Conformation and self-assembly changes of isomeric peptide amphiphiles influenced by switching tyrosine in the sequences†

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Self-assembly of peptide amphiphiles feature unique structures, high biocompatibility, and potential for various applications, and have attracted increasing interest in supramolecular chemistry, protein science and polymer science. In this paper, isomeric peptide amphiphiles derived from lauric acid and silk fibroin-based peptides with different amino acid sequences (GAGAGAY, GAGASYG, GAGYGAGA and GYGAGAGA) are investigated systematically to figure out the predominant endogenous and exogenous factors for their assembly in aqueous solution. With the position of tyrosine (Y) in the peptide segment gradually moving towards the alkane tails, the assembled peptide amphiphiles substantially change their secondary structures from the β-sheet to the disorder dominant one under neutral pH conditions, because the increase of steric hindrance induced by the position change of Y disturbs the hydrogen bonds relevant to the formation of β-sheets of (GA)n. Strong alkaline conditions are able to accelerate such a conformational change, due to the synergy of destruction of hydrogen bonds, the steric hindrance effect and electrostatic repulsion. As a consequence, the assembled peptide amphiphiles alter their nanostructures in aqueous solution from well-defined nanofibers to nanospheres with varying sizes. Therefore, it is summarized that the location of Y rather than the other effects such as pH value, etc. plays an essential role in the assembly of our isomeric peptide amphiphiles, which sheds light on the design of various isomeric peptides/peptide amphiphiles for their aggregation as well as potential functionality.

Introduction

The self-assembly of peptide/protein based biomolecules via non-covalent supramolecular interactions, such as hydrogen bonds, van der Waals interaction, electrostatic interaction, hydrophobic interaction and π/π stacking, etc., plays a critical role in their functionalities. Taking animal silks as an example, silk protein/silk fibroin chains fold into β-sheets and then aggregate in the crystalline region. The well aligned crystalline and amorphous region along with the axis of the silk filament induced by stretching or shearing force during spinning is regarded as the predominant factor for the outstanding mechanical performance of silk fibers. Recently, a large number of self-assembled peptides with a variety of nanostructures including nanofibers, nanotubes, micelles, and vesicles, etc. have attracted increasing attention showing promising potential for application as biomaterials and thermochromatic and optical materials because of their simple synthesis, high biocompatibility and distinct structures.

Among these peptides, peptide amphiphiles (PAs) are widely researched as a class of oligomeric molecules typically consisting of hydrophilic peptide segments and hydrophobic alkane chains. In the case of the peptide segments that have the ability to fold into β-sheets, the PAs usually assemble into cylinder nanofibers or ribbons in aqueous solution, instead of isotropic symmetric micelles as presented by the normal surfactants. Although it has been reported that the self-assembly of isomeric PAs with identical composition but different amino acid sequences could lead to slightly different types with regard to their nanostructures, an open question is whether dramatic changes in the conformations of PAs as well as the morphologies of those assemblies could occur via such
switching of an individual amino acid residue in the isomeric PAs or not.\textsuperscript{24} For instance, moving the isoleucine (I) residue close to the palmitoyl chain fails to trigger the nanofiber-to-
sphere alteration of self-assembled palmitoyl-AAAEEE-NH\textsubscript{2} at
any pH value, unless sterically bulky groups such as the Gd:DO\textsubscript{3}A
(Gadoteridol) moiety are decorated on the PAs.\textsuperscript{25} In this regard,
the significant influence on the conformations and the self-
assembled morphologies of the specific isomeric PAs by simply
switching the position of a single amino acid residue has not
been demonstrated yet.

In the previous work of our group,\textsuperscript{26,27} a PA, C\textsubscript{12}-GAGAGAGY
(C\textsubscript{12}: n-dodecane; G: glycine; A: alanine; Y: tyrosine), consisting
of an octapeptide hydrolyzed from Bombyx mori silk fibroin
and lauric acid was synthesized, and the pH responsive self-
assembled assembly was also revealed. As shown in Fig. S1 (ESI\textsuperscript{1}), however,
the electrostatic repulsion generated by the dissociation of both
the carboxyl group at the end of the peptide and the phenolic
hydroxyl group on Y drove the assembly of C\textsubscript{12}-GAGAGGY into
monodispersed cylindrical nanofiber networks with planar
\(\beta\)-sheets under the alkaline condition of pH = 11, which differed
from the nanofiber bundles of self-assembled C\textsubscript{12}-GAGAGAGA
or C\textsubscript{12}-GAGAGAGS (S: serine). It could also be found that the pH
value had slightly different effects on the three PAs. These
phenomena indicate that Y may play an important role in the
assembly of the corresponding PAs.

