Organogels of triterpenoid–tripeptide conjugates: encapsulation of dye molecules and basicity increase associated with aggregation†

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Two triterpenoid–peptide conjugates containing oleanolic acid and glycyrrhetinic acid were synthesized and the properties of self-assembly into organogels were studied systematically. The results showed that the xerogels could selectively adsorb dyes from water and it could be utilized in water purification by removing toxic dyes. In addition, the formation of supramolecular gels was associated to basicity increase by employing pH-indicator dyes in water.

Introduction

Supramolecular self-assembly is becoming an effective and practical strategy to fabricate different types of micro- and nanostructure materials.1 Low-molecular-weight organogelators, as one of distinct nanostructured materials, can self-assemble into various types of fibrils, strands, helicals and tapes in organic solvents via weak intermolecular interactions.2 A number of low-molecular weight organogelators have been reported in the literatures,3 because of their wide-ranging applications as template materials, drug delivery agents, cosmetics, sensors, enzyme-immobilization matrices, as well as in phase selective gelation and water purification by dye adsorption.4 Many biomolecules such as lipids, nucleic acids, steroid, proteins and peptides, can self-assemble into highly ordered supramolecular architectures.5 Due to biocompatibility, simple and effective chemical and biological modification, ability for specific molecular recognition and availability for bottom-up aggregation, the gel nanostructures fabricated from biomolecules are triggering increasing attention.6 Among them, amino acid based molecules, such as peptides offer distinct features such as highly ordered hydrogen bonds, ease of decoration with functional elements.7 The self-assembled capability of designed or extracted peptide enables them to be readily manipulated into well-defined nanostructures as building blocks with various functions. In some cases, they can offer an alternative model for gaining insight into self-assembly and protein function by imitating the behaviours of proteins.

Furthermore, inherent biological origins of peptides make them rather favourable for medical and biological applications.

Glycyrrhetinic acid (GA) 1 and oleanolic acid (OA) 2 (Fig. 1) are two typical pentacyclic triterpenoids and both possess special characteristics such as chiral rigid skeletons, relative low toxicity, and biocompatibility. They have been well known for their biological activities such as anti-inflammatory, anti-tumour, anti-virus and anti-fungal.8 Previously this kind of natural product-triterpenoids have been shown to be renewable functional nano-entities with varied rigid and flexible lengths through computations8 and used as building blocks to construct functional materials. Moreover, there have been more and more attentions focused on supramolecular self-assembly and recognition abilities of the derivatives of a few triterpenoids in recent years.9 However, there have not been reports on self-assembly of triterpenoids derivatives conjugated with peptides.

As our continuous work, here we develop a new kind of self-assembly molecules with tripeptide and triterpenoids 5–6 (Scheme 1) to impart non-covalent bond association and biocompatibility. These two molecules can form organogels in aromatic solvents and could selectively gelate aromatic solvents.

Scheme 1 Synthesis of the conjugates 5 and 6.

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3ra43068e
from water. Furthermore, the xerogels prepared from the organogels showed a striking property of adsorbing dyes such as rhodamine 6G and acriflavine from water. Therefore they could be utilized in water purification by removing toxic dyes from waste water. Additionally, we have also found that the aggregation of 6 in self-assembled fibrillar networks result in a remarkable increase of its basicity by employing different pH-indicator dyes.

Results and discussion

Peptide triterpenoid-Phe-Phe-Ala-OMe was synthesized by conventional solution-phase methodology (Scheme 1). Tripeptide Boc-Phe-Phe-Ala-OMe were synthesized by using protection of the N-terminus with the tert-butylloxycarbonyl (Boc) group and the C-terminus with methyl ester as reported in the literature. Couplings were mediated by dicyclohexyl carbodiimide/1-hydroxybenzotriazole. Then deprotection of the Boc group was conducted by treatment with trifluoroacetic acid. The triterpenoid group conjugated with succinic anhydride (3/4) was introduced into the N-terminally deprotected tripeptide NH2-Phe-Phe-Ala-OMe. All the compounds were fully characterized by mass spectrometry, 1H NMR spectroscopy (300 MHz) and 13C NMR spectroscopy (75 MHz).

