Communication

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Proximity-Induced Cooperative Polymerization in "Hinged" Helical Polypeptides

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Supporting Information Placeholder

ABSTRACT: Cooperative interactions and transitions are amongst the most important strategies utilized by biological systems to regulate a variety of physical and chemical processes. We report herein an auto-accelerated, rapid cooperative polymerization of *N*-carboxyanhydrides (NCAs) with initiators structurally as simple as linear aliphatic diamines for the synthesis of polypeptides. The polymerization initiated by diamines proceeds via the formation of "hinged" polypeptides – two blocks of helical chains connected head-to-head by the diamine molecules in the polymerization solution. The reactions follow a two-stage, cooperative polymerization kinetic; the cooperative interactions between the macrodipoles of the two hinged helical polypeptides dramatically accelerate the polymerization. Compared to the NCA polymerization initiated by the hexylamine ($CH_3(CH_2)_5NH_2$), the chain propagation rate of the NCA polymerization is increased by more than 600 times when initiated by its diamine analogue (1,6-diaminohexane, $NH_2(CH_2)_6NH_2$). This proximity-induced cooperative polymerization showcases the single helix as a remarkable cooperativity-enabling motif in synthetic chemistry.

Biological macromolecules such as actin and tubulin utilize a two-stage, nucleation-controlled cooperative polymerization mechanism to accelerate their supramolecular assembly process.[1-4] The cooperativity stems from the gain of additional interactions among the protein subunits when they organize into a helical supramolecular assembly instead of a linear assembly.[5-7] Progress has been reported for the design and utilization of synthetic subunits to carry out cooperative polymerizations for well-defined supramolecular structures and functional supramolecular materials.[8-12] Some recent studies include the realization of living supramolecular polymerization[13-14] and non-equilibrium supramolecular polymerization from synthetic molecules,[15] and the elucidation of the pathway complexity in the cooperative polymerization.[16-20] In contrast, the progress in incorporating a cooperative mechanism into covalent polymerization has been slow. While the two-stage polymerizations (nucleation-growth) have been reported in the ring-opening polymerization of *N*-carboxyanhydrides (NCAs) into α-helical polypeptides,[21-22] the acceleration of polymerization rate in the second stage (e.g., due to the formation of helical chain and additional secondary interactions) was modest. Further exploration of cooperative mechanism in the covalent polymerization process remains scarce.[23-24]

Recently, we discovered an auto-accelerated polymerization of α-helical polypeptides in the brush-like macromolecular architecture, where the cooperative interactions between the macrodipoles of neighboring helical polypeptide dramatically accelerate the ring-opening polymerization of NCAs.[25] While this brush system clearly demonstrated that the "tertiary" structure of the brush polymers could lead to a drastic acceleration in polypeptide growth, it is of great interest to identify the minimal complexity in macromolecular architectures that may still facilitate this effect. For example, if there exist only two α-helical polypeptides in proximity to one another (e.g., polymerization from two initiators linked by a molecular spacer, a "hinged" architecture), would cooperative polymerization still exist and the polymerization rates still be accelerated in this very simple, dipeptide bundle "tertiary" structure? Herein, we report the discovery of dramatic acceleration of the polymerization enabled by a motif as simple as a single polypeptide helix hinged in proximity to the propagating polypeptide chain.

Aliphatic diamines are ideal initiators to form the expected "hinged" polypeptide structures in order to test whether a single polypeptide helix has sufficient structural effect in accelerating the polymerization of the covalently connected neighboring polypeptide chain, as depicted in Figure 1a.[26-29] We used 1,6-diaminohexane (C_6-diNH_2) for the polymerization of γ-benzyl-$_L$-glutamate *N*-carboxyanhydride (BLG-NCA) in dichloromethane (DCM) (Figure 1a; entry 4, Table 1; Table S1). Under the experimental condition of $[M]_0$ = 0.10 M and $[M]_0/[I]_0$ = 50, the polymerization initiated by C_6-diNH_2 finished in 40 min (red curve, Figure 1b). In contrast, the control polymerization initiated by C_6-NH_2, the monoamine analogue of C_6-diNH_2,