Herein, isomeric PAs with four different kinds of amino acid
sequences (C\textsubscript{12}-GAGAGAGY, C\textsubscript{12}-GAGAGYGYa, C\textsubscript{12}-GAGYGAGA,
and C\textsubscript{12}-GYGAGAGA) were selected to investigate the effects of
the Y position on their assembly in aqueous solution. It should
be noted that G and other amino acid residues are usually
alternatively arranged in the sequence of Bombyx mori silk
fibroin and (GA)\textsubscript{n} is the dominant motif to form the \(\beta\)-sheet
region in the silk fiber.\textsuperscript{28,29} We expected that such an under-
standing may provide us with constructive guidance on the
relationship between sequences and the conformations of PAs
as well as the morphologies of those assemblies for various
Y-containing peptides/peptide amphiphiles, and on the design
of PA based materials for some promising applications such as
tissue repair and drug delivery.

Experimental section

Preparation and characterization of the PAs

All the PAs used in this study were synthesized via standard
9-fluorenlymethoxycarbonyl (Fmoc) solid phase peptide synthesis
on a PSI-200 peptide synthesizer (Peptide Scientific Inc.) according
to a previous report.\textsuperscript{26} Cleavage of the PAs from the Wang resin
was carried out with a mixture of TFA/TIS/H\textsubscript{2}O (Sinopharm Co.) in
a ratio of 95:2.5:2.5 for 3 h at 0°C. Excess TFA was removed by
rotary evaporation and the remaining peptide solution was titrated
with cold diethyl ether. After centrifugation, the precipitate was
dried under vacuum overnight. The crude PAs were loaded on a
preparative RP-HPLC column and purified with a linear gradient of
acetonitrile/water solution (55/45 to 45/55, containing 0.1% TFA).

The purified PAs were characterized by liquid-phase
chromatography-electrospray ionization mass spectrometry
(LC/ESI-MS) and matrix-assisted laser desorption ionization
ionization time-of-flight (MALDI-TOF). As for LC/ESI-MS (Waters
AutoPurification System) analysis, the detection wavelengths
were set at 214 nm and 254 nm, and a C18 stainless steel Waters
XBridgetm BEH300 reversed phase HPLC column was employed.
The chromatographic method was performed at a flow rate of
1 ml min\textsuperscript{-1} and 25°C, using 2:3 acetonitrile–water with 0.1%
trifluoroacetic acid as the mobile phase. MALDI-TOF analysis
was carried out on a Voyager DE-STR mass spectrometer with an
accelerating voltage of 20 kV. The sample was mixed with sinopic
acid solution and deposited on a sample plate.

PA aqueous solutions were prepared \textit{via} dissolving the
respective PAs into 0.05 M NaOH aqueous solution to the
concentration of 1 mg mL\textsuperscript{-1}. Then, the pH values of the
solutions were adjusted to 8 or 11 by 1 M HCl. It should be
noted that only NaOH and HCl were used to avoid the possible
effect on the conformation transition of PAs by other
polyvalent ions.

\textbf{pH titration of the PA solutions}

pH titration of the aqueous PA solutions was performed on
C\textsubscript{12}-GAGAGAGY and C\textsubscript{12}-GYGAGAGA by using a pH meter (Fischer
Accument) at a concentration of 1 mg mL\textsuperscript{-1} in 0.1 M KCl. The PA
solutions were added in about 1–5 \(\mu\)L increments with 0.1 M KOH
to pH 12, and then titrated in the same increments with 0.1 M HCl
to pH 2. The pH meter should be allowed to stabilize for several
seconds after each addition of HCl and KOH solutions.