The N-terminally tripeptides conjugated OA/GA-Phe-Phe-Ala-OMe (5/6) both act as excellent supramolecular organogelators to form gels in various organic solvents under suitable conditions. They form transparent gels in benzene, toluene, o-xylene, m-xylene, p-xylene (Fig. S1†). The gelation abilities of the gelator molecules were summarized in Table 1. Because of the analogous structures between 5 and 6, their organogels properties are similar with each other. However, a fine change of structures in the triterpenoid framework and variation of the functional groups may have effects on the self-assembly property of a molecule. Due to the different solubility in the solvents, there are some slight differences in MGC between 5 and 6.

To get a visual insight into the morphology of organogels, the supramolecular 3D network was inspected by scanning electron microscopy (SEM). The SEM analysis clearly revealed that the gelator facilitated the formation of network of super bundles with the micrometer range diameters and like entangled ropes (Fig. 2). A lamellar morphology was also observed through SEM (Fig. S2†), and it was consistent with X-ray powder diffraction of the xerogel (Fig. S3†).

Thus, the temperature-dependent NMR study was performed using the organogels in C6D6. As temperature increased from 25 °C to 50 °C, all the amide protons of 5 and 6 notably upfield shifted respectively (Fig. 3). These results implied that the intermolecular hydrogen bonds were present between the neighbour amide groups through a C=N−H–N linkage in the gel state. The observed upfield shift of amide protons with an increase in temperature is due to the breaking of intermolecular hydrogen bonds that leads to gel-to-sol transition.

FT-IR is another tool to investigate the different covalent interactions involved in gelation. FTIR spectra of gelators 5 and 6 were taken in the gel state and solid state to understand the intermolecular interactions between the gelator peptides. Taking 5 as an example (Fig. 4), it was observed that the amide band (NH stretching) as a broad peak centered at 3290 cm−1 for solid and 3278 cm−1 for xerogel, but no peak was observed around 3400 cm−1. It suggest all NH groups of the peptides are

| Table 1 Organogelation properties of 5 and 6 in organic solvents |
|-----------------|-----------------|-----------------|
| Solvent        | State (5/6)†    | MGC (g per 100 cm3) (5/6)‡ |
| Toluene        | G/G             | 1.4/1.4          |
| Benzene        | G/G             | 2.0/1.5          |
| o-Xylene       | G/G             | 1.6/3.5          |
| m-Xylene       | G/G             | 0.9/1.3          |
| p-Xylene       | G/G             | 1.8/1.0          |
| Mesitylene     | P/G             | —/1.2            |
| Chlorobenzene  | S/S             | —                |
| Chloroform     | S/S             | —                |
| THF            | S/S             | —                |
| DMSO           | S/S             | —                |
| Methanol       | S/S             | —                |

† G, gel; P, precipitate; S, solution. ‡ MGC, minimum gelator concentration.

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Solvents. Water and good organogelation abilities in many aromatic environments. The organogelator formation appears that of the solid and it revealed that strong hydrogen bonding promoted the organogel formation. The appearance of the C=O stretching band (amide I) at the range of 1636–1641 cm⁻¹ and the NH bending (amide II) band at the range of 1539–1566 cm⁻¹ showed that a model of aggregation similar to β-sheet arrangement found in peptides. Another peak at 1733 cm⁻¹ appeared in the solid state as well as in dilute solution in CHCl₃ (Fig. S4†), which is characteristic of the C=O stretching band of the three ester groups present in the gelator, while in the gel state, there were two C=O stretching frequencies (1758 cm⁻¹ and 1731 cm⁻¹), respectively. For the gelator 6, the same phenomenon as the gelator 5 happened to its FT-IR spectra (Fig. S5†). It certified that the stretching vibration bands of carbonyl group in L-Ala appeared at higher wavenumbers due to the hydrogen bonding involved in the gel formation, while the other carbonyl groups almost have no change between gel and solid state.

The phase-selective gelation from a mixture of solvents becomes more challenging and useful when one of the solvents is water. Interestingly, organogelators in the present study were found to be suitable for selective gelation of oil (aromatic solvent) from an oil–water mixture due to their insolubility in water and good organogelation abilities in many aromatic solvents.† In a typical procedure, 0.5 mL of toluene and 0.5 mL of water were mixed in a sample tube to which 10 mg of gelator 6 was added. The gelator 6 was then solubilized in this two-phase solution by heating and also shaken vigorously to ensure homogeneous dispersion of oil in water. After cooling the mixture to room temperature, the toluene layer was gelated, and the water layer remained intact in liquid state (Fig. S6†). Next, the organogel could be separated from the water simply by filtration.