showed less than 10% of the BLG-NCA consumption in the same period (blue curve, Figure 1b). In both reactions, the polymerization kinetics can be divided into two stages: a relatively slow "nucleation" stage in which the NCA monomers are added into short coil chains (apparent rate constants k_{app1}, Table S2), followed by a fast "growth" stage in which the monomers are added into helical chains (DP > 8~12) (apparent rate constants k_{app2}, Table S2).[30-32] The onset of the second stage always coincides with the formation of helical chains, as evidenced by the shift of IR absorbance from 1658 cm^{-1} (random coil) to 1653 cm^{-1} (α-helix) (Figure 1c and Figure S1).[33] The rate acceleration (k_{app2}/ k_{app1}) of the polymerization initiated by C_6-NH_2 was found to be modest, with a 4 fold increase in the apparent rate constant upon the formation of α-helices (entry 1, Table S2). In contrast, the apparent polymerization rate upon forming α-helices increased 38 times when C_6-diNH_2 was used as the initiator (entry 4, Table S2), forming hinged PBLGs with two growing arms. This drastic rate increase indicates strong effects on the NCA polymerization by having two helical macrodipoles located in proximity.

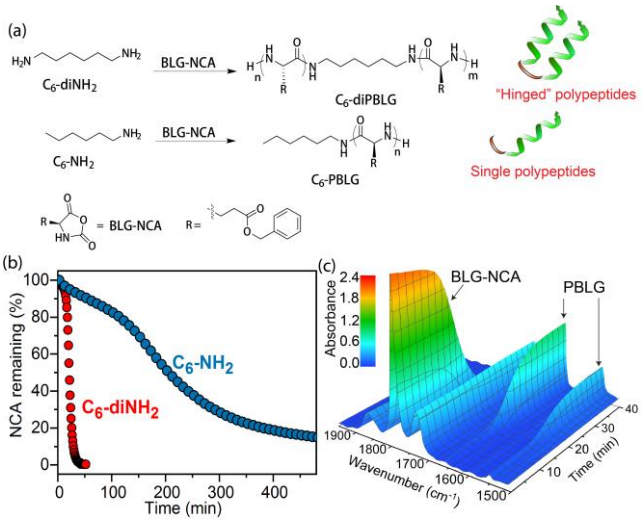

Figure 1. (a) Polymerization of BLG-NCA initiated by C_6-diNH_2 and C_6-NH_2 and schematic illustration of "Hinged" polypeptides and single polypeptides. (b) Conversion of BLG-NCA as measured by FTIR in DCM using 1,6-diaminohexane (C_6-diNH_2) and hexylamine (C_6-NH_2) as initiators ($[M]_0$ = 0.10 M, $[M]_0/[I]_0$ = 50) showing remarkable rate enhancement. (c) Changes in the concentration of BLG-NCA and PBLG in the FTIR spectrum over the duration of the polymerization of BLG-NCA initiated by C_6-diNH_2.

Table 1. Polymerization of BLG-NCA in DCM ($[M]_0$ = 0.10 M).

Entry	Initiator	$[M]_0/[I]_0$	M_n[a] (kDa)	M_w/M_n
1	C_6-NH_2	50	14.9	1.14
2	C_2-diNH_2	50	44.0	1.15
3	C_4-diNH_2	50	47.9	1.10
4	C_6-diNH_2	50	43.4	1.10
5	C_8-diNH_2	50	42.9	1.12
6	C_{10}-diNH_2	50	35.0	1.13
7	C_{12}-diNH_2	50	35.2	1.11

[a] Determined via gel permeation chromatography.

The rate enhancement is likely due to the proximity of parallel aligned two macrodipoles from the α-helices, in analogy to our recently reported polypeptide brush system.[25] To confirm this hypothesis, we investigated the polymerizations in which the helical macrodipole was either eliminated by using racemic monomers or reduced by using polar solvents with higher dielectric constant than DCM.[25] Figure 2a shows the polymerization of racemic NCA monomers (BDLG-NCA) from C_6-diNH_2. The use of racemic monomers prevents the formation of α-helix in the resulting polypeptide chains, resulting in the loss of the second-stage, accelerated polymerization. In addition, effects stemming from the electrostatic nature of the macrodipole should vary with the dielectric constant of the solvent, which is clearly evidenced in Figure 2b. Polymerization of BLG-NCA by C_6-diNH_2 in solvent with lower dielectric constants showed greater polymerization rate. In DMF, a polar solvent with high dielectric constant (ε = 38), the two-stage characteristic of the polymerization disappeared, substantiating the importance of solvent in maintaining the helical structure for the intended macrodipole which is essential to the acceleration of the polymerization.[26] The polymerization in THF, although with a lower dielectric constant (ε = 7.5) than DCM, is slower than the polymerization in DCM, likely due to the interfere of THF with the hydrogen bonds of helical polypeptide thus slowing down the NCA polymerization.

