Supramolecular hydrogels based on β-amino acid derivatives

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Here we report on a new class of supramolecular hydrogels based on dipeptides that consist of β-amino acids, which may confer proteolytic resistance to the hydrogels for biomedical applications.

Small molecules with appropriate hydrophobicity and hydrophilicity can self-assemble into nanofibers in water as the matrices of supramolecular hydrogels. Because these 3-D, fibrillar matrices may act as a versatile scaffold for biosensing, cell culturing, and incorporating therapeutic agents,[1–3] supramolecular hydrogels are attracting considerable amount of research interest. Notably, the demonstration of self-assembled oligopeptide nanofibers or nanotubes, which gel water to produce hydrogels for biomedical applications (*e.g.*, as scaffolds to promote the growth of neurons,[1] to induce biomineralization,[3] or to assist cell adhesion[4]), has stimulated the research efforts on low-molecular-weight hydrogelators.[5,6] Being used *in vivo*, these oligopeptide-based scaffolds are biodegradable through proteolytic enzymes catalyzed hydrolysis.[7,8] However, such an inherent susceptibility towards enzymes shorten the *in vivo* lifetime of these peptide-based hydrogels, thus reducing their efficacy and limiting their scope of applications when long-term bioavailability is required.

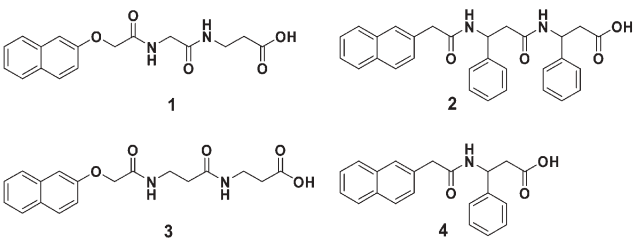
Proteolysis is a common disadvantage for peptide-based therapeutic agents. Therefore, many efforts have focused on designing and synthesizing non-peptide molecules that mimic the structures and functions of peptides or proteins to achieve prolonged or controlled stability and bioavailability of those molecules.[7] Among the peptidomimics,[9] β-peptides, which contain β-amino acids, have received the most intensive attention due to their improved biostability.[7,10] Despite the rapid progress in the design and synthesis of β-peptides, the application of β-amino acids for controlling the bioavailability of supramolecular hydrogels remains unexplored because whether a β-amino acid derivative would act as a hydrogelator remains unknown (although Hanabusa *et al.* have shown that some β-amino acid containing alkylamides form organogels[11]). In this paper, we presented the first synthesis and characterization of hydrogelators that are β-amino acid derived peptidomimics.

Compound **1** ($C_{10}H_7OCH_2CONH$-α-Gly-β^3-HAla-COOH) is composed of a α-amino acid (glycine) and a β-amino acid (β^3-alanine), and compound **2** ($C_{10}H_7CH_2CONH$-β^3-HPhe-β^3-HPhe-COOH) of two β-amino acids (β^3-phenylalanine). Because large amounts of other β-amino acids are readily available and the conformation of β-amino acid containing peptidomimics can be calculated with relative accuracy, the confirmation of β-amino acids based hydrogelators should provide a new way to tailor the stability of hydrogels in biological environment and ultimately expand the ranges of applications of the hydrogels as biomaterials.

Scheme 1 illustrates the chemical structures of the two hydrogelators, which are dipeptidic mimics linked to naphthalene groups *via* amide bonds. The syntheses of both compounds are simple and straightforward: The N-hydroxyl succinimide (NHS) activated ester of 2-(naphthalen-2-yloxy)acetic acid reacts with glycine to afford 2-(2-(naphthalen-2-yloxy)acetamido)acetic acid (**5**), and 2-(naphthalen-2-yl)acetic acid reacts with β^3-phenylalanine to afford 3-(2-(naphthalen-2-yl)acetamido)-3-phenylpropanoic acid (**4**), respectively. The NHS assisted coupling between **5** and β^3-alanine gives **1** in 67% yield, and the coupling between **4** and β^3-phenylalanine affords **2** in 72% yield.

Both **1** and **2** act as hydrogelators under proper conditions (*e.g.*, concentration, temperature, and pH). After 5 mg of **1** is suspended in 1.0 mL of water, the adjustment of the pH value of the suspension to 4.8 results in a clear solution. To adjust the pH value of the solution back to 4.3 gives a transparent hydrogel (Gel I, Fig. 1A). A gel–sol phase transition happens at 45 °C, and cooling the solution back to room temperatures regenerates Gel I. These two procedures can be repeated for many times without affecting the quality of the hydrogel. Similarly, 5 mg of **2** in 1.0 mL of water also forms a slightly opaque hydrogel (Gel II, Fig. 1B) by carefully adjusting pH or temperature (the pH range and temperature of gel–sol phase transition are 6.2–6.5 and ~48 °C, respectively). Both Gels I and II are stable at room temperature for several months.