\textbf{Structural and morphological detection of the assembled PAs}

Circular dichroism (CD).

The PA solutions with a concentration of 1 mg mL\textsuperscript{-1} at pH 8 or pH 11 were detected on a JASCO
J810 CD spectropolarimeter by using a quartz cell with 1 mm
path length at 25°C. Each spectrum was a mean of the results of
ten scans from 190 nm to 260 nm at a bandwidth of 1 nm.

Fourier transform infrared (FT-IR) spectroscopy. FT-IR spec-
tra were acquired on a Nicolet Nexus-470 spectrometer with a
resolution of 4 cm\textsuperscript{-1}. PA solutions at pH 8 or pH 11 were spread
on a CaF\textsubscript{2} plate and vacuum dried before the test. Each
spectrum was an average value of 64 consecutive scans.

Transmission electron microscopy (TEM). Samples for TEM
observation were prepared by pipetting one droplet of the PA
solution (1 mg mL\textsuperscript{-1}) at different pH values onto copper grids
at high magnification.

Atomic force microscopy (AFM). The PA solution (1 mg mL\textsuperscript{-1})
was pipetted onto a freshly cleaved mica substrate and dried at room temperature before detection. The AFM images were collected in tapping mode on a Nanoscope IV
equipped with a 10 \(\mu\)m \(\times\) 10 \(\mu\)m scanner at a scanning speed
with a line frequency of 1 Hz, and then were saved at a resolution
of 512 \(\times\) 512 points.
**Results and discussion**

First of all, LC/ESI-MS detection clearly presents the molecular mass of 805.6 (m/z, [M + H]+) for all PAs synthesized, in agreement with the molecular weight ([M]) of C_{12}-G_{4}A_{3}Y (Fig. S2, ESI†). Furthermore, the MALDI-TOF mass spectrum exhibits four main fractions of each sample, corresponding to the molecular mass of [M + H]+ (805.5), [M + Na]+ (827.5), [M + K]+ (843.5) and [M – H + 2Na]+ (849.5) (Fig. S3, ESI†), respectively. Considering that the polarity of the stationary phase is less than that of the mobile phase because a C18 reversed phase HPLC column was employed in LC/ESI-MS, a sample with high hydrophobicity would take a long time to be eluted. It can be seen in Fig. S2 (ESI†) that the PA becomes increasingly hydrophobic with the Y residue moving towards the alkyl tails, as its corresponding elution time is slightly extended. These measurements not only indicate the high purity of the PAs, but also further confirm the chemical structures of C_{12}-GAGAGAGY, C_{12}-GAGAGYGA, C_{12}-GAGYGAGA, and C_{12}-GYGAGAGA listed in Fig. S4 (ESI†).

To compare the secondary structures, either in aqueous solution or in the dry state, of these four PAs, CD and FT-IR were separately employed. At the pH value of 8, the CD spectra of C_{12}-GAGAGAGY, C_{12}-GAGAGYGA, and C_{12}-GAGYGAGA exhibit obviously positive and negative Cotton effects around 195 and 215 nm (Fig. 1a, black, green and blue curves), respectively, indicating the high purity of the PAs, but also further confirm the chemical structures of C_{12}-GAGAGAGY, C_{12}-GAGAGYGA, and C_{12}-GAGYGAGA listed in Fig. S4 (ESI†). At pH 11, C_{12}-GAGYGAGA is free from the difference of pH values, indicating that the content of disordered conformation for C_{12}-GYGAGAGA is nearly 4 times the counterpart of C_{12}-GAGYGAGA at pH 8 (Table 1). On the other hand, for the same PA, e.g. C_{12}-GAGYGAGA, the content of disordered conformation at pH 11 is determined by the negative band at around 200 nm, with Y becoming increasingly close to the alkyl tail. The results obtained from deconvolution of the corresponding amide I band (Fig. S5, ESI†) show that the content of disordered conformation for C_{12}-GYGAGAGA is nearly 4 times its value at pH 8, indicating the acceleration of strong alkali conditions to such conformational transition. However, the ratio of the β-sheet to disordered conformation of C_{12}-GYGAGAGA is free from the difference of pH values, indicating that Y’s position has a high priority over the pH value in affecting the conformation of these isomeric PAs. In all, the PAs substantially change their secondary structures from the β-sheet to the disordered dominant one under neutral pH conditions with the position of Y in the peptide segment gradually moving towards the alkane tails, possibly because the increase of steric hindrance induced by the position switching of Y disturbs the hydrogen bonds relevant to the formation of β-sheets of (GA)$_n$.22 According to the results of the titration experiment shown in Fig. S6 (ESI†), the pKₐ values of –COOH and –Ph–OH in the PAs are about 3.1 and 9.8, respectively. Therefore, the dissociation of both –COOH and –Ph–OH occurs at pH 11. Obviously, –Ph–O$^-$ brings an additional electrostatic repulsion in the system and is able to push this series of changes to more easily take place in comparison with the situation at pH 8.

