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Primary ammonium/tertiary amine-mediated controlled ring opening polymerisation of amino acid *N*-carboxyanhydrides†

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Stable commercial primary ammonium chlorides were combined with tertiary amines to initiate the controlled ring opening polymerisation of amino acid *N*-carboxyanhydrides to yield polypeptides with defined end group structure, predetermined molar mass and narrow molar mass distribution.

Achieving good control over polymerisation reactions is essential for the synthesis of well-defined polymers. Typically, anionic, cationic, controlled radical and ring opening polymerisation (ROP) techniques are applied to synthesise polymers with predetermined composition, functionality, molar mass, and low dispersity.[1] These properties are essential in the fields of self-assembly and biomimicry. Self-assembled and biomimetic supramolecular assemblies, such as micelles, vesicles, hydrogels and hierarchical scaffolds, are often developed for biomedical or materials science applications.[2–7]In this context, polypeptides are very interesting polymers, not only because they can be designed to be biocompatible and biodegradable, but also because they can be synthesised in a controlled manner by ROP of amino acid *N*-carboxyanhydrides (NCAs).[8,9]

The non-metal catalysed ROP of NCAs is known to proceed *via* two distinct pathways, namely the normal amine mechanism (NAM) and the activated monomer mechanism (AMM) (Scheme 1a and b).[10] The NAM is favoured by the use of nucleophilic initiators such as primary amines and yields well-defined polypeptides. The AMM is favoured by bases, such as tertiary amines, and yields polypeptides with high molar mass and dispersity. Although the choice of initiator can influence the NCA polymerisation pathway, it is challenging to completely suppress the AMM. Over the past two decades, considerable advances in controlled NCA polymerisation have been realised. The effort was mostly aimed at the elimination of side reactions, notably the AMM, by using transition metal catalysts,[11] silazane[12] and ammonium salts[13] as initiators, by lowering the reaction temperature[14] and by applying high vacuum techniques.[15] Also primary/tertiary amine organocatalytic systems have been introduced promoting an accelerated amine mechanism through monomer activation (AAMMA).[16]

Ammonium salts are attractive alternatives to amines as initiators for ROP of NCAs as they are more stable, easier to handle and to purify. Schlaad *et al.*[13] postulated that the ammonium-mediated ROP mechanism may involve an equilibrium between dormant (ammonium) and active (amine) ω-chain ends (Scheme 1c), leading to a controlled propagation like in living cationic polymerisation or nitroxide-mediated radical polymerisation. It was suggested that the protons introduced *via* the ammonium salts would protonate NCA anions and thereby suppress the AMM.[13,17,18] However, this technique proved ineffective for hydrophobic NCAs, possibly as a result of this equilibrium being too far shifted to the ammonium side due to a more apolar reaction medium.[19,20] For instance, γ-benzyl-L-glutamate (BLG) NCA could only be polymerised by a mixture of the ammonium salt and its corresponding primary amine,[17] thus somehow defeating the initial purpose of using the sole ammonium salt as an initiator. Being able to establish an alternative ammonium-mediated ROP without the need for the corresponding amine would be extremely beneficial because – aside from the aforementioned advantages – ammonium salts, especially the chlorides, are easier to synthesise and more readily available for purchase than their amine counterparts.

Since the use of a primary amine in combination with its corresponding ammonium salt for the polymerisation of BLG–NCAs seems to only serve the purpose of shifting the dormant–active equilibrium to allow the polymerisation to proceed (Scheme 1c), we questioned whether a catalyst could serve the same purpose. In an effort to establish a more versatile variant of the ammonium-mediated polymerisation, we decided to investigate catalysts that could be universally used in combination with any ammonium salt initiator. Since tertiary amines

