COMMUNICATION

Macroscopic chiral symmetry breaking in gelation of Fmoc-amino acids. Homochiral selective secondary nucleation promoted by the choice of solvent or stirring

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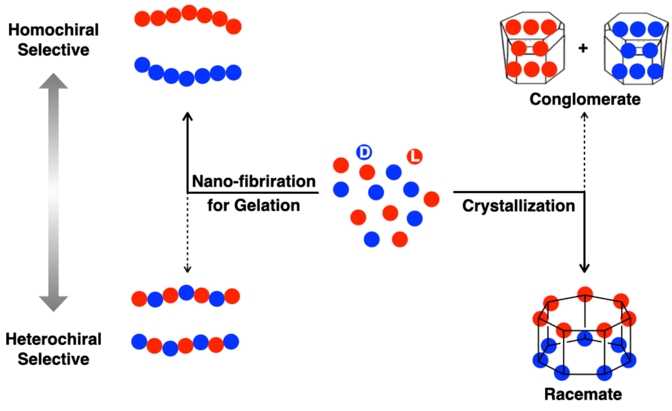
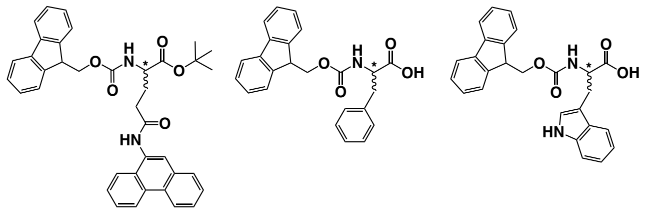
A racemic mixture of *N*-Fmoc-protected phenylalanine (2) or tryptophan (3) was found to spontaneously afford a gel enriched in either L- or D-form of their enantiomers stochastically. Homochiral selective secondary nucleation, promoted by the choice of solvent or stirring, was suggested as the key process for the observed phenomenon.

Introduction

When a chiral compound undergoes self-assembly, its stereochemistry is known to provide significant effects on the direction of the processes and the stability or morphology of the resultant nanostructures.[1–4] Among diverse self-assembling processes of chiral compounds, crystallization[5–7] and gelation[8–10] are the representative examples that have been deeply and lengthily investigated from not only fundamental science but also industrial points of view. If a stoichiometric mixture of enantiomers crystallizes, heterochiral selective assembly dominantly takes place to afford racemic crystals, suggesting that these crystals tend to be thermodynamically more advantageous than the corresponding homochiral crystals, i.e., conglomerates (Scheme 1).[11–13] In contrast, a gel obtained from a racemic mixture of enantiomers mostly exhibits inferior thermal or mechanical stability than that obtained from the corresponding single enantiomer.[14] Such an intriguing contrast in the effects of chirality on the stability of crystals and gels inspires a hypothetical idea that, in gelation, homochiral selective assembly of enantiomers might be the predominant event (Scheme 1).[15,16] This idea also allows to expect that chiral symmetry breaking could be observed with a higher probability in gelation rather than in crystallization, in the latter of which the spontaneous formation of conglomerate crystals from a racemic mixture of enantiomers has been regarded as a quite minor scenario (< 10%).[11,17] In accordance with these hypothetical assumptions, we have recently revealed that macroscopic chiral symmetry breaking really occurs in gelation of an *N*-9-fluorenylmethyloxycarbonyl (Fmoc)-protected glutamate derivative (**1**; Fig. 1), whose racemic mixture (rac-**1**) was found to afford organogels enriched in either of its enantiomers stochastically.[18] Contrary to the self-assembling behaviour of rac-**1** in gelation, its crystallization under a



Scheme 1. Schematic representation of the tendencies of a racemic mixture of enantiomers that prefers homo- and heterochiral selective assembly at its gelation and crystallization, respectively.



1 2 3

Fig. 1. Molecular structures of Fmoc amino acid-based gelators **1**, **2** and **3**.

