VIP Supramolecular Chirality

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Self-Assembly of Racemic Alanine Derivatives: Unexpected Chiral Twist and Enhanced Capacity for the Discrimination of Chiral Species**

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Chiral nanostructures, such as double helices, twists, and rolled-up nanotubes, have been attracting great interest owing to their fascinating structural features, their relationship to biological structures, and possible applications in chiral sensing and separation.[1,2] Chiral nanostructures are mostly fabricated from chiral building blocks, whereby the molecular chirality of the building blocks determines the supramolecular chirality of the nanostructures.[3–8] In comparison with the rich knowledge that has been gathered with regard to chiral self-assembly, less is known about the supramolecular chirality of self-assembled systems based on achiral or racemic building blocks, although these systems are also very important for the understanding of self-assembly processes, symmetry breaking, and the development of new functions.[9] It has been revealed that achiral building blocks, either intrinsically achiral molecules or a racemate (an equimolar mixture of enantiomers), can occasionally self-assemble into chiral nanostructures. However, even when chiral nanostructures are obtained, both the left- (*M*) and right-handed (*P*) chiral structures are usually equally distributed, and these self-assembled systems are generally circular-dichroism (CD) silent or without any macroscopic chirality.[10–20] It remains an interesting challenge to discover such macroscopic chirality and even to develop new applications of systems self-assembled from achiral molecules or racemates.

Herein, we report an interesting system self-assembled from racemic alanine derivatives. The racemic mixture self-assembled into a chiral twist, whereas the individual enantiomers could only form flat nanostructures. Unexpectedly, the chiral twist formed showed macroscopic chirality: a slight enantiomeric excess in the twist resulted in remarkable enhancement of the CD spectra. Moreover, the twist showed a high capacity to discriminate various amino acid derivatives and could even be used to determine the *ee* value of a mixture of amino acid derivatives. To the best of our knowledge, the ability to use chiral nanostructures self-assembled from a racemate for chiral sensing has not been described previously.

Figure 1 A shows the structure of the enantiomeric molecules used, termed L-AlaC17 and D-AlaC17. These derivatives of L- and D-alanine contain an *N*-fluorenyl-9-methoxycarbonyl (Fmoc) moiety and a long alkyl chain. Upon gel formation in various organic solvents, the enantiomers self-assembled into fibrous or flat nanostructures, as shown by SEM (Figure 1 B; see also Figure S1 in the Supporting Information). Although L-AlaC17 and D-AlaC17 each formed a transparent gel in hexane, initially as fibrous nanostructures (Figure 1 B), which were transformed into nanobelts upon aging for a few hours (see Figure S2), no apparent chiral structure was obtained from the enantiomers in any solvent. However, when an equimolar mixture of L-AlaC17 and D-AlaC17 was heated until transparent and then cooled in hexane, a white suspension rather than a gel was obtained. By SEM, uniform twisted ribbons with a width of about 1 μm and a pitch of more than 5 μm were observed (Figure 1 C,D). These twisted ribbons stacked into broomlike bundles, and all twists in a bundle appeared with the same handedness, which suggests that the formation of these twists is a nucleation-controlled process.[16,21] Though both left-handed and right-handed chiral twists were observed, one of the two chiral nanostructures was predominant in a given batch.

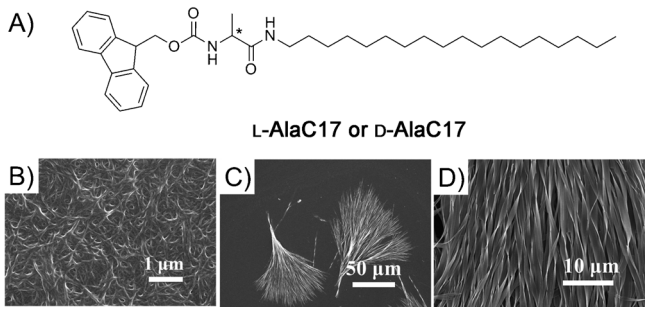

Figure 1. A) Molecular structure of the enantiomeric alanine derivatives L-AlaC17 and D-AlaC17. B) SEM image of the transparent hexane gel formed by L-AlaC17. C, D) SEM images of the suspension formed by the racemic mixture at different scales.

