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Catalytic dephosphorylation of adenosine monophosphate (AMP) to form supramolecular nanofibers/hydrogels†

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The use of enzyme to instruct the self-assembly of the nucleoside of adenosine in water provides a new class of molecular nanofibers/hydrogels as functional soft materials.

This communication reports the use of enzyme to instruct the self-assembly of the nucleoside of adenosine in water for developing supramolecular nanofibers/hydrogels as a novel type of soft biomaterials. Hydrogels, composed of a solid network in water, have been a type of fascinating materials that find many useful applications. Although the network usually is made of covalently crosslinked polymers, certain small molecules (referred as hydrogelators) are able to self-assemble in water to form nanofibers. When their density is sufficiently high, the nanofibers entangle to form the network and result in hydrogels, which is called supramolecular hydrogels.[1] A variety of small molecules can act as hydrogelators, including the derivatives of nucleosides. Nucleosides, consisting of a nucleobase and a sugar (*i.e.*, ribose or deoxyribose), are the building blocks of nucleic acids (*i.e.*, RNA or DNA).[2] Because of their biological relevance and importance, they have attracted considerable research efforts in the area of using nucleosides as the building blocks for generating hydrogels as a new type of biomaterials. For example, Shimizu *et al.* first reported the hydrogels made of nucleotide-appended bolaamphiphiles. They found that nucleotides connected by long spacers are capable of gelling water effectively through spontaneous formation of a fibrous network. They also suggested that hydrogen bonds involving the 5′-hydroxyl group of the deoxyribose moiety, hydrophobic interaction between the long oligomethylene chains, and π–π stacking of the thymine residues result in the effective hydrogelation.[3] After this early report, several other groups also have developed hydrogels built upon nucleosides.[4–10] Among them, Barthelemy *et al.* prepared a family of uridine phosphocholine amphiphiles that self-assemble to form supramolecular structures or materials including vesicles, fibers, hydrogels, and organogels.[4] Oda and co-workers have developed cationic gemini surfactants that have nucleotides as the counterions and form hydrogels upon the addition of complementary nucleoside bases.[6] Kim *et al.* used click chemistry to connect benzyl groups to nucleoside derivatives for making hydrogelators.[8] Jamieson *et al.* demonstrated that the addition of potassium ions into the mixture of guanosine gelator and a non-gelator guanosine leads to a very stable hydrogel.[9] Recently, Barthelemy *et al.* reported the self-assembly of a glycosyl-nucleoside-lipid to form nanofibers and nanotubes and suggested that the carbohydrate moieties provide a suitable environment to deliver nucleic acids into human cells.[10] These developments clearly highlight the promising opportunity presented by the hydrogelators made of nucleosides.[5]

The above developments not only validate the suitability of nucleosides to serve as a motif in hydrogelators, but also illustrate several ways to trigger the formation of those hydrogels, such as the change of temperatures,[4] the addition of ions,[6] or the adjustment of pH.[3] Despite the effectiveness of these methods for identifying hydrogelators made of nucleosides, a biocompatible method to generate the hydrogels would be more beneficial for the applications of the hydrogelators of nucleosides in a biological environment. Thus, we intend to use an enzyme-catalysed reaction to form the hydrogels of nucleosides because (i) enzyme-catalyzed hydrogelation is able to take place *in vitro* and *in vivo*;[11] (ii) the exceptional selectivity and high efficiency offered by enzyme-catalysed reactions allow more precise control of hydrogelation; (iii) enzymatic hydrogelation of nucleosides is yet to be explored. Among the possible candidates of nucleosides and enzymes, we choose adenosine[2] and alkaline phosphatase (ALP)[2] as the nucleoside and the enzyme, respectively, for this investigation of the enzymatic formation of supramolecular nanofibers/hydrogels of nucleosides. Scheme 1A shows the process of catalytic dephosphorylation of adenosine monophosphate to form supramolecular nanofibers and hydrogels. Adenosine is the nucleobase in several important bioactive molecules,[12] including adenosine-5′-triphosphate (ATP), a multifunctional nucleotide used in cells as a coenzyme, and cyclic adenosine monophosphate (cAMP), a second messenger molecule.[13] ALP, a readily available hydrolase, is able to remove phosphate groups from a wide range of substrates, including alkaloids, nucleotides, and proteins. Thus, the use of ALP to trigger the formation of supramolecular nanofibers/hydrogels consisting of an adenosine motif may offer new opportunity to explore the promise of the hydrogelators of nucleosides.

