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Small molecule hydrogels based on a class of antiinflammatory agents

Zhimou Yang,[a] Hongwei Gu,[a] Yan Zhang,[a] Ling Wang[a] and Bing Xu*[ab]

[a] *Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China*

[b] *Bioengineering Program, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China. E-mail: chbingxu@ust.hk; Fax: 852 2358 1594; Tel: 852 2358 7351*

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Here we report small molecule hydrogels formed by the combination of two *N*-(fluorenyl-methoxycarbonyl) amino acids, which belong to a novel class of antiinflammatory agents.

Hydrogels, usually formed by using natural or synthetic polymers, represent an important class of biomaterials because they resemble the extracellular matrix (ECM) of the body and show biocompatibility, thus offering scaffolds for tissue engineering and providing matrices for drug delivery.[1] Despite the successes in the research of low molecular weight organogels,[2] small molecule hydrogels[3–5] have only received attention recently and have just emerged as biomaterials (*e.g.*, hydrogels based on oligopeptides have been used as scaffolds to grow neurons[3]). This paper describes a new hydrogel formed by the combination of two *N*-(fluorenyl-methoxycarbonyl) amino acids—NPC 15199 (**1**) and Fmoc-L-lysine (**2**), which belong to a novel class of antiinflammatory agents reported by Burch *et al.*[6] (Scheme 1). In the hydrogels, NPC 15199 not only serves as the structural component, but also offers antiinflammatory function since it exhibits activity in animal models of inflammation.[6] The approach of the direct use of drug molecules to form a hydrogel can lead to a new type of hydrogels as potential biomaterials,[5] which could function as "self-delivery" systems. In addition, this hydrogel allows other therapeutic agents to be incorporated. A hydrogel with self-rendered antiinflammatory activity could be beneficial when it serves as a carrier for other therapeutic agents because the arrest of inflammatory response is one of the important prerequisites for biomaterials.

Scheme 1 illustrates the typical process for the gelation of the mixture of **1** and **2**. Both **1** and **2** are amphiphilic molecules: **1** carries a carboxylic acid group, and **2** an amino acid moiety. They both bear a hydrophobic fluorene fragment that allows π–π interaction. Neither **1** nor **2**, however, forms a hydrogel independently because of their limited solubility in water (0.0064 g/100 g H_2O for **1** and 0.037 g/100 g H_2O for **2** at 25 °C, which correspond to 0.18 and 1.0 mM, respectively). Addition of one equiv. of Na_2CO_3 to the suspension of either **1** or **2** turned it into a clear solution. The pH values of the solution of **1** and the solution of **2** are about 8.3 and 9.8, respectively. Adding one equiv. of **1** (in solid or suspension form) to the solution of **2** or one equiv. of **2** to the solution of **1** afforded a solution at 40–48 °C. Cooling the solution to room temperature led to gelation within 3 minutes (Fig. 1A). The formed hydrogel appears semi-transparent, indicating that a small amount of **1** or **2** exists as microparticles in the hydrogel. The pH value of the resulting hydrogel is ~9.1, and the minimum concentration of **1** or **2** required for gelation is 10 mM, corresponding to the ratio of the gelator to water at 1 : 5500. The use of the same procedure, except doubling the amount of Na_2CO_3, produces a clear hydrogel (Fig. 1B, pH ~10.4), which exhibits gel–sol transition when the pH > 11.4.

Despite the gelation of the mixture of **1** and **2** requiring the assistance of a base, the formed hydrogels collapsed neither in neutral nor in acidic conditions (*e.g.*, the hydrogels are stable at a pH value as low as 2.0). Raising the temperature above 51 °C induced the gel–sol phase transition of the hydrogels, and the solutions changed back to the hydrogels upon cooling. Such a cycle can be repeated many times without affecting the gelation ability.

These hydrogels can act as carriers for other bioactive agents by simply adding them into the solution of **2** or **1**. For example, when 3.1 mg of 5-fluoro-2′-deoxyuridine (**3**), an antineoplastic agent,[7] were added into 0.4 ml of the solution of **2** (formed by adding one equiv. of Na_2CO_3), the hydrogel formed (Fig. 1C) after the suspension of **1** was added into the solution of **2** and **3**. When two equiv. of Na_2CO_3 were used, the amount of **3** that could be incorporated into the hydrogels increased to 4 mg and the hydrogel remained transparent (Fig. 1D).

