a highly potent class of materials for biomedical applications. Previous synthetic methodologies have been limited in terms of tunability of the reaction parameters, preventing the development of new α-amino acid-based copolymers. This work provides new opportunities for the synthesis of polymer architectures that have previously been difficult to access through metal-mediated processes. We believe that the mild and selective ROCOP method presented herein will advance the development of α-amino acid-based copolymer materials.

4. Experimental

4.1. Materials and methods

Glass-wares were oven-dried at 110 °C for a minimum of two nights prior to use. Air- or moisture sensitive compounds were manipulated under nitrogen atmosphere using Schlenk techniques or in a nitrogen-filled Mbraun MB 150-GI glovebox. L-alanine (> 98%, Sigma-Aldrich, Sweden), 2-chloropropionyl chloride (97%, Sigma-Aldrich, Sweden), acetic acid (AcOH) (glacial, Sigma-Aldrich, Sweden), phosphorous pentoxide (P2O5) (> 98%, Sigma-Aldrich, Sweden) were used as received. Benzyl alcohol (BnOH) (Reagent Grade, Acros Organics, Germany), 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD) (98%, Sigma-Aldrich, Sweden), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (98%, Sigma-Aldrich, Sweden), p-toluene sulfonic acid monohydrate (PTSA) (> 98.5%, Sigma-Aldrich, Sweden), dichloromethane (DCM) (anhydrous, ≥ 99.8%, Sigma-Aldrich, Sweden), toluene (anhydrous, ≥ 99.8%, Sigma-Aldrich, Sweden), 1-methyl-2-pyrrrolidinone (NMP) (anhydrous, ≥ 99.5%, Sigma-Aldrich, Sweden), N,N-dimethylacetamide (DMA) (anhydrous, ≥ 99.8%, Sigma-Aldrich, Sweden) were brought inside the glovebox under inert conditions prior to unsealing them. L-lactide (LLA) (GMP grade, PUR-Sorb® L, Corbion purac) was recrystallized from anhydrous toluene twice. LLA and DMMD were dried over P2O5 for a minimum of one week prior to bringing them inside the glovebox.

4.2. Instruments and measurements

Nuclear magnetic resonance (NMR) spectra were obtained in CDCl3 or D2O at 20 °C on a Bruker Avance UltraShield™ spectrometer (1H: 400.13 MHz, 13C: 100.62 MHz). The chemical shifts and coupling constants are reported in ppm (δ) and Hz (J) respectively, and referenced to the residual solvent peak relative to tetramethyl silane (1H: δ 7.26; 13C: δ 77.16). Spectra were recorded using Bruker TopSpin v3.5 pl 7 and data processed by TopSpin v3.5 pl 7 or MestReNova v9.0.0. For isolated polymers, 5 mg mL−1 (1H NMR) and 40 mg mL−1 (13C NMR) were used.

Number average and weight average molecular weights (Mn and Mw) and molecular weight dispersions (D = Mw/Mn) were measured by size exclusion chromatography (SEC) and determined from a GPCMAX system equipped with a RI Detector and two linear mixed bead columns (LT4000L). The samples were injected using an autosampler from Malvern Instruments and tetrahydrofuran was used as mobile phase (1 mL min−1, 35 °C). The calibration was created using polystyrene standards (160–371,000 g mol−1) with narrow molecular weights. Corrections for the flow rate fluctuations were made using toluene as an internal standard. For isolated polymers, 2 mg mL−1 was used.

4.3. Monomer synthesis

The synthesis of 3S,6S-dimethylmorpholine-2,5-dione was performed via a two-step procedure similar to the previously reported route [24].

4.3.1. Shottlen-Baumann N-acylation

To a stirring solution of L-alanine (17.8 g, 0.2 mol) in Et2O/H2O (1:1 v/v, 75 mL) was added 4 M aqueous NaOH (50 mL) at −5 °C. 2-Bromo propionyl bromide (23.0 mL, 0.22 mol) in Et2O (25 mL) was added dropwise to the reaction mixture together with additional 4 M aqueous NaOH (75 mL) over a period of 20 min. After 3 h, the reaction was quenched by the addition of 4.0 M aqueous HCl until pH 1 was reached. The mixture was extracted with EtOAc × 3, washed over brine and the combined organic phases dried over MgSO4. Removal of the solvent afforded white crude crystals that were recrystallized from hot acetone. The product was obtained as white crystals in 87% yield (31.23 g). 1H NMR (400 MHz, CDCl3) δ 4.58 (1H, q, 3JH-H = 6.8 Hz, −CH2(CHOH)(Br)), 4.39 (1H, quint, 3JH-H = 7.4 Hz, −CH2CH2(NH)(−)), 1.65 (3H, d, 3JH-H = 6.8 Hz, −CH2CH2(NH)(−)). 13C NMR (101 MHz, CDCl3) δ 178.7 and 178.6 (C=O(OH)), 175.1 and 170.5 (−NH(C(O)=O)), 55.6 and 55.5 (−CH2CH2(NH)(−)), 51.6 and 51.5 (−CH2CH3(Br)), 23.6 and 23.5 (−CH3(CH2)(Br)), 18.4 and 18.3 (−CH3(C(O)=O)).
4.4. General polymerizations

All catalysts, initiators and monomers used during polymerization were stored in a nitrogen filled glovebox prior to use. Stock-solutions were prepared fresh inside the glovebox and any injections or sampling outside of the glovebox were performed with disposable syringes which were cleared of air and moisture prior to use by 3 repetitive vacuum/nitrogen cycles. In a typical experiment, oven-dried glassware was charged with DMM (200 mg, 1.4 mmol) and LLA (807 mg, 5.6 mmol) inside a nitrogen-filled glovebox. The round-bottom flask was fitted with a rubber septum and brought out of the glovebox. Through nitrogen filled syringes, anhydrous DCM (5 ml, final total initial monomer concentration = 1.0 M) was added to dissolve the monomers and the flask was immerged in a thermostated oil-bath preheated to 30 °C (±0.2 °C). Initiator (0.14 mmol from a 0.14 M stock solution in DCM) and catalyst (0.14 mmol from a 0.14 M stock solution in DCM) were added consecutively. At suitable time intervals, an aliquot of 0.1 ml reaction mixture was withdrawn from the reaction solution and quenched with AcOH or TEA (5 equiv.) depending on the catalytic system employed, and subsequently analyzed by 1H NMR spectroscopy and SEC. For monomer conversion, the ratio of the methine-proton giving rise to the quartet at 4.92 ppm (monomer) was used in relation to the new broader peak at 4.62 ppm (polymer) for DMMD. For thine-proton giving rise to the quartet at 7.39 ppm (monomer) was used in relation to the new broader peak at 4.62 (polymer) for DMMD.

CRediT authorship contribution statement

Tove Kivijärvi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization, Writing - review & editing. Daniella Pappalardo: Supervision, Writing - review & editing. Peter Olsen: Writing - review & editing. Anna Finne-Wistrand: Conceptualization, Supervision, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

Appendix A. Supplementary material

Supplementary data can be found at https://doi.org/10.1016/j.eurpolymj.2020.109703.

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