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† Electronic supplementary Information available: Preparation and analyses of the assemblies of **2** and **3** together with the evaluation of enantiomeric excess values of these compounds in the assemblies. See DOI: 10.1039/x0xx00000x

different solvent condition proceeds heterochiral selectively, leaving only racemic crystals. These observations raise questions if other chiral compounds also exhibit similar chiral symmetry breaking behaviour in gelation, and if it is the case, what are the important factors for this phenomenon to emerge. Here commercially available two amino acid-based gelators (Fmoc phenylalanine **2** and tryptophan **3**; Fig. 1) were picked up for the surveys particularly to see if not only recently designed specific molecular structures such as **1** but also gelators already well-explored commonly share the same feature.

Results and discussion

Although gelation of several Fmoc amino acids as a form of single enantiomer has been reported so far,[19–21] little has been known in the case of their racemic mixtures. Fmoc phenylalanine **2** is one of the few examples whose racemic mixture (rac-**2**) was found to afford gels.[20] With this literature information in mind, solvent conditions reported for rac-**2** gelation such as phosphate buffer (PB, 50 mM, pH = 7.4) and PB/DMSO (9/1, in vol.) mixture were applied for racemic mixtures of other Fmoc amino acids. While most of the tested racemic Fmoc amino acids/solvent combinations turned out to be unsuitable to obtain gels, racemic form of Fmoc tryptophan (rac-**3**) in PB/DMSO showed a sign of gelation. Both of rac-**2** and **3** showed inferior gelation capability than the corresponding their single enantiomers (Table S1†), as is the usual case of chiral molecular gelators including previously reported **1**.[14,18] rac-**2** in PB (5.2 mM) afforded mechanically the least tolerant assembly among them (Fig. S1†), while its xerogel displayed entangled nano-fibrous morphology in scanning electron microscopy (Fig. S2†).

With these basic data, rac-**2** or **3** was subjected to the gelation under the above-mentioned solvent conditions to seek the possibility of the production of chiral symmetry-broken states macroscopically. A mother solution of rac-**2** in PB/DMSO ([**2**] = 15.5 mM) was divided into eight portions, individual of which was kept in a vial at 20 °C for more than 4 days to obtain a weak gel or sol–gel mixture. These gels showed a stochastic fluctuation in their ee values from –21 to 26% (Figs. 2a purple and S4†), demonstrating that **2**, as similarly to **1** (Fig. 2d)[18] also exhibits macroscopic chiral symmetry breaking phenomenon in its gelation. In sharp contrast, all the gels obtained from rac-**2** in PB (5.2 mM) showed only small deviations from zero in their ee values (less than 2% ee; Figs. 2b purple and S5†), proving that macroscopic chiral symmetry breaking in gelation of rac-**2**, if any, takes place inefficiently in this solvent. rac-**3** in PB/DMSO ([**3**] = 30.9 mM) also behaved similarly to rac-**2** in PB, as seen in Figs. 2c purple and S6†. Since heterochiral selective nano-fibrillation of these gelators (Scheme 1) could be a one of the possible reasons for the absence of chiral symmetry breaking in their gelation, a scalemic mixture of L- and D-enantiomers of **2** (L-**2** and D-**2**, respectively) or **3** (L-**3** and D-**3**, respectively) was subjected to the gelation to see if the ratio of the major enantiomer in the resultant gel is less than that in the starting mixture. However, this possibility was clearly excluded for both of **2** and **3**, as the gelation of a scalemic mixture was always accompanied with the enrichment of the major enantiomer in the gel. When a L-rich mixture of L-**2** and D-**2** was subjected to the gelation ([**2**] = 15.5 and 5.2 mM in PB/DMSO and PB, respectively), a sol–gel mixture resulted with the enrichment of the major L-**2** enantiomer in the gel. The enantiomeric excess (ee) values of **2** in the start/gel/sol were evaluated to be 31/73/22 and 33/74/19% in PB/DMSO and PB, respectively, by means of chiral HPLC (Figs. S7a–f and S9†). While the difference in ee values of the L-enantiomer among the start/gel/sol was less for **3** in PB/DMSO (39/48/35%, Figs. S7g–i and S9†) than those observed for **1** (32/72/8%,[18] Fig. S9†) and **2**, **3** also showed the enrichment of the major enantiomer through gelation, suggesting that homochiral selective assembly commonly takes place in the gelation of **2** and **3** as previously observed for **1** (Scheme 1). As expected, the D-enantiomer was enriched in the gel when starting with a D-rich mixture for all of **1**–**3** (Figs. S8 and S10†). In the previous work, comparison with the crystal structures of an analogue of **1**[18,22] allowed to estimate that **1** in its gelation undergo homochiral selective assembly to afford 1D columns through π-stacking and hydrogen bonding (HB) interactions.[18] Three crystal structures of L-**2** with and without solvents have been reported by Adams *et al.*[23] and Nilsson *et al.*,[24] all of which commonly show similar 1D columns of L-**2** assembled via Fmoc–Fmoc and benzyl–benzyl *π*-stacking as well as carbamate–carbamate HB interactions. Such homochiral columnar assemblies of **2** are the most plausible structural candidates that exist in the gels of **2**. Those intra-columnar interactions between the neighbouring L-**2** give a rationale why homochiral selective assembly of the enantiomers of **2** can be more preferred than their heterochiral selective assembly in gelation as the perfect overlap between non-superimposable L- and D-**2** along with the column axis is hard to occur. DMSO in