To gain further insight into these nanostructures, we analyzed them by X-ray diffraction (XRD), UV/Vis spectroscopy, and CD spectroscopy (Figure 2). Bragg peaks were found at 2θ values of 3.4 and 2.3° for the twist (from the

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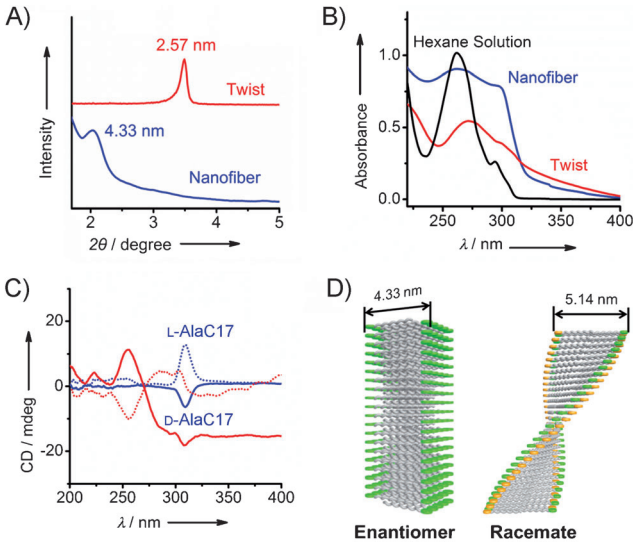

Figure 2. A) XRD, B) UV/Vis, and C) CD spectra of the enantiomeric gels (blue) and the racemic assemblies (red). Different CD signals were observed for assemblies of the racemate in different experiments. D) Schematic illustration of the molecular packing for a single enantiomer and the racemate. The green and yellow heads represent enantiomers with opposite chirality. Only one bilayer is shown for simplicity.

racemate) and nanofibers (from the enantiomers), respectively. According to the Bragg equation, the *d* spacing of the L-AlaC17 or D-AlaC17 xerogel was estimated to be 4.33 nm. This value is larger than one but less than twice the molecular thickness (about 2.8 nm; see Figure S4) and thus suggests the formation of an interdigitated bilayer structure in the assemblies. For the twist structure, the calculated *d* value of 2.57 nm is shorter than the length of a molecule and therefore also indicates a bilayer structure, but a layer distance of 5.14 nm is adopted (Figure 2D). These different packing arrangements were further confirmed by FTIR measurements (see Figure S5). The N–H stretching band of the nanofibers and the twist was centered at 3299 and 3305 cm^{-1}, respectively. This observation suggests the existence of stronger hydrogen bonds in the nanofiber. The asymmetric and symmetric CH_2 stretching bands of the alkyl group appeared at 2920 and 2851 cm^{-1} for the nanofibers and were shifted to 2917 and 2848 cm^{-1} for the twist. These IR bands indicate more ordered packing of the alkyl chain in the racemic assemblies.[22]

The UV/Vis spectrum of a hot solution of L-AlaC17 in hexane showed an intense absorption at 262 nm with a shoulder at 294 nm. For the transparent hexane gel, the shoulder became more obvious, and the peak at 262 nm broadened, but without any shift. For the twist obtained from the racemic mixture, the band was centered at 272 nm. It was thus red-shifted by 10 nm relative to that found for L-AlaC17 and suggested the formation of a head-to-tail π–π stacking arrangement of the chromophores.

