Response speed control of helicity inversion based on a “regulatory enzyme”-like strategy

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In biological systems, there are many signal transduction cascades in which a chemical signal is transferred as a series of chemical events. Such successive reaction systems are advantageous because the efficiency of the functions can be finely controlled by regulatory enzymes at an earlier stage. However, most of artificial responsive molecules developed so far rely on single-step conversion, whose response speeds have been difficult to be controlled by external stimuli. In this context, developing artificial conversion systems that have a regulation step similar to the regulatory enzymes has been anticipated. Here we report a novel artificial two-step structural conversion system in which the response speed can be controlled based on a regulatory enzyme-like strategy. In this system, addition of fluoride ion caused desilylation of the siloxycarboxylate ion attached to a helical complex, resulting in the subsequent helicity inversion. The response speeds of the helicity inversion depended on the reactivity of the siloxycarboxylate ions; when a less-reactive siloxycarboxylate ion was used, the helicity inversion rate was governed by the desilylation rate. This is the first artificial responsive molecule in which the overall response speed can be controlled at the regulation step separated from the function step.

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Results and Discussion

Requirements for the F⁻-triggered helicity inversion in this system is that the carboxylate ions before and after the desilylation should induce opposite helicities of the $\text{LZn}_3\text{La}$. Thus, we investigated the CD spectra of $\text{LZn}_3\text{La}$ in the presence of several chiral carboxylic acids ($\text{S}_1\cdot\text{H}$, $\text{S}_2\cdot\text{H}$, $\text{H}_1\cdot\text{H}$, and $\text{H}_2\cdot\text{H}$) and 3 equiv of DABCO.

![Figure 1](image1.png)

Figure 1. Concept and design of responsive functional systems based on a regulatory enzyme-like strategy. (a) One-step structural conversion for responsive functions. (b) Multi-step structural conversion for responsive functions. The function activity (reaction rates) may be controlled at an earlier step called the regulation step. (c) Design of a new artificial system for helicity inversion mediated by desilylation of the coordinating siloxycarboxylate ions at the regulation step.

![Figure 2](image2.png)

Figure 2. CD spectra of $\text{LZn}_3\text{La}$ (0.20 mM, acetonitrile/chloroform, 9:1, path length 1 mm, 295 K) in the presence of 3 equiv of chiral carboxylic acids ($\text{S}_1\cdot\text{H}$, $\text{S}_2\cdot\text{H}$, $\text{H}_1\cdot\text{H}$, and $\text{H}_2\cdot\text{H}$) and 3 equiv of DABCO.

...substances. In the present $\text{LZn}_3\text{La}$ system, the helicity is sensitively affected by structural differences in the chiral carboxylate ions, whereas the helicity inversion rate is not significantly affected (thus called the intrinsic helix inversion rate, hereafter). These facts inspired us to design a system in which helicity inversion is driven by a slow chemical transformation in the coordinating carboxylate ions. In fact, there have been several helical metal complexes that can change their helix inversion rates by replacing the central metal ion. The time-programming in these systems needs to change the intrinsic helix inversion rates, whereas the helix inversion rates of the present system can be controlled at the regulation step without changing the intrinsic helix inversion rates. We now report this new type of two-step structural conversion in which the response speed of the helicity inversion at the final function step was effectively controlled at the regulation step using siloxycarboxylate ions with different reactivities.
Therefore, we expected that, if the silyl group in S1⁻ and S2⁻ is removed by the reaction with fluoride ion, a responsive helicity inversion should take place.

Indeed, the addition of fluoride ion caused significant changes in the CD spectra. While the siloxycarboxylate ion S1⁻ induced a negative Cotton effect at 350 nm attributable to the (M)-helicity of LZn3La (Fig. 3a,i), the Cotton effect started to immediately decrease after the addition of 3 equiv of fluoride ion. The intensity decreased with approximate first-order kinetics and turned positive after 30 min. The spectral changes were almost completed after 100 min (Fig. 3b,i) to result in a CD spectrum similar to that of the (P)-helical LZn3La in the presence of H1-H and DABCO (Fig. 2). This suggested that the siloxycarboxylate ion S1⁻ coordinating to LZn3La was converted into the desilylated derivative H1⁻. This was clearly evidenced by the ESI-MS peak (m/z = 611.0 for [LZn3La + H1⁺]) observed in the solution after reaction with the fluoride ion (Supplementary Fig. S3).