As shown in Fig. 2A, the scanning electron micrograph (SEM) of the cryo-dried hydrogel that consists of **1**, **2**, and Na_2CO_3 with a

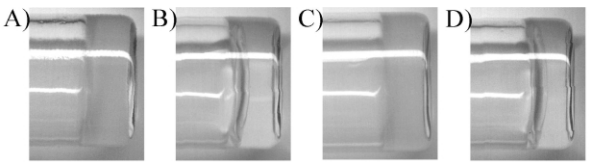


Fig. 1 The optical images of the hydrogels formed by A) 3.5 mg of **1**, 3.7 mg of **2**, and 1.1 mg of Na_2CO_3 in 0.4 ml H_2O; B) 3.5 mg of **1**, 3.7 mg of **2**, and 2.2 mg of Na_2CO_3 in 0.4 ml H_2O; C) 3.5 mg of **1**, 3.7 mg of **2**, 1.1 mg of Na_2CO_3, and 3.1 mg of **3** in 0.4 ml H_2O; and D) 3.5 mg of **1**, 3.7 mg of **2**, 2.2 mg of Na_2CO_3, and 4 mg of **3** in 0.4 ml H_2O.

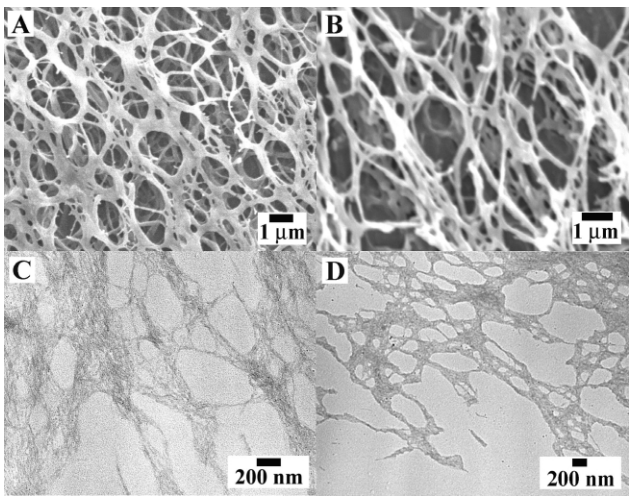
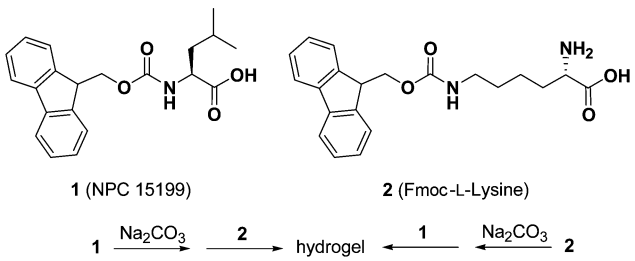


Fig. 2 (A, B) SEM images and (C, D) TEM images of the hydrogels in Fig. 1B and Fig. 1D, respectively.



Scheme 1 The chemical structures of the two components used in the hydrogels and the schematic gelation process.

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mole ratio of 1 : 1 : 2 exhibits entangled irregular fibers (width of 120–500 nm), which provide the matrix for the hydrogel. The addition of **3** induced slightly different morphology—the fibers, compared to without **3**, become thicker and have a width of 70–300 nm (Fig. 2B). The fibrous matrices, however, are preserved, which is consistent with the formation of the hydrogel shown in Fig. 1D. Transmission electron micrographs (TEM) reveal that the fibers consist of fibrils (~11 nm, Fig. 2C). Upon the addition of **3**, the fibrils have greater width (~20 nm, Fig. 2D). Both results indicate that the nanofibers observed in SEM are bundles of supramolecular polymer chains, which should result from the intermolecular interactions between **1** and **2**, or among **1**, **2**, and **3**.