Both the enantiomeric and the racemic assemblies showed CD signals, but with different shapes. A positive or negative CD signal centered at 309 nm was observed for the D-AlaC17 and L-AlaC17 assemblies, respectively. An exciton-couplet Cotton effect was observed for the racemic assemblies, with a positive peak centered at 255 nm and two negative valleys at 294 and 308 nm. A cross-over was observed at 270 nm, which is very close to the maximum absorption of the racemate. However, CD signals also appeared at higher wavelengths. These signals could be assigned to chiral scattering of the light as a result of the interaction of the light with the chiral nanostructures.[21] Interestingly, whereas the same CD spectra were obtained for the individual enantiomers in many experiments, the sign of the CD spectra for the racemate could be different from batch to batch. It is well-known that the emergence of a couplet Cotton effect is usually associated with coupling between the chromophores owing to π–π stacking. Our results above indicate that the π–π stacking of the Fmoc groups is stronger in the twist structures than in the fibers. The weaker interaction between the Fmoc groups in the enantiomeric assemblies could be explained by the greater overlap of the alkyl chains in the nanofiber and thus their separation of the neighboring chromophores (Figure 2D). This hypothesis is in accordance with the conclusions reached on the basis of the UV/Vis spectra and the XRD results.

On the other hand, the uncertain appearance of intense CD signals in spectra of the racemic mixture is a surprise. This unique phenomenon could be explained as follows: Since an exact 1:1 molar ratio of L-AlaC17 to D-AlaC17 can hardly occur at a molecular level, we suspect that the slight excess of one enantiomer in the racemic mixtures led to the random distribution of CD signals for the different batches of the twist samples. We therefore carried out the following experiments.

We changed the molar ratio of the two enantiomers (L-AlaC17/D-AlaC17) and investigated the resulting self-assembled nanostructures as well as their CD spectra. For the comparison of different samples, we used the G value, the ratio of the CD signal to the UV/Vis absorbance, as a function of wavelength to evaluate their differences.[23] We plotted the G value for each sample with different mixing ratios (Figure 3A). Three distinct features were observed: First, determined CD signals were always obtained for the mixtures with different ratios. An excess of L-AlaC17 resulted in a negative Cotton effect at 255 nm and a positive Cotton effect at 309 nm, whereas mirror-image CD spectra were obtained for mixtures with an excess of D-AlaC17. Second, the CD signals observed were much more intense for a non-equimolar mixture of the enantiomers than for the pure enantiomers (see Figure S6). Intense CD spectra with the peaks in the predicted positions were even detected for mixtures with 0.2 or −0.2% *ee*. The CD signals centered at 309 and 255 nm were strongest for mixtures with an *ee* value of 2% (or −2%) and 4% (or −4%), respectively. This result indicated that the self-assembly of the racemic mixture is very sensitive to a slight enantiomeric excess and supports our deduction above. Third, the CD measurements are in accordance with the SEM observations. We found that twists obtained from the mixture containing L-AlaC17 with 2% *ee* were exclusively right-handed, whereas the presence of D-AlaC17 with 2% *ee* led to the formation of exclusively left-handed twists (Figure 3B). Both the CD spectra and the handedness of the chiral twist follow the majority rules.[24]