TEM images of the PA assemblies are consistent with what has been revealed by CD and FT-IR. At pH 8, C_{12}-GAGAGAGY PAs assemble into nanofiber-structured networks with a width of about 10 nm and a length of several microns (Fig. 2a1). The width of a nanofilament is almost double the length of a C_{12}-GAGAGAGY chain, which is about 4.5 nm calculated from energy minimum modelling by Hyperchem. Some twisted nanofibers are also observed in the TEM images (Fig. S7, ESI†), as displayed (Fig. 1b, green curve).33 Nevertheless, the disorder dominated conformation of C_{12}-GAGYGAGA and C_{12}-GYGAGAGA at pH 11 is determined by the negative band at around 200 nm (Fig. 1b, blue and pink curves).

The FT-IR spectra also reveal the secondary structural changes of these PAs via switching the position of Y (Fig. 1c and d). The amide I band located at about 1625 and 1650 cm$^{-1}$ can be ascribed to β-sheet and disordered conformations, respectively.34 The gradual conformational changes from the β-sheet to the disordered one in the PAs are confirmed by the rise of the band at 1650 cm$^{-1}$, with Y becoming increasingly close to the alkyl tail. The results obtained from deconvolution of the corresponding amide I band (Fig. S5, ESI†) show that the content of disordered conformation for C_{12}-GYGAGAGA is nearly 4 times the counterpart of C_{12}-GAGYGAGA at pH 8 (Table 1). On the other side, for the same PA, e.g. C_{12}-GAGYGAGA, the content of disordered conformation is about 60% at pH 11, which is 3.5 times its value at pH 8, indicating the acceleration of strong alkali conditions to such conformational transition. However, the ratio of the β-sheet to disordered conformation of C_{12}-GYGAGAGA is free from the difference of pH values, indicating that Y’s position has a high priority over the pH value in affecting the conformation of these isomeric PAs. In all, the PAs substantially change their secondary structures from the β-sheet to the disordered dominant one under neutral pH conditions with the position of Y in the peptide segment gradually moving towards the alkane tails, possibly because the increase of steric hindrance induced by the position switching of Y disturbs the hydrogen bonds relevant to the formation of β-sheets of (GA)$_n$.22 According to the results of the titration experiment shown in Fig. S6 (ESI†), the pKₐ values of –COOH and –Ph–OH in the PAs are about 3.1 and 9.8, respectively. Therefore, the dissociation of both –COOH and –Ph–OH occurs at pH 11. Obviously, –Ph–O$^-$ brings an additional electrostatic repulsion in the system and is able to push this series of changes to more easily take place in comparison with the situation at pH 8.

![Fig. 1](image-url) Characterization of the secondary structures of self-assembled PAs. (a and b) CD spectra of C_{12}-GAGAGAGY (black), C_{12}-GAGAGYGA (green), C_{12}-GAGYGAGA (blue), and C_{12}-GYGAGAGA (pink) at pH 8 and 11, respectively. The concentration of each sample is 1 mg mL$^{-1}$. (c and d) FT-IR spectra of C_{12}-GAGAGAGY (black), C_{12}-GAGAGYGA (green), C_{12}-GAGYGAGA (blue), and C_{12}-GYGAGAGA (pink) at pH 8 and 11, respectively.