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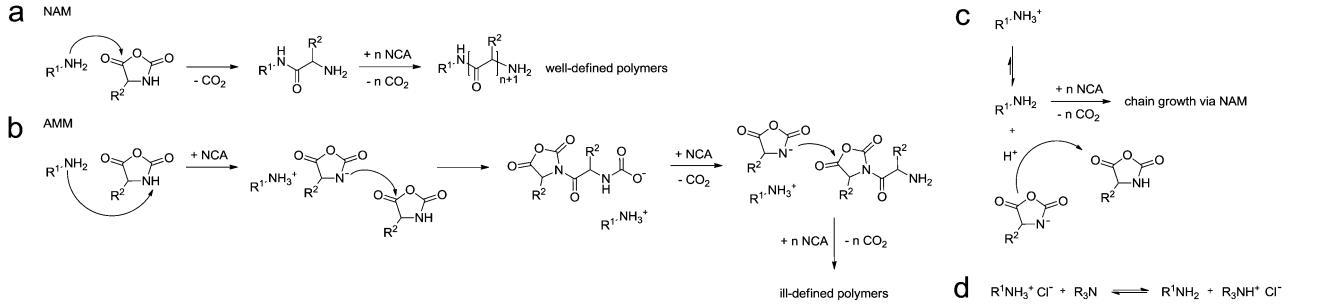

Scheme 1 (a) Normal amine mechanism (NAM), (b) activated monomer mechanism (AMM), (c) proposed mechanism for the ammonium-mediated ring opening polymerisation[13] and (d) primary/tertiary amine–ammonium equilibrium.

are less good nucleophiles than they are basic,[21] we set out to investigate mixtures of primary ammonium salts and tertiary amines (Scheme 1d). As mentioned earlier, tertiary amines like triethylamine (TEA) are used as catalysts to promote an uncontrolled polymerisation of NCAs *via* the AMM to obtain long polypeptides within very short timeframes; but as a drawback, such polypeptides also exhibit high dispersity, typically greater than 2, and no defined end groups.[8,22] To our surprise, we obtained well-defined polymers with narrow molar mass distributions and predefined end groups. We, therefore, sought to establish the robustness of this new controlled ROP of NCAs.

1,2,3-Tris(aminomethyl)benzene (TAB) is an amine that can be used as a trifunctional initiator for ROP of NCAs but is only available for purchase in the form of its trihydrochloride salt (TAB·3HCl).[23] It was, therefore, chosen as a candidate to test our primary ammonium/tertiary amine-mediated ROP of NCAs. We initially studied the efficacy of TAB·3HCl as an initiator for the polymerisation of BLG–NCA. We followed monomer conversion and dispersity by SEC and molar mass by ^{1}H-NMR (ESI†). We found that the polymerisation was very slow with only 13% conversion after seven days at room temperature (r.t.) (Fig. 1a), and 48% conversion after seven days at 50 °C (Fig. 1b). We then used a 1 : 0.5 mixture of TAB·3HCl and TEA for the polymerisation of BLG–NCA (Fig. 1c). The resulting polypeptide had a low dispersity (1.08) and 67% conversion was achieved after five days at room temperature. The reaction was stopped after seven days and the polymers were worked up and analysed by ^{1}H-NMR. End group analysis showed that the number-average molar mass (M_n) closely matched the targeted molar mass (ESI†). In order to assess whether TEA was solely responsible for this faster and controlled ROP, we followed a 'control' polymerisation by SEC (Fig. 1d) and ^{1}H-NMR. For this control reaction, we used a solution of TEA to initiate the polymerisation of BLG–NCA. The SEC traces and ^{1}H-NMR spectra showed that the polymerisation was clearly uncontrolled (M_n > 77 kDa, dispersity > 2) and concluded that the AMM was the dominant mechanism (Scheme 1b). These results confirmed that TAB·3HCl and TEA, when used as an initiator mixture, have a kind of synergistic effect on the polymerisation of BLG–NCA in that it proceeds in a controllable fashion. It appears that the NAM was most likely the dominant mechanism of the primary ammonium/tertiary amine-mediated ROP of NCAs, however, as suggested by Scheme 1d, not excluding the occurrence of the AMM (*vide infra*).