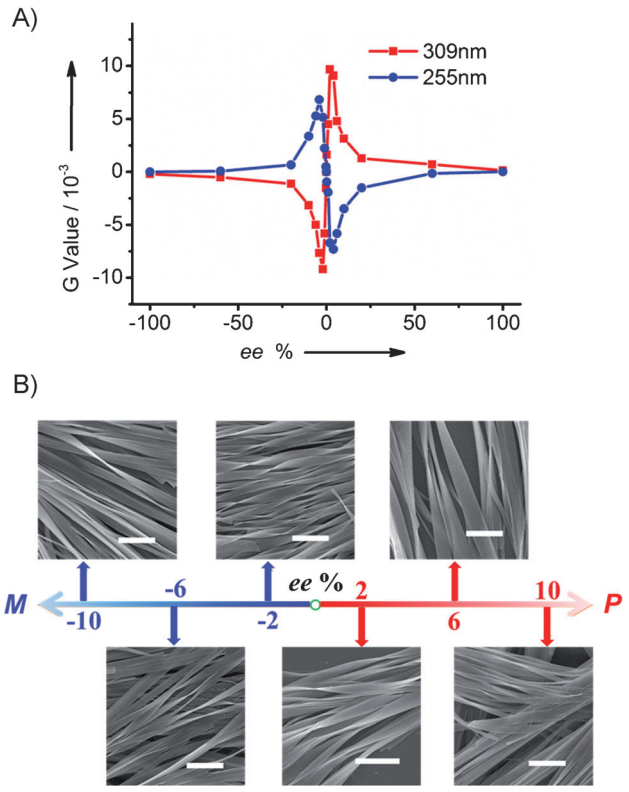

Figure 3. A) G values centered at 309 and 255 nm as a function of the *ee* value of non-equimolar mixtures of L-AlaC17 and D-AlaC17. The G value of the racemate was set to zero. B) SEM images of the nanostructures formed from mixtures of L-AlaC17 and D-AlaC17 with various *ee* values (*ee* = ([L-AlaC17]−[D-AlaC17])/([L-AlaC17]+[D-AlaC17])×100%). Scale bars: 5 μm.

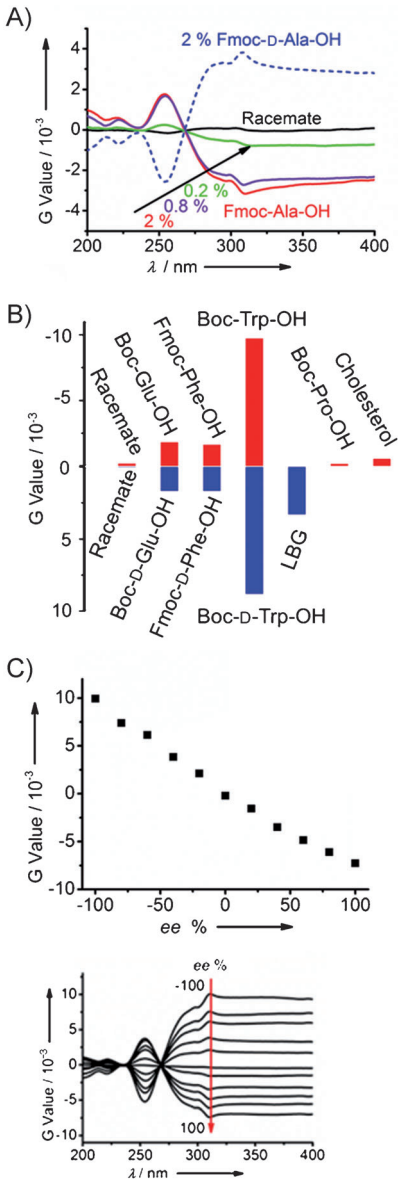

Figure 4. A) CD spectra of the racemate upon the addition of Fmoc-Ala-OH or Fmoc-D-Ala-OH at various concentrations. B) Intensity of the CD signals (centered at 309 nm) of the racemate upon the addition of 2 mol% of various amino acid derivatives. LBG is a glutamic acid derivative with two long alkyl chains.[32] C) G value (centered at 309 nm) of the racemate upon the addition of 1 mol% of a mixture of Boc-Trp-OH and Boc-D-Trp-OH with various *ee* values. The lower graph shows the corresponding spectroscopic changes as the enantiomeric excess is increased in steps of 20%. Boc = *tert*-butoxycarbonyl.