Fig. 2 shows the rheological data of Gel I and Gel II with dynamic frequency sweep. The values of their storage moduli (G′)



Scheme 1 The chemical structures of the β-amino acid derivatives synthesized in this work. Among them, **1** and **2** are hydrogelators.

Fig. 1 Optical images of the hydrogels formed by (A) **1** and (B) **2**.

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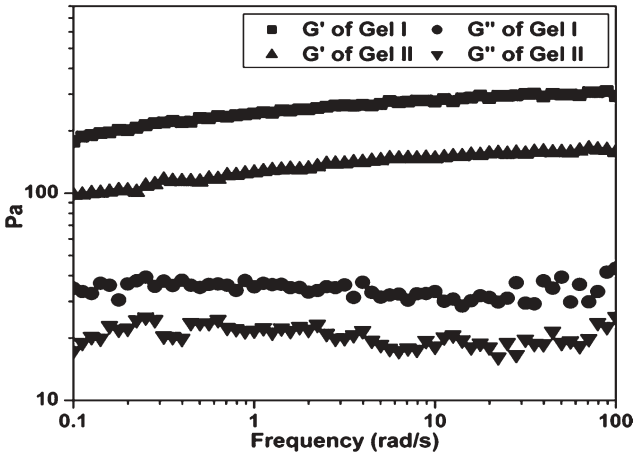

Fig. 2 Frequency dependence of the dynamic storage moduli (G′) and the loss moduli (G″) of Gel I and Gel II.

exceed that of their loss moduli (G″) by a factor of 5–6, indicating that these two samples are hydrogels.[12] Both the values of G′ of the hydrogels exhibit weak dependence on frequency (from 0.1 to 100 rad s^{-1}) at the stress above 20 Pa, suggesting that the matrices of these two gels have good tolerance to external force. The comparison of the G′ values of the hydrogels indicates that the storage modulus of Gel II is slightly larger than that of Gel I, suggesting that Gel II is elastically stronger than Gel I, which agrees with the stronger π–π interactions offered by β^3-phenylalanine. In addition, Gels I and II display relatively weaker elasticity than that of the hydrogels formed by Fmoc-amino acid derivatives.[6] This observation indicates the weaker π–π interaction of naphthalene groups than that of Fmoc groups and weaker hydrogen bond networks due to the conformational flexibility associated with β-amino acids. To further study the microstructure of Gels I and II, we obtained the transmission electron micrograph (TEM) images of the hydrogels. As shown in Fig. 3A, uniform sized and well-dispersed small fibrils (about 25 nm) constitute the matrices for Gel I. The sizes of fibrils in Gel II, unlike in Gel I, are less uniform (ranging from 20 to 80 nm), and the small fibrils exhibit tendency to tangle with each other and form large bundles, which further confirms the stronger π–π stacking ability of β^3-phenylalanine and agrees with higher elasticity of Gel II.

We obtained the emission spectra of **1** and **2** in solution phase and gel phase for investigating the molecular arrangements in the hydrogels. Both compounds exhibit broad emission peaks in solution phase with the maxima at 357 nm for **1**, and 330 nm for **2**. The asymmetric shapes of the emission peaks of **1** and **2** in solution phase and the extensions of their tails in the longer wavelength region indicate that both **1** and **2** have strong tendency to form dimers or oligomers even in diluted solution phase ($<10^{-5}$ M), which should contribute to the hydrogelation of **1** or **2**. In the gel phases, the centers of the emission peaks show considerable red-shifts (from 357 to 382 nm for **1**; from 330 to 350 nm for **2**), suggesting that **1** and **2** form efficient π–π stacking in their respective gel phases. The pronounced emission above 400 nm for Gel I suggests that the π–π interaction of naphthalene groups in Gel I is stronger than that of Gel II (because Ueno *et al.* recently showed that caged excimer of naphthalene group to give a broad emission peak over 400–550 nm[13]). This result also agrees well with

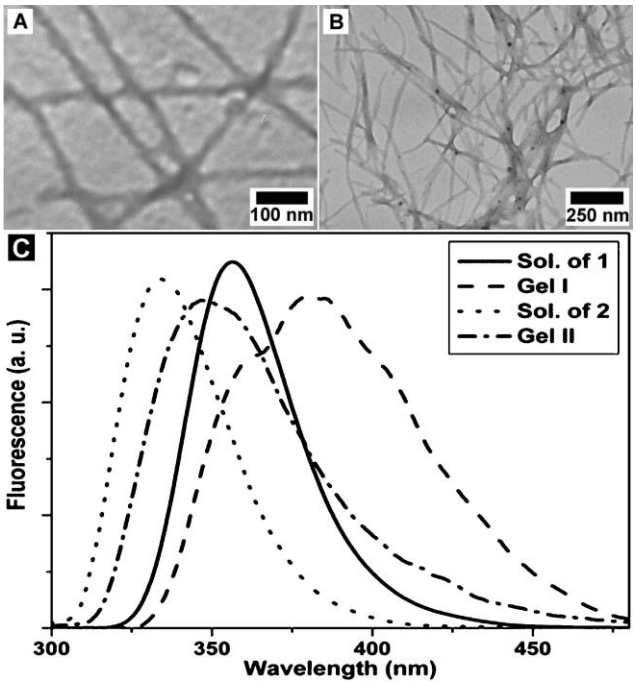

Fig. 3 TEM images of (A) Gel I and (B) Gel II and (C) emission spectra of compound **1** or **2** in solution and gel phase ($\lambda_{ex.}$ = 272 nm).

the aggregation behavior of **1** and **2** in solution phases, as indicated by the emission spectra of **1** and **2**.