To further understand the molecular arrangement of **1** and **2** in the hydrogels, we used circular dichroism (CD)[8] and fluorescent spectroscopy to characterize the solution and the hydrogels of **1** and **2**. As shown in Fig. 3A, when one equiv. of Na_2CO_3 is used, the Cotton effect at 224 nm (nπ* transition) indicates superhelical arrangements of the L-Leu or L-Lys residues, which induce the helical orientation of the fluorenyl groups (the Cotton effect at 280–311 nm (ππ* transition)) in the hydrogel. The increase of the amount of Na_2CO_3 to two equiv. leads to the blue-shift of the corresponding peaks: the nπ* transition shifts to 221 nm, and the ππ* transition to 259–309 nm, which is consistent with the increase of the ionic strength and rigidity of the hydrogel. In solution, **1** and **2** give an emission peaking at 309 nm, which shifts to ~323 nm in the hydrogel containing one equiv. of Na_2CO_3, which suggests that the two fluorenes of **1** and **2** overlap in an antiparallel manner.[9] In the hydrogel containing two equiv. of Na_2CO_3, the emission maximum presents at 330 nm, and a broad phosphorescence peak[9] centers at 455 nm, implying that fluorene groups overlap more efficiently and move less freely in the hydrogel. The small shoulders around 381 nm in both hydrogels indicate that a small amount of the fluorenes overlap[9] in a parallel manner.

Based on the above information, we suggest that the π–π interactions between the fluorenyl groups provide part of the linkages required for forming extended chain structures. The hydrogen bond networks (one example of them is shown in Scheme 2) offer the rest of the force needed for the gelation. As shown in Scheme 2, the change of pH would exert minimal disruption on the hydrogen bond networks; therefore, the hydrogels were stable upon change of pH within a certain range. The irregularity of the morphology of the fibrous matrix suggests that there should be more than one type of arrangement of the hydrogen bond networks, resulting in relatively large cavities for the storage of other small molecules.

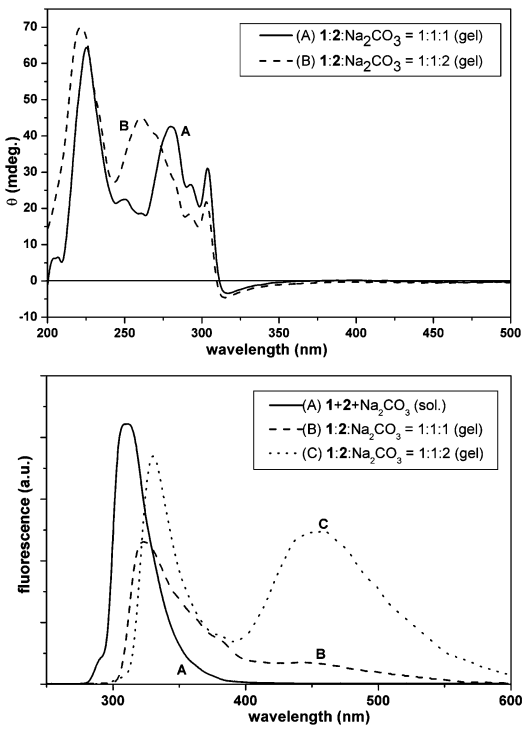
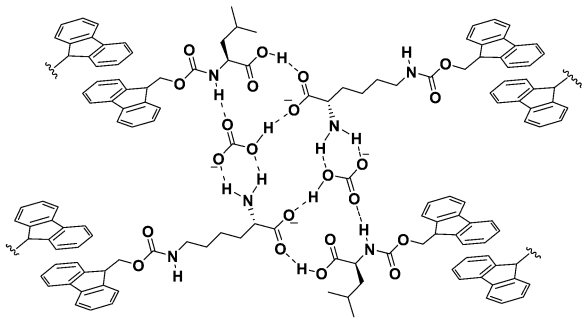


Fig. 3 The circular dichroism† of the hydrogels (above) and the emission spectra of **1** and **2** in solution and in hydrogels.



Scheme 2 One example of the possible hydrogen bond networks and π–π interactions that would promote gelation (Na^+ ions are omitted for clarity) when the molar ratio of **1**, **2**, and Na_2CO_3 is 1 : 1 : 1.

In summary, we have used a potential antiinflammatory agent to form hydrogels, which also allow other drug molecules to be incorporated. Future work will focus on the rheological study of this type of hydrogels and the evaluation of their *in vitro* or *in vivo* activity for the development of new biomaterials.

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Notes and references

† The solubility of **1** and **2** in solution was too low to give a measurable CD spectrum.

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