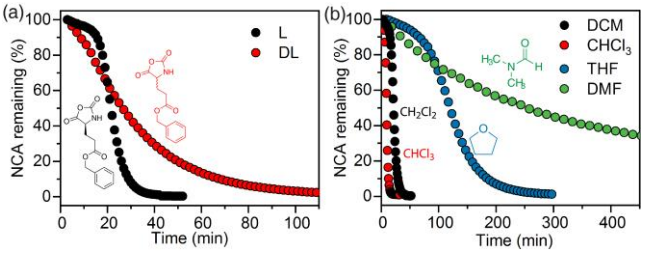

Figure 2. (a) Conversion of BLG-NCA and BDLG-NCA as measured by FTIR in DCM using C_6-diNH_2 as initiators ($[M]_0$ = 0.10 M, $[M]_0/[I]_0$ = 50). (b) Conversion of BLG-NCA as measured by IR in DCM (dielectric constant ε = 9.10), chloroform (ε = 4.81), and DMF (ε = 38) initiated by C_6-diNH_2 ($[M]_0$ = 0.10 M, $[M]_0/[I]_0$ = 50).

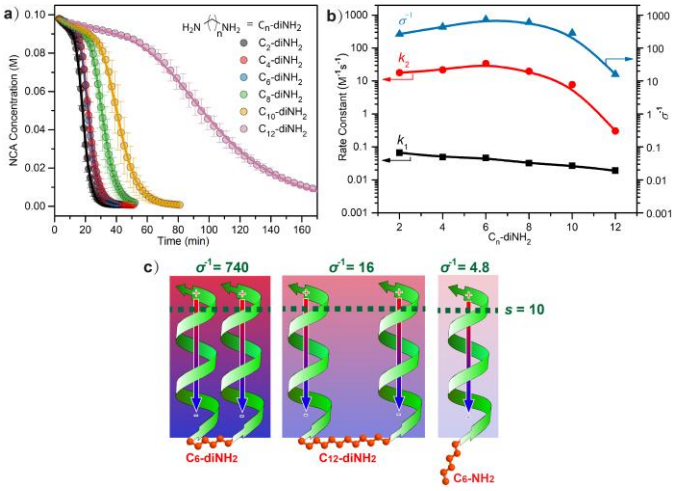

Figure 3. (a) Kinetic data (circles) obtained from the polymerization of BLG-NCA using C_n-diNH_2 (n = 2, 4, 6, 8, 10, 12) as initiators at $[M]_0$ = 0.10 M and $[M]_0/[I]_0$ = 50 is fitted with the two-stage kinetic model (solid lines) at s = 10. Error bars represent standard deviations from three independent measurements. (b) Extracted k_1, k_2 and calculated σ^{-1} for different C_n-diNH_2. (c) Representation of the proximity of macrodipoles induced polymerization of α-helical polypeptides by C_6-diNH_2, C_{12}-diNH_2 and C_6-NH_2 after reaching critical chain length s = 10. The positive pole is located at the actively growing N-terminus and the direction of growth is shown as the green arrow at the end of growing polypeptides.

To elucidate the effect of proximity between amino groups and hence α-helices on the polymerization rate, diamines with various spacer lengths were examined (entries 2-7, Table 1; Figure 3a; Table S1). Two-stage kinetics and significant rate enhancement in the second stage were observed in all diamine initiators with the length of spacer (n) from 2 to 12 (entries 2-7, Table 1). The apparent propagation rate constants (k_{app2}, Table S2) of the polymerization initiated by different diamines were well correlated with the length of spacers, and the proximity effect starts to disappear quickly after n > 10. This result indicates that the proximity of the two growing helical polypeptides in the same macromolecule plays an essential role in the rate enhancement of polymerization. The dependence of chain propagation rate on the formation of and the distance between helical macrodipoles in the proximity of active chains reveals a characteristic cooperative behavior in the polymerization of these "hinged" polypeptides.