In order to study the robustness and limits of the process, a series of polymerisations of BLG–NCA were initiated with mixtures of 1-pyrenemethylamine hydrochloride (PyA·HCl) (1 equiv.) and TEA (0 to 1.5 equiv.) at different ratios. The results, shown in Table 1, reveal that the rate of polymerisation increases with increasing amount of TEA, as expected. For all PyA·HCl/TEA ratios, except 1 : 1.5, the polypeptides exhibited very low dispersities (<1.1) suggesting that the primary ammonium/tertiary amine-mediated polymerisations proceeded in a controlled manner. Moreover, SEC analysis with (RI and) UV at λ = 340 nm (Fig. 2) allowed to conclude – on a qualitative basis – that all polypeptide fractions carried a pyrene unit (the only species absorbing at this wavelength),

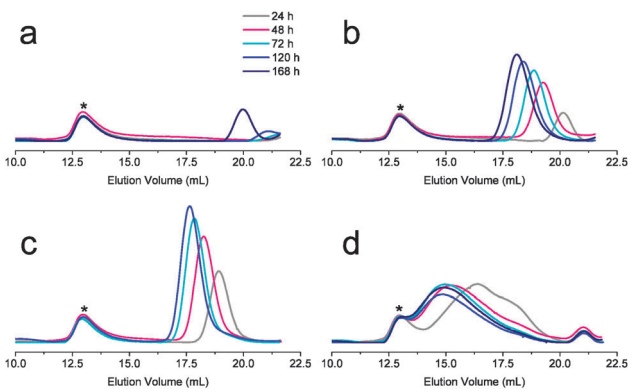

Fig. 1 SEC traces of PBLGs obtained during the polymerisations of BLG–NCA (150 equiv.) in DMF initiated by (a) TAB·3HCl (1 equiv.), r.t.; (b) TAB·3HCl (1 equiv.), 50 °C; (c) TAB·3HCl/TEA (1 : 0.5 equiv.), r.t.; and (d) TEA (0.5 equiv.), r.t.; the peak (*) is a high molar mass polystyrene (PS2M; 2 MDa) used as internal standard for calculating the monomer conversion (ESI†).

Table 1 Results of the polymerisations of BLG–NCA at room temperature initiated by PyA·HCl/TEA (molar ratio 1 : *x*, *x* = 0 to 1.5)

PyA·HCl/TEA	1 : 0	1 : 0.2	1 : 0.5	1 : 0.7	1 : 0.9	1 : 1.1	1 : 1.5
24 h							
Conversion	—	37%	50%	70%	80%	88%	94%
Dispersity	—	1.12	1.07	1.07	1.07	1.07	1.90
120 h							
Conversion	25%	77%	86%	95%	96%	99%	100%
Dispersity	1.06	1.10	1.08	1.07	1.08	1.08	1.90

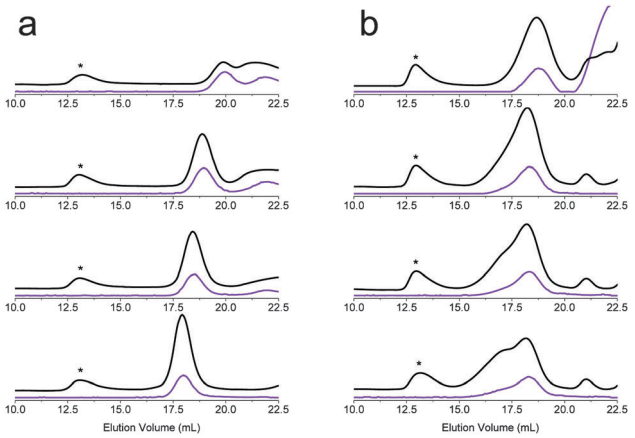

Fig. 2 SEC traces (RI in black, UV_{340nm} in purple) of polymerisations of BLG–NCA (150 equiv.) in DMF initiated by (a) PyA·HCl/TEA (1 : 1.1) at (top to bottom) 2 h, 6 h, 10 h, 24 h; (b) PyA·HCl/TEA (1 : 1.5) at (top to bottom) 2 h, 6 h, 8 h, 24 h; the peak (*) is a high molar mass polystyrene (PS2M, 2 MDa) used as internal standard for calculating the monomer conversion (ESI†).