Interestingly, the structures of the siloxycarboxylate ions significantly affected the response speeds of the helicity inversion. We similarly prepared the (M)-helic LznLa complex by using the mandelate-based siloxycarboxylate ion S2⁻ in place of the lactate-based S1⁻. This helical complex, LznLa with S2⁻, also showed a gradual decrease in the CD intensity after the addition of 3 equiv of fluoride ion, but the reaction was so slow that the CD signal did not turn positive even after 720 min (Supplementary Fig. S5). When the amount of fluoride ion was increased from 3 equiv to 4 equiv (Fig. 3a,ii), the helicity was inverted as observed for LznLa with S1⁻. However, the reaction was still significantly slow compared to the LznLa−S1⁻ system; the CD signal turned positive after 120 min, but it took 650 min to complete the reaction (Fig. 3b,ii). The resultant CD spectrum (+23.7 mdeg at 350 nm, Fig. 3a,ii) was very similar to that of LZn3La in the presence of H2-H and DABCO (+23.7 mdeg at 350 nm, Fig. 2). This indicated that the siloxycarboxylate ion S2⁻ coordinating to LznLa underwent desilylation to give the hydroxycarboxylate H2-. This was confirmed by the ESI-MS peak (m/z = 641.9 for [LznLa + H2⁺]) (Supplementary Fig. S6).

As already described, it is clear that the helicity inversion of LznLa was triggered by the fluoride ion via the desilylation of S1⁻ or S2⁻ coordinating to the LznLa helical complex. However, the LznLa−S1⁻ system showed significantly faster response than the LznLa−S2⁻ system. This difference should mainly arise from the different reactivity of the silyl groups in the carboxylate ions S1⁻ and S2⁻ toward the fluoride ion. In the case of the lactate-based S1⁻, the silyl group was completely removed within 3 min (Supplementary Fig. S4), which was evidenced by the ¹H NMR analysis. Since the observed half-life of the CD intensity changes (t½ ≈ 20 min) was much longer than that of the desilylation (t½ < 1 min), the response speed of the helicity inversion should be governed by the intrinsic helix inversion rate of the LznLa scaffold⁴,⁵ (Fig. 4a). On the other hand, the ¹H NMR analysis indicated that the desilylation of S2⁻ was very slow; the unreacted S2⁻ still remained even after 120 min (Supplementary Fig. S7). It should be noted that the observed response speed of the helicity inversion is much slower than the intrinsic helix inversion rate of the LznLa scaffold. Obviously, the observable overall response speed of the helicity inversion is controlled at the desilylation step (Fig. 4b). Therefore, the helicity inversion of LznLa was triggered by the fluoride ion, and the response speed was controlled at the regulation step of the signaling cascade by using the siloxycarboxylate ion without changing the intrinsic helix inversion rate.

In summary, we have developed a new artificial signal transduction cascade system for controlling the helicity inversion speeds. The fluoride ion triggered two successive chemical events, e.g., desilylation of the siloxycarboxylate ions followed by helicity inversion of the LznLa dynamic helix. The overall response speed was efficiently controlled at the regulation step of the signaling cascade, just like regulatory enzymes in biological systems, by using the slower desilylation of the siloxycarboxylate ions without changing the intrinsic helix inversion rates. Before this study, the control of the response speeds of functional molecules had been believed to require modification of their parent molecular framework. Our research of the function tuning at the regulation step in a signal transduction cascade could be applied to a variety of functional molecular systems that can control the response speed without altering the intrinsic nature of the functional molecules. In addition, this fine-tuning of the response speeds would open the way to new chemistry in which molecular machinery motions and chemical functions are controlled in a time-programmable fashion.

**Methods**

**General procedures.** All chemicals were reagent grade and used without further purification. Column chromatography was performed with Kanto Chemical silica gel 60 N (spherical, neutral). ¹H NMR spectra
were recorded on a Bruker AVANCE600 spectrometer (600 MHz), a Bruker DPX400 (400 MHz), or a Bruker AVANCE400 spectrometer (400 MHz). In NMR measurements, tetramethylsilane was used as an internal standard (0 ppm). CD spectra were recorded on a JASCO J-820 spectropolarimeter at 295 K. Mass spectra (ESI-TOF, positive mode) were recorded on an Applied Biosystems QStar Pulsar i spectrometer.