A cooperative growth mechanism[2] (Oosawa's model developed for actin polymerization) was adapted to analyze this class of covalent cooperative polymerizations that consist of two successive growth stages. Distinct two-stage feature is observed after the growing chains reach a critical length and form helices which possess secondary interactions between monomer units and stronger macrodipoles. In this two-stage model, reaching the critical degree of polymerization, s, causes the propagation constant to change from k_1 to k_2 due to the folding of the coil into an α-helix. The stepwise addition of monomer, M, onto the active chains in the first and second stage are described in equation (1) and (2), respectively. We denote an active polymer of degree of polymerization i by M_i^*, where * represents the reactive end. The kinetic cooperativity factor, following the convention in supramolecular polymerization, can be defined as the dimensionless ratio $\sigma = k_1/k_2$ where a very small value of σ ($\ll 1$) implies a highly cooperative reaction.

$$M_i^* + \mathrm{M} \xrightarrow{k_1} \mathrm{M}_{i+1}^* \quad 1 \le i < \mathrm{s} \quad \text{(coil state)} \quad (1)$$

$$M_i^* + \mathrm{M} \xrightarrow{k_2} \mathrm{M}_{i+1}^* \quad i \ge \mathrm{s} \quad \text{(helix state)} \quad (2)$$

The two-stage, cooperative growth model was applied to the data obtained from the polymerization of BLG-NCA using C_6-NH_2 and C_n-diNH_2 (n = 2, 4, 6, 8, 10, 12) as initiators at $[M]_0$ = 0.10 M and $[M]_0/[I]_0$ = 50. The optimized fits for the data (solid lines in Figure 3a) were obtained with the critical degree of polymerization, s, to be 10 for all the samples. The rate constants (k_1 and k_2) as well as the inverse of the kinetic cooperativity factor σ^{-1} ($\sigma^{-1} = k_2/k_1$) for each polymerization initiated by diamine as a function of spacer length are summarized in Figure 3b. Compared to C_6-NH_2, k_2 of the polymerization initiated by C_6-diNH_2 is increased dramatically (Figure S3). By shortening the spacers from C_{12}-diNH_2 to C_2-diNH_2, k_1 only slightly increases, while k_2 increases 112 times, from 0.304 M^{-1} s^{-1} of C_{12}-diNH_2 to 34.0 M^{-1} s^{-1} of C_6-diNH_2 (entries 4 and 7, Table S3). Furthermore, σ^{-1} varies between 270 and 740 when the diamine hydrocarbon spacer length ranges from 2 to 10 (C_2-diNH_2 to C_{10}-diNH_2) with highest cooperativity occurring at C_6-diNH_2, and then decreases rapidly to 16 with the use of C_{12}-diNH_2. (entries 2-7, Table S3) The result shows that appropriate proximity of the helical macrodipoles dictates the rate enhancement in the polymerization. We note that even in C_{12}-diNH_2, the acceleration of the chain growth rate (σ^{-1} = 16) is still much larger than that in monoamine C_6-NH_2, in which less than 5-fold acceleration (σ^{-1} = 4.8) was found (entry 1 and 7, Table S3). C_6-diNH_2 peaks the overall proximity effect and shows the second-stage chain propagation rate of the NCA polymerization is 613 times fast than that of the polymerization initiated by C_6-diNH_2 (entry 4, Table S3). It is remarkable that such a strong cooperative behavior on chain propagation can be induced by simply having two growing chains located in proximity.

Precise control and quantitative regulation of molecular cooperativity is very important in biology and chemistry but is yet to be achieved. In this study, we demonstrate the important role of the cooperative behaviors in the synthesis of polypeptides, in which the ring-opening polymerization of NCAs can be drastically accelerated simply by using aliphatic diamines as initiators in dichloromethane. The fact that the interaction of simply two helical chains are sufficient to facilitate strong cooperative behavior in the polymerization suggests this type of proximity-induced cooperative polymerization may be utilized in the synthesis of a variety of macromolecules involving the formation of strong macrodipoles. The simple design in this study also provides an ideal system to calibrate the cooperative strength and precisely determine parameters that matter in the cooperative reaction, potentially allowing in-depth, mechanistic studies on the cooperative covalent polymerization. Furthermore, bundling of helix (e.g., three- or four-helix bundles and coiled coils) forms important protein structural motif, which have regulated numerous biological activities and shown profound impact on the ubiquitous cooperativity observed in biology. In this study, we unfolded the importance of helix as one of the remarkable cooperativity-enabling elements in synthetic chemistry, by demonstrating that this structural motif can self-catalyze its own formation by packing two helices in proximity.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Experimental procedures, simulations with kinetic model, GPC-LS traces and predicted DP from a modified two-stage model of polypeptides (PDF).

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Notes

The authors declare no competing financial interests.

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