<table>
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<tr>
<th>PAs</th>
<th>pH 8 (%)</th>
<th>Disordered (%)</th>
<th>pH 11 (%)</th>
<th>Disordered (%)</th>
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<tr>
<td>C_{12}-(GA)$_3$GY</td>
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<td>—</td>
<td>100</td>
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<tr>
<td>C_{12}-(GA)$_3$GYGA</td>
<td>100</td>
<td>51</td>
<td>49</td>
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the pH in this case is close to the reported transition value from the untwisted to the twisted structure for C12-GAGAGY.26 The specific negative band of 219 nm for the twisted β-sheet may be obscured by the untwisted 215 nm band in the CD spectrum (Fig. 1a) due to the limited amount of twisted fibers, and thus only the TEM image is able to reveal the existence of this morphology. Both the aggregated C12-GAGAGYGA and C12-GAGYGAGA isomers at pH 8 present a nanofibrous structure without a twisted morphology (Fig. 2b1 and c1), because the increased steric hindrance of C12-GAGAGYGA and C12-GAGYGAGA by switching of the Y’s position makes the PAs only possess untwisted morphology. Interestingly, the nanofibers assembled from C12-GAGAGYGA still exhibit an average length of about several microns, whereas the average length of the nanofibers of self-assembled C12-GAGYGAGA decreases to hundreds of nanometers. Moreover, the assembly of C12-GYGAGAGA exhibits an expected nanospherical structure with diameters ranging from 30 to several hundred nanometers (Fig. 2d1), which can be ascribed to its disorder dominant conformation leading to a propensity to form a spherical morphology.35 Based on the deconvolution result of FT-IR listed in Table 1, these spheres may still consist of well-ordered crystalline regions with a β-sheet conformation.35

However, a strong alkali condition (pH 11) delivers two main influences on the morphologies of these assembled PAs. C12-GAGAGAGY only possesses an untwisted nanofiber structure (Fig. 2a2), similar to the majority of peptide amphiphiles under neutral pH conditions. When the location of Y starts to switch, the self-assembled nanospheres emerge (Fig. 2b2), and become an exclusive morphology (Fig. 2c2 and d2). This trend can be attributed to the strong electrostatic repulsion induced by dissociation of –COOH and –Ph–OH groups at pH 1127 and the location of Y. Both effects disturb the hydrogen bonds relevant to the formation of the β-sheet of (GA)n, which is the driving force of the main structure of these nanofibers. Thus, the assembled PAs with significant disordered conformation prefer a spherical morphology to realize a stable surface energy. In addition, the nanospheres display a rugged and hydrophilic surface (Fig. S8, ESI†), demonstrated by the protuberances and the lattice spacing of 0.338 and 0.316 nm, consistent with the d-spaces of staining orthorhombic C6H4O6U·2H2O in the (002) and (310) planes, respectively.36 It is worth noting that the self-assembled C12-GAGAGYGA or C12-GAGYGAGA exhibits only nanofiber morphology at pH 8 and either presents the coexistence of or even complete nanospheres at pH 11, while those of C12-GAGAGAGY and C12-GYGAGAGA are pH independent in terms of their shapes (Fig. 2), consistent with the constant ratio of β-sheet to disordered conformation. Obviously, it also suggests that the location of Y is the major concern for the morphological changes of these isomeric PAs rather than the pH value of the solution, which plays an accessorial role.

More specific information about the morphologies of the assembled nanofibers and nanospheres was acquired by AFM. As shown in Fig. 3a and b, nanofibers assembled by C12-GAGAGAGY at pH 8 possess an average width of about 10 nm, which is in agreement with the results of the relevant TEM images. The heights

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**Fig. 2** Nanostructures of the self-assembly of the PAs: TEM images of self-assembled C12-GAGAGAGY, C12-GAGAGYGA, C12-GAGYGAGA, and C12-GYGAGAGA at pH 8 (a1–d1) and 11 (a2–d2), respectively. The inset in d2 shows the TEM image of the corresponding nanospheres at high magnification.