When one enantiomer was present in excess with up to 2% *ee*, the twisted structure as well as a large CD response were observed. However, a continued increase in the *ee* value to more than 10% lead to a dramatic decrease in the CD intensity and significant relaxation of the twist structure (Figure 3B). In most previously studied self-assembly systems that obey the majority rules, the enantiomers generally showed larger G values than the mixtures of the enantiomers, and tangentlike curves were obtained. Normally one enantiomer needed to be present with 20% *ee* or even higher to roughly control the chirality of the supramolecular systems.[25–30] In the present case, only a slight excess of one enantiomer, at even as low as 0.2% *ee*, could significantly alter and determine the chirality as well as the structures of the racemic assemblies. Our results demonstrate a new form of majority rules in self-assembled systems: that a slight excess of one enantiomer could be more effective.[31]

The high sensitivity of the racemic assemblies to a slight enantiomeric excess can be exploited for ultrasensitive chiral sensing by replacing the excess enantiomer with another chiral species. We tested some chiral analogues of the enantiomers and obtained satisfactory results with great detection power. First, analogous alanine derivatives were chosen. Figure 4A shows the changes in the CD signals upon the addition of Fmoc-Ala-OH or Fmoc-D-Ala-OH at various concentrations. The introduction of 2 and 0.8 mol% of the chiral dopant induced significant enhancement of the CD signals. The resulting CD spectra are similar to those of the racemate with a slight excess of one enantiomer, and the mirror-image CD spectrum was obtained when the amino acid with the opposite chirality was used. Moreover, 0.2 mol% of Fmoc-Ala-OH was enough for clear changes to be observed in the CD spectrum. The total concentration of L-AlaC17 and D-AlaC17 used in these experiments was

5 mg mL^{-1}, which means that the detection limit for the chiral dopant is no more than 1.8×10^{-5} mol L^{-1}.

So far, most chiral sensing systems have been based on noncovalent interactions between an achiral host and an enantiomeric guest at a molecular level.[33–37] In this study, we discovered a highly efficient supramolecular chiral sensing system based on the interaction between a chiral nanostructure and chiral molecules in which the chiral nanostructure serves to express as well as amplify the molecular chirality.

This discrimination ability was extended to other chiral species to prove the generality of the method (Figure 4B). After the introduction of 2 mol% of the chiral dopant, remarkable CD signals were obtained, the sign of which corresponded to the particular enantiomer. Amino acid derivatives without the Fmoc group, the long alkyl chain, or the alanine moiety could also be detected in the CD spectra. However, a proline derivative and cholesterol failed to be recognized although they are also chiral molecules. It seems that the existence of amide groups adjacent to the asymmetric center is crucial for an effective interaction between the amino acid derivative and the racemate. Hydrogen bonding plays a key role in such discrimination.[38]

The sensing of chiral molecules was further extended to the determination of the *ee* value of the racemic system. Figure 4C shows the spectroscopic changes observed for the twists upon the addition of tryptophan derivatives with various *ee* values. The total concentration of the enantiomers was 1 mol% with respect to the racemate. Upon the addition of the chiral mixtures, intense CD signals were obtained. An excess of the L-type amino acid induced a similar CD spectrum to that of the individual L-type amino acid, and mirror-image CD spectra resulted when an excess of the D-type amino acid was present. By plotting the G value as a function of the *ee* value, we could construct a standard curve. Moreover, the induced CD intensity was approximately proportional to the *ee* value.[34–37] Thus, without any complicated procedures, we can determine the enantiomeric excess by comparison with the standard curve.

In conclusion, we unexpectedly found that macroscopic chirality as well as beautiful twist ribbons rather than flat structures were obtained when racemic alanine derivatives underwent self-assembly in hexane. The handedness of the twist was extremely sensitive to the enantiomeric excess, according to a new form of majority rule. Upon the addition of a small amount of an amino acid derivative to the racemic assemblies, remarkable enhancement of the CD signals was observed. Thus, the racemic assemblies can be used for the detection of a broad range of chiral amino acid derivatives. The recognition is extremely sensitive and can also be used to determine the enantiomeric excess of a mixed system. In other words, we were able to develop an efficient chiral sensor for the discrimination of chiral species. The research reported herein represents an important step toward the understanding of the relationship between molecular chirality and supramolecular chirality, and the application of chiral nanostructures.

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