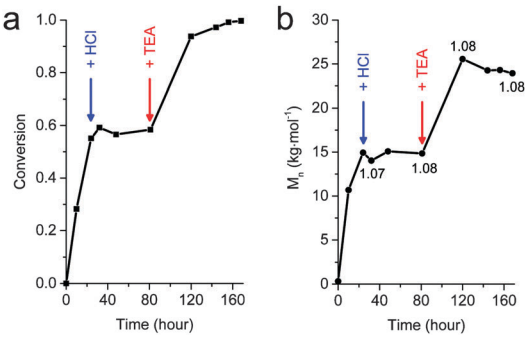

Fig. 3 Polymerisation of BLG–NCA (150 equiv.) in DMF initiated by PyA·HCl/TEA (1 : 0.5 equiv.), paused at 24 h by adding HCl and resumed at 81 h by adding TEA. (a) time-conversion plot, (b) number-average molar mass and dispersities (at 24 h, 81 h, 120 h, and 168 h) determined by SEC.

which is supportive of the NAM. In the case of the uncontrolled polymerisation, *i.e.* PyA·HCl/TEA = 1 : 1.5, the polypeptide chains, but not all, were labelled with pyrene (Fig. 2b).

Interestingly, for all PyA·HCl/TEA initiator mixtures ranging from 1 : 0 to 1 : 1.1, a secondary UV_{340nm} (+RI) absorption peak was generally observed at high elution volumes (> 20 mL) and disappeared with time, as the polymerisation proceeded (Fig. 2a). This observation could be explained by the coexistence of both NAM and AMM, as a consequence of the equilibrium shown in Scheme 1d, where the AMM plays a determining role in the early stages of the polymerisation and the NAM would progressively take over, provided that the initial TEA/PyA·HCl ratio is under a certain limit (< 1.5). More precisely, the tertiary amine would initially generate *N*-acylated NCA oligomers through the 'fast' AMM (Scheme 1b) but the primary ammonium chlorides, present in greater quantities (Scheme 1d), would regulate the propagation by (i) imposing the NAM, causing the *N*-acylated NCA oligomers to be progressively incorporated at the ω-end of other growing chains (hence the disappearance of secondary RI peak), and (ii) providing protons to prevent the AMM from dominating throughout the propagation (hence the final monomodal and narrow molar mass distribution), thereby ensuring rapid incorporation of any unreacted primary amine either *via* 'normal' initiation or *via* the reaction with α-ends of *N*-acylated NCA oligomers (hence the disappearance of the secondary UV_{340nm} peak over time).

The underlying primary ammonium/tertiary amine equilibrium (Scheme 1d) suggests that the ratio of total amines (both primary and tertiary) to HCl (amine/HCl) should affect the polymerisation rate. The larger the amine/HCl ratio, the higher the concentration of active amine chain ends and with it the polymerisation rate. However, at a too large amine/HCl ratio the controlled nature of the reaction will be lost. Likewise, the lower the amine/HCl ratio, the higher the concentration of dormant ammonium chain ends, ultimately leading to an inhibition of the polymerisation.

In order to validate this dormant–active species model, we repeated the PyA·HCl/TEA (1 : 0.5) initiated polymerisation of BLG–NCA, and added HCl (1 equiv. with respect to TEA) 24 h following the initiation. At that stage, the amine/HCl ratio was hence adjusted to 1, thereby shifting the equilibrium to the dormant side. At 81 h, we added TEA (1 equiv. with respect to previously added HCl), and let the reaction run for another 87 h. SEC analysis showed that the polymerisation was 'paused' following the addition of HCl at 24 h as neither the monomer conversion (Fig. 3a) nor the molar mass of the polymer (Fig. 3b) had increased between 24 h and 81 h. After 81 h, both molar mass and conversion started to increase again, indicating that the polymerisation resumed following the addition of TEA. These results not only support the dormant–active mechanism for the ammonium-mediated ROP of NCAs, but also suggest that the correlation observed between polymerisation rates and TEA/PyA·HCl ratios (Table 1) may in fact result from the aforementioned cause-effect relationship between amine/HCl ratios and polymerisation rates.