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**Fig. 3** Section analysis of the AFM images of nanofibers and nanospheres. (a and b) AFM image and height profile analysis of the nanofibers assembled by C12-GAGAGAGY at pH 8. (c and d) AFM image and the height information of the selected nanospheres, i.e. sphere 1 (black), 2 (red), 3 (blue) and 4 (green), formed via the self-assembly of C12-GYGAGAGA at the pH value of 11, respectively.
of the nanofibers are around 7 nm which can be attributed to the water evaporation involved morphological alteration. On the other side, nanospheres assembled by \( \text{C}_{12}-\text{GAGYGAGA} \) at pH 11 or by \( \text{C}_{12}-\text{GYGAGAGA} \) in the pH range from 4 to 11 display a wide distribution of their sizes. For example, Fig. 3c and d show that the sizes of the 4 representative \( \text{C}_{12}-\text{GYGAGAGA} \) nanospheres range from around 60 nm to 320 nm. It should be noted that the shapes of the AFM height profiles (Fig. 3d) are nearly hemispherical, and there is no obvious hollow structure in their HRTEM images (Fig. 2d2 and Fig. S8a, ESI†), indicating that these nanospheres are solid particles rather than hollow ones or vesicles. As most of the PAs with disordered conformation randomly distribute within the nanospheres, the absence of ordered force to confine the size along the assembling direction of PAs eventually endows the nanospheres with various diameters, in comparison with the regular cylinder fiber structure.

Dynamic light scattering (DLS) measurement also manifests the existence of these self-assembled nanospheres with a wide size distribution mainly ranging from 70 nm to about 600 nm (Fig. S9, ESI†), which are reasonably larger than those observed by TEM and AFM. As listed in Table S1 (ESI†), the average size (Z-average) values of assembled \( \text{C}_{12}-\text{GAGYGAGA} \) at pH 11 and \( \text{C}_{12}-\text{GYGAGAGA} \) at both pH 8 and 11 are about 290 nm, 260 nm and 310 nm, respectively, and are independent of the concentration of PAs.

Moreover, the macroscopic aggregation behaviours of the representative isomeric PAs (\( \text{C}_{12}-\text{GAGAGAGY} \) and \( \text{C}_{12}-\text{GYGAGAGA} \)) in aqueous solutions (1 mg mL\(^{-1}\)) at different pH values were observed (Fig. S10, ESI†). The stable and transparent \( \text{C}_{12}-\text{GAGAGAGY} \) solution with good fluidity at pH 11 turns to a translucent fluid as the pH decreased to 8, and finally forms an opaque gel at pH 4, indicating that a solution to gel transition occurs due to the decreasing pH induced successive protonation of \(-\text{Ph-O}^+\) and \(-\text{COO}^-\) and the hierarchical aggre- gation of the nanofibers. In addition, the FT-IR spectrum of self-assembled \( \text{C}_{12}-\text{GAGYGAGA} \) at pH 4 also exhibits the \( \beta \)-sheet dominated conformation (Fig. S11, ESI†). As \( \beta \)-sheet nanofibers are formed at pH 8 for \( \text{C}_{12}-\text{GAGAGAGY} \), \( \text{C}_{12}-\text{GAGAGYGA} \) and \( \text{C}_{12}-\text{GYGAGAGA} \), the protonation of \(-\text{COO}^-\) at pH 4 brings an increased \( \beta \)-sheet content in the PAs and is beneficial for these PAs to preserve the nanofiber morphology which further aggregates into bundles in the same way reported previously. 27 However, the \( \text{C}_{12}-\text{GYGAGAGA} \) solution retains its stability, transparency and fluidity throughout the pH change process, as such nanospheres are nearly identical at different pH values.