Silylation of ethyl lactate (Fig. 5). Under nitrogen atmosphere, tert-butyldimethylchlorosilane (10.0 g, 66.3 mmol) was added to a solution of (S)-ethyl lactate (7.2 mL, 63 mmol) and imidazole (5.15 g, 75.6 mmol) in dry dichloromethane (40 mL). The mixture was stirred for 2 h at room temperature. After addition of water, the mixture was extracted with dichloromethane. The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The crude oily product was purified by column chromatography (silica gel, ethyl acetate/hexane, 2:100) to give ethyl (S)-2-((tert-butyldimethylsilyloxy)propanoate (E1) (15.6 g, quant.) as colorless oil, 1H NMR (400 MHz, CDCl3) δ 0.07 (s, 3 H), 0.10 (s, 3 H), 0.91 (s, 9 H), 1.28 (t, J = 7.1 Hz, 3 H), 4.14–4.21 (m, 2 H), 4.31 (q, J = 6.8 Hz, 1 H).

Preparation of a stock solution of (S)-2-((tert-butyldimethylsilyloxy)propanoic acid (S1·H; Fig. 5). An aqueous solution of lithium hydroxide monohydrate (49.3 mg, 1.17 mmol in 4 mL of water) was added dropwise to a solution of ester E1 (119 mg, 0.510 mmol) in THF (4 mL) at 0 °C. The mixture was stirred for 4 h at room temperature and then concentrated. The solution was acidified to pH 4–5 with aqueous KHSO4 solution (1 M) and extracted with chloroform. The combined organic layer was dried over anhydrous sodium sulfate and filtered. The product S1·H was stored as chloroform solution, because S1·H gradually decomposes without solvent. 1H NMR (400 MHz, CDCl3) δ 0.07 (s, 3 H), 0.94 (s, 9 H), 1.46 (d, J = 7.1 Hz, 3 H), 4.31 (q, J = 6.8 Hz, 1 H).
Silylation of mandelic acid (H2·H; Fig. 5). Under nitrogen atmosphere, tert-butylidimethylchlorosilane (307 mg, 2.04 mmol) was added to a solution of (S)-mandelic acid (H2·H; 96.0 mg, 0.631 mmol) and imidazole (190 mg, 2.79 mmol) in dry DMF (2 mL) at 0 °C. The mixture was stirred for 32 h at room temperature. After addition of water, the mixture was extracted with diethylether. The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. The crude oily product was purified by column chromatography (silica gel, ethyl acetate/hexane, 3:7) to give ethyl (S)-2-[(tert-butylidimethylsilyloxy)-2-phenylacetate (E2)48 (230 mg, 0.605 mmol, 95%) as pale yellow oil. 1H NMR (400 MHz, CDCl3) δ 0.01 (s, 3 H), 0.11 (s, 3 H), 0.14 (s, 3 H), 0.19 (s, 3 H), 0.82 (s, 9 H), 0.91 (s, 9 H), 5.14 (s, 1 H), 7.26–7.33 (m, 3 H), 7.44–7.47 (m, 2 H).

Preparation of a stock solution of (S)-2-[(tert-butylidimethylsilyloxy)-2-phenylacetate (S2·H; Fig. 5). A solution of potassium carbonate in 50% aqueous methanol (1 M, 30 mL) containing ester E2 (116 mg, 0.305 mmol) was heated to reflux for 1 h. After cooling to room temperature, the solution was concentrated. The residue was acidified to pH 4–5 with diluted hydrochloric acid (0.5 M) and the solution was extracted with chloroform. The combined organic layer was dried over anhydrous sodium sulfate and filtered. The product S2·H48 was stored as chloroform solution, because S2·H gradually decomposes without solvent. 1H NMR (400 MHz, CDCl3) δ −0.02 (s, 3 H), 0.13 (s, 3 H), 0.94 (s, 9 H), 5.20 (s, 1 H), 7.34–7.43 (m, 5 H) (Supplementary Fig. S2).

Helicity inversion by F− addition. A chloroform solution of the siloxycarboxylic acids (S1·H or S2·H, 3 equiv) was added to an acetonitrile solution of [ZnL40 in the presence of DABCO (3 equiv). After 5 min, an acetonitrile solution of DABCO (2 equiv) was added to an acetonitrile solution of tetrabutylammonium fluoride (3 or 4 equiv) was added to the solution and the time course of the CD spectral changes was investigated. The solvent ratio of the solution was adjusted to be acetonitrile/chloroform = 9:1.

Data availability. Data supporting the findings of this study are available within the article (and its Supplementary Information files) and from the corresponding author on reasonable request.

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**Author Contributions**
S.S. Conducted all of the synthesis and characterization of the materials as well as spectroscopic measurements. S.A. initiated and guided this work discussing with T.N. All three authors participated in the writing and editing of the manuscript.

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