Although the results support a dormant–active species equilibrium mechanism, the complete mechanism is likely to be more complex. As suggested by the secondary UV_{340nm} peak in the SEC results (Fig. 2) and the uncontrolled polymerisation initiated by PyA·HCl/TEA 1 : 1.5, the chain growth may be the result of both NAM and AMM. In addition, the predominance of one mechanism over the other may not only vary with the primary to tertiary amine and amine/HCl ratios, but may also vary throughout the chain growth process. It should also be noted that the amine–ammonium equilibrium depends greatly on solution pH, solvent polarity and total concentration, making a prediction of the reaction kinetics difficult (work in progress).

In order to demonstrate the general applicability and versatility of this new technique, we tested it with another bulky tertiary amine, *i.e.* diisopropylethylamine (DIPEA, Hünig's base), and two other hydrophobic NCAs, *i.e.* L-leucine (LLeu) and L-phenylalanine (LPhe) NCAs (see ESI† and Fig. 4). As a representative example, the time-conversion plot for the polymerisation of LLeu–NCA (and BLG–NCA, for comparison, Fig. 4a) initiated by benzylamine (BnA), benzylamine hydrochloride (BnA·HCl), BnA·HCl/TEA, and TEA is shown in Fig. 4b. Most importantly, the

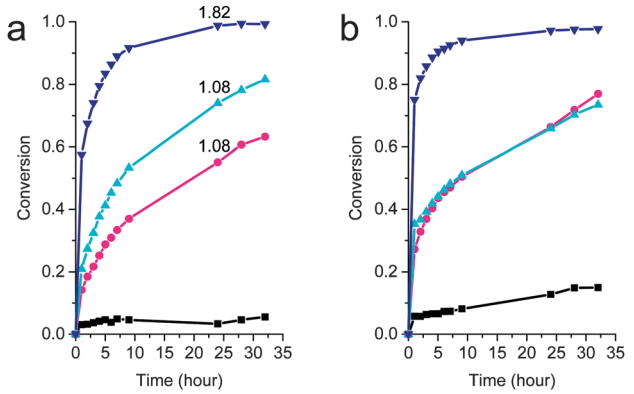

Fig. 4 Time-conversion plots for the polymerisations of (a) BLG–NCA (100 equiv.) and (b) LLeu–NCA (100 equiv.) in DMF initiated by ■ BnA·HCl (1 equiv.), ● BnA·HCl/TEA (1 : 0.5 equiv.), ▲ BnA (1 equiv.), and ▼ TEA (0.5 equiv.). Monomer conversions were determined by FT-IR spectroscopy (see ESI†); numbers in (a) are the polymer dispersities determined by SEC.

polymerisations of LLeu–NCA and BLG–NCA with BnA·HCl were very slow (<15% conversion after 32 h), as expected, and were both considerably faster with BnA·HCl/TEA (respectively, 63% and 77% conversion after 32 h), almost or as fast as with BnA. The PBLGs exhibit low dispersities, except for the sample that was initiated by TEA. PLLeu could not be analysed by SEC due to poor solubility in organic solvents.

In summary, we reported a novel versatile way of controlling ROP of NCAs (BLG–, LLeu or LPhe–NCA) by using an initiator system composed of primary ammonium salt (TAB·3HCl, PyA·HCl or BnA·HCl) and tertiary amine (TEA or DIPEA) (which is different to the primary/tertiary amine organocatalytic system proceeding by the AAMMA). The rate of polymerisation could be controlled by varying the primary ammonium salt to tertiary amine ratio, based on an active (amine)–dormant (ammonium) equilibrium (Scheme 1d), and the polymerisation could even be paused and resumed by the addition of HCl and TEA, respectively. Despite proceeding through competing chain growth mechanisms, NAM and AMM, the polymerisation was controlled and afforded polypeptides with defined end groups and molar mass and low dispersity. Current work is devoted to a detailed kinetic and mechanistic analysis of the primary ammonium/tertiary amine-mediated ROP of NCAs.

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