Hitherto, a reasonable mechanism for the self-assembly of isomeric \( \text{C}_{12}-(\text{GA})_3\text{GY} \) in aqueous solution at pH values of 8 and 11 is proposed. As illustrated by Scheme 1, the position of Y may act as the predominant endogenous factor for the substantial transformation of both the conformations and nanostructures of the self-assembled isomeric PAs. For the assembly of \( \text{C}_{12}-\text{GAGAGAGY} \), the hydrophobic non-covalent interaction drives the PA to form micelles, and hydrogen bonds between peptide segments break the spherical symmetry, leading to a \( \beta \)-sheet conformation. Along with the electrostatic repulsion derived from the dissociation of \(-\text{COOH}\) and \(-\text{Ph-OH}\) under different pH conditions, the self-assembled \( \text{C}_{12}-\text{GAGAGAGY} \) eventually takes a cylindrical nanofiber morphology. For the other isomers, firstly, as Y intrinsically has a steric hindrance effect to disturb the hydrogen bonds relevant to the formation of \( \beta \)-sheets of \((\text{GA})_n\), the enhancement of this effect as Y moves towards the alkyl tails significantly disrupts the \( \beta \)-sheet conformation.

Scheme 1 Schematic illustration of the mechanism of the different self-assembly processes of \( \text{C}_{12}-\text{GAGAGAGY} \), \( \text{C}_{12}-\text{GAGYGAGA} \) (or \( \text{C}_{12}-\text{GAGAGYGA} \)), and \( \text{C}_{12}-\text{GYGAGAGA} \) induced by the different positions of the Y residue and accelerated by strong alkaline conditions. Red, blue, and cyan patterns indicate the GY and GA segments and the alkyl chain, respectively.
Secondly, as the position of Y becomes increasingly close to the alkane chains, the hydrogen bonds located at the amino acid residues near the alkyl tails are broken, thus leading to the destruction of the β-sheets. The subsequent transformation of the β-sheet to a disorder dominated conformation finally pushes the aggregated C12-GYGAGAGA to form nanospherical structures as shown in Scheme 1 and Fig. S12 (ESI†). In addition, strong alkaline conditions have the ability to accelerate this conformational and morphological transformation when the effect of Y’s position is not strong enough to disrupt the β-sheets. The existence of –COO⁻ and –Ph–O⁻ derived from the completely dissociated –COOH and –Ph–OH generates strong electrostatic repulsion to hinder the formation of hydrogen bonds between peptide segments, thus breaking the β-sheets and resulting in a nanospherical structure composed of a disordered dominated conformation. While at pH 8, neither C12-GAGAGYGA nor C12-GAGYGAGA is able to generate strong enough hindrance to disrupt the β-sheet conformation, thus the relevant self-assembled PAs still exhibit a cylinder nanofiber structure.

The mechanism we summarized here differs from the previously reported sterically bulky moiety assisted nanofiber-to-sphere alteration in the PAs without Y residues.25 Introducing sterically bulky moieties is unnecessary in our case, as the –Ph–OH group in Y provides different steric effects based on its position in the sequences of isomeric PAs. On the other hand, it has been reported that the amino acid residue which is close to the alkyl tail in PA has a significant influence on the formation of the β-sheet and its subsequent nanofiber morphology.22 Steric effects induced by the Y close to the alkyl tail in our case may greatly increase the space between two peptide sections, thus disturbing the H bonds relevant to the formation of the β-sheet.

Conclusions

By investigation of the self-assembly of four isomeric PAs, C12-GAGAGGY, C12-GAGAGYGA, C12-GAGYGAGA, and C12-GYGAGAGA, we reveal that the different positions of Y in the sequences play a predominant role in the conformations and assembly of these PAs. Generally, the conformations of the PAs change from β-sheet to disordered, resulting in substantial morphological alteration of the assemblies from uniform cylinder nanofibers to nanospheres with varying sizes, as Y is moved closer to the hydrophobic alkane chain, because it significantly disturbs the hydrogen bonds relevant to the formation of β-sheets of [G]ₙ. Strong alkaline conditions generate electrostatic repulsion via the dissociation of –COOH and –Ph–OH, which is able to accelerate such changes along with the position of Y. Furthermore, the results not only indicate that the position of a typical amino acid residue with a large side group may indeed induce the nanofiber-to-sphere alteration of the PA assembly, but also provide an example to show the undefined relationship between the chemical composition and aggregation/assembly of PAs because of the crucial role the conformation plays in the assembly of PAs. Therefore, this may provide a new avenue to guide us to a comprehensive understanding of the relationship between sequences and nanostructures for various isomeric peptides/peptide amphiphiles and the design of related materials for a number of promising biomedical applications in the future.

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Notes and references