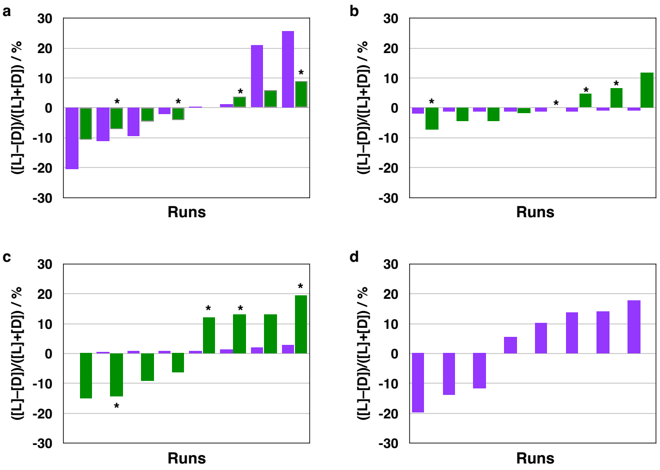
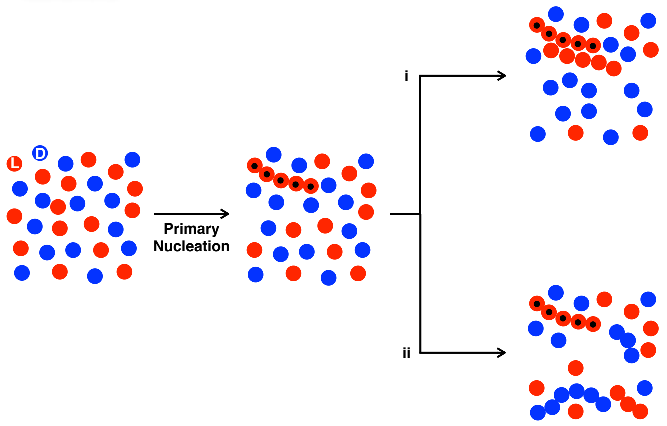


Fig. 2. Enantiomeric excess values of gels obtained from (a) rac-**2** in PB/DMSO (9/1, in vol.; [**2**] = 12.9 and 15.5 mM for with and without stirring, respectively), (b) rac-**2** in PB ([**2**] = 4.4 and 5.2 mM for with and without stirring, respectively), (c) rac-**3** in PB/DMSO (9/1, in vol.; [**3**] = 26.3 and 30.9 mM for with and without stirring, respectively) and (d) rac-**1** in acetonitrile ([**1**] = 10.2 mM). Values in green and purple were obtained with and without stirring the solutions for gelation, respectively. Values with asterisk (*) were obtained by stirring the corresponding solutions in clockwise (cw) and then counterclockwise (ccw) directions, while other values in green were obtained with opposite order (ccw –> cw).† Results for rac-**1** in acetonitrile reported in reference 18 are included as (d) for comparison.



Scheme 2. Schematic representation of the nanofibril formation for gelation in a racemic solution of **2** or **3** that is governed by (i) homochiral selective secondary or (ii) random primary nucleation.