Based on the above rationale, we made the hydrogelator (**3**) and the corresponding precursor (**4**) by connecting adenosine

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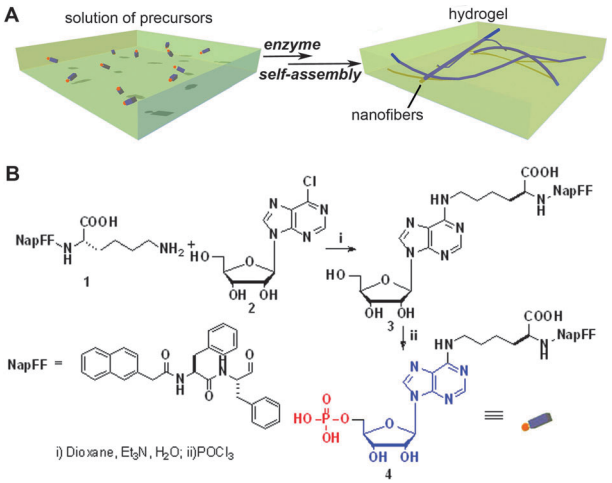

Scheme 1 (A) The illustration to show the concept of enzyme-catalyzed formation of nanofibers and hydrogels. (B) The synthetic route of the hydrogelator of adenosine (**3**) and the corresponding precursor (**4**).

and adenosine monophosphate (AMP), respectively, with a small-molecule hydrogelator naphthalene-Phe-Phe-Lys (NapFFK, **1**), a tripeptide derivative made by the conjugation of 2-(naphthalen-2-yl)acetic acid with Phe-Phe-Lys, because **1** forms a hydrogel at a low critical gelation concentration (0.8 wt%).[14] ALP dephosphorylates **4** to generate **3**, which has a minimum gelation concentration of 0.8 wt%. Transmission electron microscopy (TEM) reveals that the molecules of **3** self-assemble in water to form long and uniform nanofibers; rheology study confirms that the enzyme triggers the hydrogelation of **3**. This preliminary study not only opens a new way to synthesize supramolecular nanostructures of nucleosides, but also has potential to provide the assembly of nucleosides for a range of possible new applications.

Scheme 1 shows the synthetic route and structure of the hydrogelator (**3**) and the precursor (**4**). According to our previously reported procedure, we synthesized **1** using solid phase peptide synthesis (SPPS).[15] Nucleophilic substitution of the chloride on **2** yields **3**, which is phosphorylated at the 5′-hydroxyl group of the ribose of adenosine to afford **4**. Dissolving well in water (Fig. 1A), **4** exhibits excellent stability in water and hardly shows any dephosphorylation in the absence of alkaline phosphatase (ALP).

After the successful synthesis of **4**, we tested enzymatic hydrogelation using **4**. After dissolving 7.0 mg of **4** in 200 μL of buffer at pH = 7.4 to make a clear solution (Fig. 1A), we added 10 units of alkaline phosphatase (ALP) into the solution. As shown in Fig. 1B, a transparent gel forms after the addition of ALP, indicating that the ALP converts the precursor **4** to the desired hydrogelator **3**, which self-assembles in water to form the nanofibers and affords the hydrogel. LC-MS also confirms the conversion of **4** to **3**. We also found that **3** can self-assemble in water and form a weak hydrogel (Fig. 1C) at the concentration of 2.0 wt% and pH 7.4. The addition of oligomeric nucleic acid poly(T)$_{10}$ to the hydrogel of **3** results in a new hydrogel (Fig. 1D) that exhibits significantly increased elasticity, suggesting that the Watson–Crick interaction between A (on **3**) and T (on poly(T)$_{10}$)

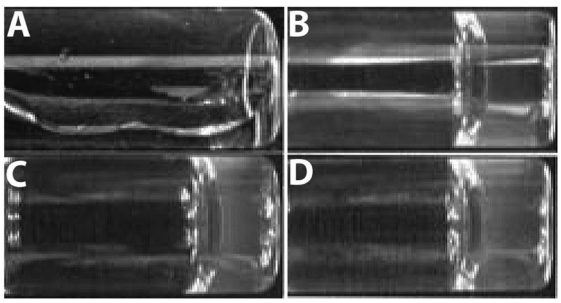

Fig. 1 Optical images of (A) the solution of **4** (7.0 mg of **4** in 200 μL of water at pH 7.4); (B) the gel formed by adding alkaline phosphatase to the solution of **4**; (C) the gel of **3** (4.0 mg of **3** in 200 μL of water at pH 7.4); (D) the gel of **3** treated by adding 1 eq. poly(T)$_{10}$ (2.0 wt%, pH 7.4).