Removal of toxic dyes from waste water is important for environmental improvement. Ideally, dye-adsorbing agents should have the amphipathicity domains for efficient dye removal. Recently the use of gels for dye removal has also been reported and they have showed the property of dye adsorption through purely physical or chemically interactions between dyes with the gelator aggregates.† The adsorption of four different compounds into the gel was studied, which are commercial dyes, including two positively charged aromatic compounds as rhodamine 6G and acriflavine and two anion dyes as fluorescein disodium and cresol red. In the adsorption studies, the solutions containing the different guests were deposited on xerogel of 6 in toluene. As shown in Fig. 5, the xerogel was extremely selective on the adsorption of the positively charged dyes such as acridine yellow and rhodamine 6G, and 1 mL of xerogel (1.5 g per 100 cm³) is able to completely decolour a 5 mL solution dyes with a dye concentration 10 mg L⁻¹. After 24 h, more than 95% of acridine yellow and 92% of rhodamine 6G were removed by the xerogel monitored by UV-visible spectroscopy (Fig. 6) of dyes in aqueous solution, which revealed that the dye molecules were transferred from water to xerogel leaving clear water (Fig. 5B). Conversely, the solid state of 6 didn’t decolour any dye solution under the same condition (Fig. S7†). The facts of selective absorption cation dyes suggest that there be electrostatic interactions between the cation dye molecules and several electron-rich atoms in peptides.† In addition, the self-assembly structure of the xerogel facilitates the interactions with dye molecules.

**Fig. 4** IR spectra of 5 in solid and xerogel (1.0 g per 100 cm³) made from toluene.

**Fig. 5** (A) Photograph of dye solution of (a) rhodamine 6G, (b) fluorescein disodium, (c) acriflavine and (d) cresol red. (B) Photograph of dye solution and xerogel 6 after adsorption.

**Fig. 6** UV/vis spectrum of aqueous solution of dye solution (a) rhodamine 6G, (b) fluorescein disodium, (c) acriflavine and (d) cresol red and absorbed by xerogel 6 after 24 h.
As we know, cresol red is a pH-indicator dye in aqueous solution, which means its unionized form shows yellow and the deprotonated form changes to purple. At the same time, its absorption wavelength changes in the UV spectra accordingly. Interestingly, the xerogel didn’t decolour solution of negatively charged cresol red, while it changed its colour from yellow to purple immediately (Fig. 5a). Furthermore, UV-visible spectroscopy illustrated that the absorption peak shifted from 435 nm to 573 nm (Fig. 6d), which indicated cresol red was deprotonated after interacting with the gel. It suggest that the supramolecular gels showed an amazing basicity increase in the aggregated gel state and could capture protons from cresol red. It was probably due to the close proximity of –NH groups on the gel fibers that could provoke the cooperative assistance of several basic groups through water or cresol deprotonation (Scheme 2).

Generally, phenolic hydroxyl (pK_a = 9.95) is more acidic than H_2O (pK_a = 15.9), so the self-assembly aggregates captured protons from phenolic hydroxyl of cresol red more easily than from water. In addition, cresol red solution can contact within the surfaces of gel fully to promote the interaction between dye molecules and the assembly gelators. However, compound 6 as the solid state didn’t make that change. Actually, Miravet and co-workers reported that L-proline derivatives showed an amazing basicity increase in the aggregated state which can be used as basic catalysts in aldol reaction. As expected, the xerogel of 6 changed its colour from yellow to blue and it was identified with the changed absorption wavelength in the UV spectra (Fig. 7).

Conclusions
A new kind of tripeptide conjugated triterpenoids have been synthesized and found to be capable of forming robust organogels in some aromatic solvents through hydrogen bonding and van der Waals interactions. In addition, these organogels could selectively gelate aromatic solvents from their mixtures with water. Due to the fact that the self-assembly nanostructures of gelators interact selectively with positively charged dyes, the xerogels showed a striking property of adsorbing cation dyes such as rhodamine 6G and acriflavine from water. The dye adsorption ability of gels made them potentially utilized in water purification by removing toxic dyes. To the best of our knowledge, this is the first report of the self-assembly of triterpenoid-based conjugates with peptide and the characteristics of the conjugates would give some clues of potential applications in nanoscience and nanotechnology.

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Notes and references