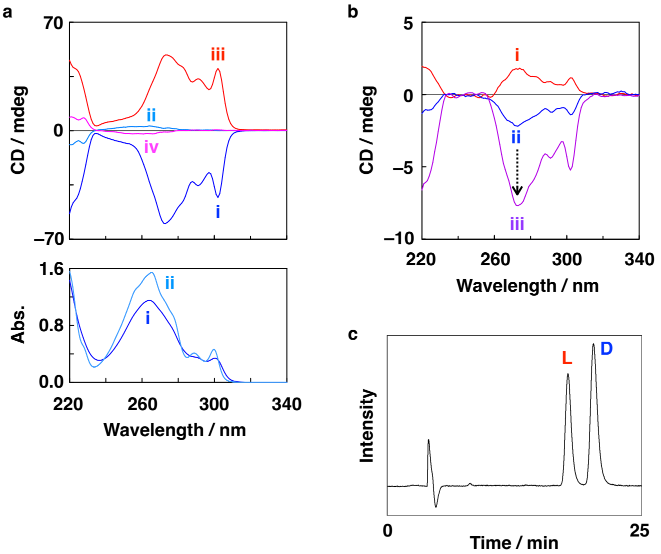
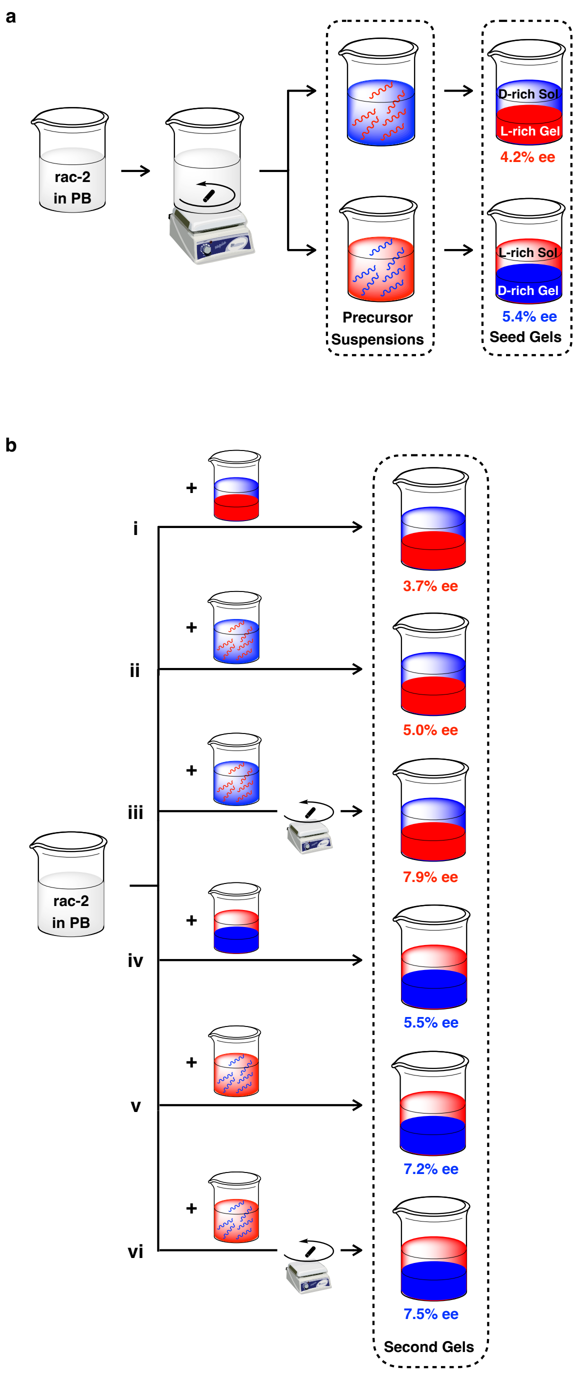


Fig. 3. (a) CD spectra of gelling (i) D-**2** and (iii) L-**2** and solutions of (ii) D-**2** and (iv) L-**2** in PB together with the corresponding absorption spectra of the (i) gel and (ii) solution of D-**2** at 20 °C. Gels (7.8 mM) and solutions (0.78 mM) were subjected to the measurements with quartz cells having optical lengths of 0.1 and 1 mm, respectively. (b) CD spectra of (i and ii) two solutions of rac-**2** in PB after stirring for 2 h and (iii) the sample affording CD (ii) after gelation for additional 22 h. (c) Chiral HPLC chromatogram of **2** in the gel that exhibiting CD (b-iii).