To explore the structural parameters of the β-amino acid derivatives for hydrogelation, we also synthesized compounds **3** and **4** and tested if **3** or **4** would form a hydrogel. Neither **3** nor **4** acts as a hydrogelator. The structural difference between **1** and **3** is that β^3-HAla replaces the glycine, which obviously renders **3** more hydrophobic because of the introduction of CH_2 moiety. As the consequence of increased hydrophobicity, **3** fails to act as a hydrogelator. Under the same condition for **1** forming a hydrogel, **3** forms metastable vesicles to give a milky solution, which turn into a precipitate overnight at room temperature. On the other hand, **4** is one β^3-HPhe less than **3**. In addition to the decrease of the π–π interactions, the structural removal of phenylalanine reduces the topological polar surface area (tPSA)[14] up to 30%. Hence, **4** fails to form hydrogels but gives a precipitate. The above results further confirm that the balanced hydrophobic interactions and hydrogen bonding play key roles in the supramolecular hydrogels of **1** or **2**.

Thus, we proposed a possible hydrogen bond network and π–π interactions that sustain the 3-D fibril network (Fig. 4). For Gel I, the hydrogen bonds are likely to form between two layers of molecules of **1**. One molecule of **1** connects with at least two other molecules of **1** through hydrogen bonds from amide groups (Fig. 4A, B). This supramolecular arrangement should allow the partial overlap of two naphthalene groups to explain the broad emission peak centered at 400 nm of Gel I. It also permits the formation of nanofibers of **1**. For Gel II, the hydrogen bonds between the carboxylic acid group and the amide group next to the naphthalene may provide the necessary interaction to form a superhelical structure (Fig. 4C, D). In this superstructure, the overlap between naphthalenes is less dominant, which also explains why the emission spectra of Gel II only display relatively weak

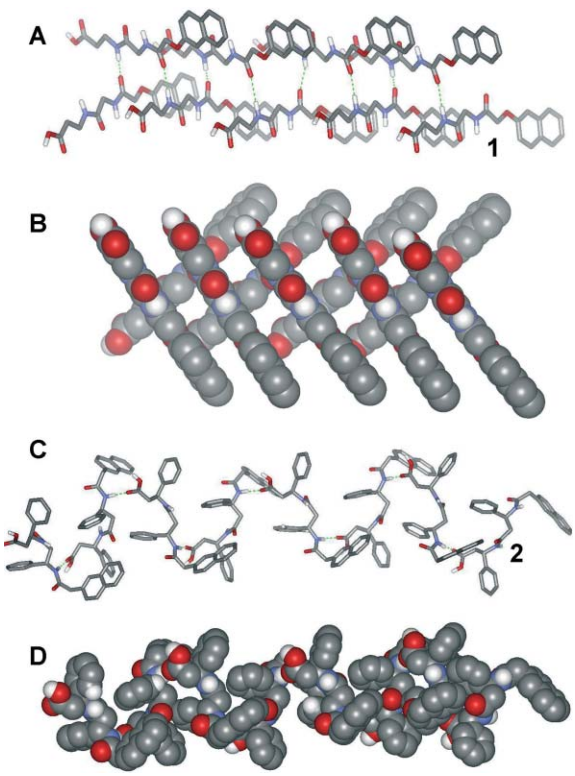

Fig. 4 Possible molecular arrangements in Gel I and Gel II. The possible hydrogen bonds would allow supramolecular chains of (A) **1** and (C) **2**; CPK model of the supramolecular chains of (B) **1** and (D) **2**.

intensity above 400 nm. The chain of **2** showed in Fig. 4C, D should also be hydrophobic and tends to aggregate into nanofibers with a range of widths. These supramolecular chains in Gel I or Gel II further grow into a 3-D fibrillar matrix *via* self-assembly, which traps the water molecules and leave spaces for the incorporation of drug molecules within it and stopped the flow of the liquid.

In summary, we have developed two novel hydrogels which formed by self-assembly of the β-amino acid derivatives. In a biological environment, this type of hydrogels should have prolonged bioavailability in comparison with the hydrogels formed by α-amino acid derivatives. Further work will focus on the use of these hydrogels to incorporate and deliver therapeutic agents and the examination of their stabilities *in vitro* and *in vivo*.

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