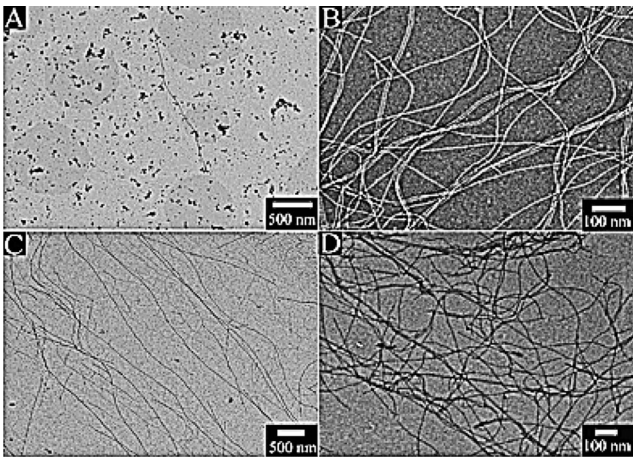

Fig. 2 Transmission electron microscope (TEM) images, obtained by negative staining,[16] of (A) the solution of **4**; (B) the gel formed by the addition of the phosphatase; (C) the gel of **3** formed at pH 7.4; (D) the gel of **3** treated by adding 1 eq. poly(T)$_{10}$.

strengthens the nanofiber networks that serve as the matrices of the hydrogel.

As shown in the TEM images, the aqueous solution of the precursor (**4**) hardly shows any well-formed nanofibers (Fig. 2A). After the treatment of ALP, the formed hydrogel contains long nanofibers with diameters of 11–17 nm and lengths beyond several microns (Fig. 2B). Some of the nanofibers appear to form bundles that consist of 2–3 individual nanofibers. Unlike the hydrogel formed by the addition of ALP, the TEM of the hydrogel of **3** formed by the change of pH shows low-density of nanofibers and relatively low degree of crosslinking (Fig. 2C). Notably, the intermolecular interaction through the interbase binding between **3** and poly(T)$_{10}$ results in a more effective cross-linked network that consists of well-dispersed nanofibers (Fig. 2D), whose diameters range from 12 to 24 nm. This result indicates that the use of the single strand nucleic acids may be an effective approach for modulating the mechanical properties of the hydrogels of nucleosides.

One characteristic property of gels is their viscoelasticity, which reflects the mechanical properties of the hydrogels. According to the rheological measurement, the strain sweep and frequency sweep provide sufficient data for studying the

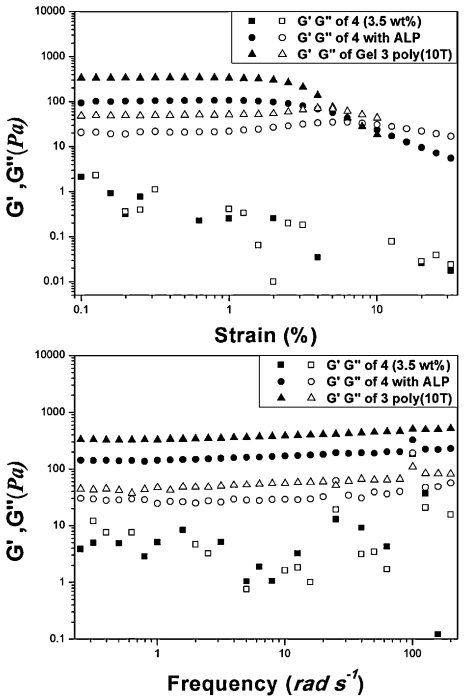

Fig. 3 Strain and frequency dependence of the dynamic storage moduli (G') and the loss moduli (G'') of the solution of **4** (3.5 wt%), the gel formed by the addition of ALP into the solution of **4**, and the gel formed by the mix of poly(T)$_{10}$ and **3**.

deformation and flow of materials, especially in gel states. As shown in Fig. 3, during strain sweep, the value of the dynamic storage modulus (G') of the solution of **4** at the concentration of 3.5 wt% almost equals to its loss modulus (G''), about 1 Pa, which indicates a liquid-like state. After the treatment with ALP, the mixture exhibits much higher G' than G'', and the value of G' increases from 1 to 100 Pa, indicating that the sample behaves as a viscoelastic material. The addition of poly(T)$_{10}$ to the hydrogel of **3** results in an increase in G' up to at least 3 times higher than that of **4** treated with ALP. This result clearly indicates that the oligonucleic acid likely enhances the cross-linking of the nanofibers of **3**. Moreover, the value of G' of the hydrogel, in frequency sweep, exhibits little dependence on the frequency, suggesting that the matrix of the hydrogel has good tolerance to external shear force. All of these results indicate that the hydrogel of **3** and poly(T)$_{10}$ consists of a more complex network structure, which provides effective cross-linking and increases the elasticity of the hydrogel. This result also agrees with the morphology as revealed by TEM (Fig. 2D).

In conclusion, we have successfully demonstrated that, with proper molecular design, the integration of enzymatic reaction and self-assembly provides a feasible approach to create the molecular hydrogels of adenosine. Of all the nucleosides and nucleotides, the recognition of adenosine 5′-monophosphate (AMP) is vital[17] since nucleotide phosphates such as AMP are important because of their role in bioenergetics, metabolism, and transfer of genetic information. This work, thus, provides new, biofunctional supramolecular assemblies that contribute to the development of novel biomaterials, which certainly deserve further exploration. In addition, this approach should be applicable for other nucleosides, such as GMP, cAMP, ADP or ATP, that are substrates of an array of enzymes and carry important functions.

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