the media for the crystallization of L-**2** was found to co-crystallize with L-**2** by filling the inter-columnar spaces in the crystalline lattice.[24] This observation provides a speculation that molecular shape of DMSO might have an advantage to promote homochiral selective secondary nucleation when **2** self-assembles into 1D nanostructures (Scheme 2-i). This hypothetical idea allows to explain why macroscopic chiral symmetry breaking in gelation emerges for **2** in PB/DMSO (Scheme 2-i) but not for **2** in PB and **3** in PB/DMSO (Scheme 2-ii).

Given that homochiral selective assembly is suggested to occur in gelation of **2** and **3**, it would be curious to conduct their gelation under unprecedented conditions that possibly promote homochiral selective secondary nucleation. One of the candidates worthy to be tested is stirring the mother solution for the assembly, as its drastic effect on the direction of the self-assembly was observed in the crystallization of sodium chlorate.[25–27] In this self-assembling process, while standing a solution of $NaClO_3$ afforded statistically equal numbers of L- and D-crystals, stirring the same solution selectively gave either of L- or D-crystals, which was ascribed to the promotion of homochiral selective secondary nucleation upon stirring. Although the degree of chiral symmetry breaking from a racemic mixture such as rac-**2** or **3** could be less significant than that from an achiral state as is the case of $NaClO_3$, this report is still inspiring to introduce a stirring step in the gelation process to seek the possibility of macroscopic chiral symmetry breaking in gelation of **2** in PB and **3** in PB/DMSO. In fact, stirring a solution of rac-**2** at the initial stage of gelation has great effects on the following gelation behaviour. When a freshly prepared rac-**2** solution in PB ([**2**] = 4.4 mM) was divided into eight portions and each of them was gently stirred for 2 h at 20 °C (see supporting information on page S5†), the resultant samples apparently looked homogeneous solutions. However, standing for additional 16 h at 20 °C after stop stirring allowed all of them to turn into weak gels or sol–gel mixtures. In contrast, no sol–gel transformation was observed under the same concentration of rac-**2** for more than two weeks without stirring the solutions, which means the successful suppression of undesired random primary nucleation (Scheme 2-ii) for the emergence of macroscopic chiral symmetry breaking. More interestingly, the ee value of the gels showed a clear stochastic fluctuation from −7 to 12% (Figs. 2b green and S12†), unambiguously demonstrating the emergence of macroscopic chiral symmetry breaking in gelation of **2** in PB.[28] In order to gain more insights on the role of the stirring step, a stirred solution of rac-**2** was subjected to DLS analyses in which a scattering peak due to the assemblies of **2** was observed at a hydrodynamic diameter of 55 nm (Fig. S18†).[29] It would be noteworthy that macroscopic chiral symmetry was already broken at this stage as the stirred solutions became CD active (Fig. 3b-i or ii). Their spectral patterns are similar to that of either L- or D-**2** gel, which showed mirror-imaged CD spectra of one another having three positive and negative peaks at 273, 291, and 302 nm, respectively (Fig. 3a-i and iii).[30] Since the corresponding solutions of these enantiomers displayed only negligibly small signals in 240–320 nm (Fig. 3a-ii and iv), the CD signals of gelling L- and D-**2** in this region are most likely originating from their chiral supramolecular structures such as helical nanofibers reported by Roy and Banerjee.[19] The intensity of CD signal corresponding to D-**2** rich assembly was getting stronger along with the progress of gelation (Fig. 3b-iii), where the ee value of the major enantiomer of **2** in the resultant gel reached up to 19%, as determined by chiral HPLC (Fig. 3c). These observations demonstrated that stirring a rac-**2** solution allows the emergence of chiral symmetry broken state in the assembled **2**, which determines the enantiomer enriched in the following gel. Since the effects of stirring on the macroscopic chiral symmetry breaking in crystallization of $NaClO_3$ and



Scheme 3. Schematic representations of (a) the preparation of a seed gel and its precursor suspension by stirring a rac-**2** solution in PB and (b) seeding of the seed gel or its precursor suspension into a rac-**2** solution in PB to afford the second gel. A rac-**2** solution in PB seeded with (i) a L-rich gel, (ii, iii) its precursor suspension, (iv) a D-rich gel or (v, vi) its precursor suspension was subjected to the gelation (iii, vi) with or (I, ii, iv, v) without stirring.

gelation of **2** look alike, those observed in the latter could be explained by the similar mechanism as the former where stirring the solution promotes rapid production of secondary nuclei from a primary nucleus homochiral selectively (Scheme 2-i). In fact, seeding a chiral symmetry broken gel or its precursor, i.e., a stirred solution of rac-**2**, into another rac-**2** solution perfectly determined the direction of the enrichment of the enantiomer in the following gelation homochiral selectively (Scheme 3). rac-**2** solutions in PB ([**2**] = 4.4 mM) were subjected to the gelation procedure with stirring to afford gels enriched in either of the enantiomers as confirmed by chiral HPLC (Scheme 3a and Fig. S14a,b†). The resultant L- (4.2% ee) or D-enriched (5.4% ee) gel (seed gel, 0.15 mL) was suspended in a freshly prepared rac-**2** solution (1.5 mL) and the heterogeneous mixture was standing at 20 °C. In 24 h, both of the two mixtures seeded with L- or D-enriched gel resulted partial gelation (second gel, Scheme 3b-i and iv, respectively), which should be triggered by the seeded gel since no gelation takes place in rac-**2** solution alone under that concentration ([**2**] = 4.4 mM) as described previously. The enantiomers enriched in the seed and second gels showed a good agreement, where the ee values of the second gels (3.7 and 5.5% for L- and D-enriched gels, Scheme 3b-i and iv, respectively, and Fig. S14c,d†) were comparable to those of the seed gels. Of interest, the precursor suspensions for the seed gels showed a superior capability than the corresponding seed gels to afford larger ee values in the second gels (5.0 and 7.2% for L- and D-enriched gels, Scheme 3b-ii and v, respectively, and Fig. S14e,f†) when they were used for seeding instead of the corresponding seed gels. Further increase of the ee value (7.9 and 7.5% for L- and D-enriched gels, Scheme 3b-iii and vi, respectively, and Fig. S14g,h†) was observed when the rac-**2** solutions seeded with the precursor suspensions were subjected to the gelation with stirring, in accordance with the expectation that secondary nucleation should be promoted upon stirring. Stirring also worked in the gelation of rac-**3** in PB/DMSO ([**3**] = 26.3 mM), where the ee value of the gels displayed an even larger fluctuation from –15 to 20% (Figs. 2c green and S13†) than that observed for rac-**2** in PB with stirring (Fig. 2b green), suggesting that this sample treatment works for a range of gelators/conditions. On the other hand, gelation of rac-**2** in PB/DMSO ([**2**] = 12.9 mM) with stirring resulted a fluctuation from –10.7 to 8.9% in the ee value of the gels (Fig. 2a green and S11†), which is comparable to that for rac-**2** in PB with stirring (Fig. 2b green) but smaller than that for rac-**2** in PB/DMSO without stirring (Fig. 2a purple). Overall, macroscopic chiral symmetry breaking in gelation was proved to emerge in all the attempted gelator/solvent combinations (**2**/PB, **2**/PB/DMSO and **3**/PB/DMSO) by offering suitable treatments and conditions for the promotion of secondary nucleation, unveiling a certain degree of generality of this phenomenon in gelation of Fmoc-amino acids.

Conclusions

In conclusion, this work revealed that two Fmoc-amino acids undergo homochiral selective assembly and macroscopic chiral symmetry breaking in their gelation. Solvent conditions as well as stirring at the start of gelation were found to play the key roles for the effective operation of homochiral selective secondary nucleation to emerge macroscopic chiral symmetry breaking.[31] Surveys on the preferred behaviour of chiral compounds other than amino acids in gelation is now on-going to answer the query if a wide range of chiral compounds commonly show the same tendencies as observed for **1**–**3** when they afford gels.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

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Data availability

The data supporting this article have been included as part of the Supplementary Information.

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