

## Gel–Flocculation Transition of a Supramolecular Hydrogel Induced by Depletion Effect of Polymers

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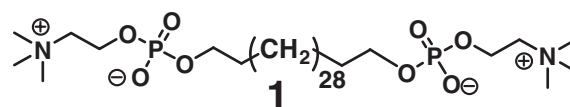
Upon addition of hydroxyethyl cellulose, a supramolecular hydrogel from a bolaamphiphilic molecule showed significant flocculation. Microscopic and spectroscopic observations revealed that the phenomenon was ascribable to polymer-induced depletion flocculation of the self-assembled fibril networks into the tape bundles.

Supramolecular gels consist of self-assembled fibrillar networks (SAFIN), in which low molecular-mass organic gelators (LMOGs), self-assemble into physically cross-linked fibrous structures.<sup>1</sup> In the network structures, the LMOGs gelate a wide variety of organic and aqueous solvents. Because of the diversity in molecular design, supramolecular gels have also been examined in terms of applications to a matrix for bioanalysis,<sup>2</sup> controlled release and drug delivery,<sup>3–5</sup> a scaffold for cell engineering,<sup>6</sup> and so on.

One remarkable property of the supramolecular gels is that the systems can exhibit three switchable states: a molecularly dissolved solution (sol), swelled SAFIN formation (gel), and deswelled SAFIN formation (flocculation). So far, most studies have focused on switching of the sol–gel phase transition triggered by thermal,<sup>5</sup> photo,<sup>7</sup> pH,<sup>8</sup> enzyme,<sup>9</sup> and molecular stimuli.<sup>10</sup>

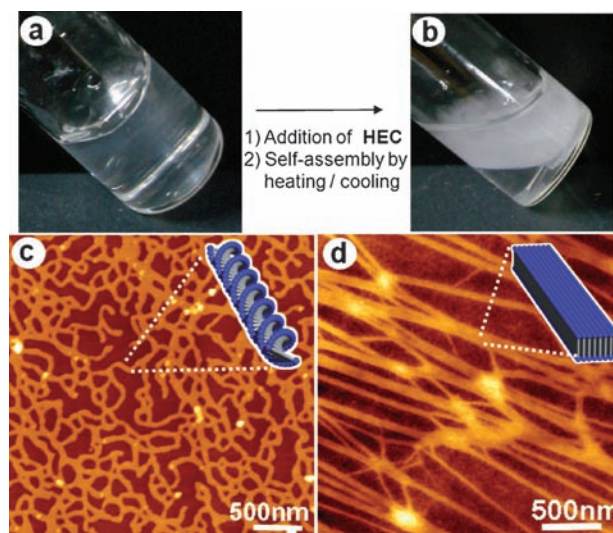
To achieve gel–flocculation transition of a supramolecular gel, we utilized depletion interaction,<sup>11</sup> i.e., an attractive force between colloid objects produced by osmotic pressure of non-adsorbing polymers. Depletion force induces various colloidal particles<sup>12</sup> and biomacromolecules,<sup>13</sup> such as proteins, DNAs, and viruses, to flocculate or crystallize upon addition of polymers. Utilization of the polymers as stimuli for gel phase transformation seems more advantageous to biological applications because one can utilize a variety of biopolymers dissolved in vivo (20–40 wt %) as stimuli. In addition, gel–flocculation transition can expel water and small molecules from the gel without further decomposition of the SAFIN.

In this study, we found that the depletion effect stemming from polymers induces hierarchical aggregation of the molecular fibril self-assembled from, dotriacontan-1,1'-diylbis[2-(trimethylammonio)ethyl phosphate], **1** to give a bundle of tape structures of up to several hundred nanometers in width (Scheme 1). The aggregation eventually causes “gel–flocculation phase transition.” To the best of our knowledge, there has been no report of gel–flocculation transition of supramolecular gels triggered by depletion flocculation.



Scheme 1.

Blume et al. reported that on cooling from hot aqueous solution, bolaamphiphilic hydrogelator **1** gave a transparent hydrogel (Figure 1a).<sup>14,15</sup> They estimated that **1** self-assembled to form helical single fibers 6–7 nm wide and the fibers physically cross-linked to form the gel. In fact, atomic force microscopic (AFM) observation indicated **1** formed nanofibers even below critical gelation concentration (CGC), (0.05 wt %) (Figure 1c together with estimated molecular packing). Further condensation of the solution hampered the AFM observation due to overlapping of the nanofibers.



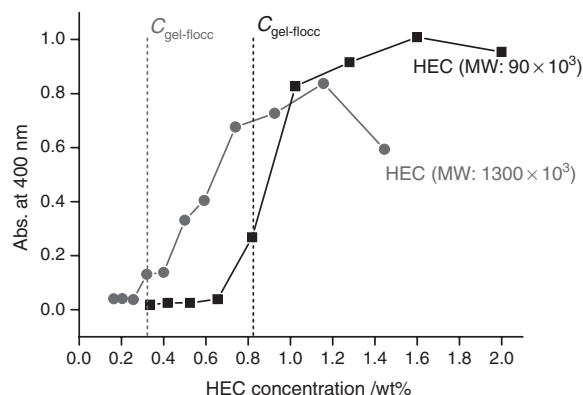
**Figure 1.** Photo of (a) a hydrogel self-assembled from 0.5 wt % **1** in a vial and (b) flocculation of the hydrogel upon addition of HEC (MW  $1300 \times 10^3$ , 0.5 wt %) and subsequent self-assembly by heating to 90 °C and cooling to 20 °C. Tapping mode AFM images of (c) dilute hydrogel of pure **1** (0.03 wt %) and (d) the flocculated tape from mixture of **1**/HEC (0.5 wt %/0.5 wt %, MW of HEC  $1300 \times 10^3$ ). Insets were schematic illustrations of estimated molecular arrangement within the assemblies.

Interestingly, addition of hydroxyethyl cellulose (HEC, MW:  $1300 \times 10^3$ ) to the hydrogel showed a remarkable effect on the phase behavior. When a hot mixed solution of **1**/HEC (0.5 wt %/0.5 wt % in water) was cooled to 20 °C which is well below gel formation temperature of **1** ( $T_{\text{gel}} = 48$  °C), the solution became opaque; the system exhibited substantial flocculation irrespective of the gel formation (Figure 1b). Other polymers examined such as ethylhydroxyethyl cellulose (EHEC, MW:  $390 \times 10^3$ ) and linear poly(*N*-isopropylacrylamide) (poly-NIPAM) also induced a similar flocculation phenomenon under the same conditions (Figure S6).<sup>16</sup> Optical microscopic<sup>16</sup> and AFM observation indicated that the **1**/HEC mixture formed bundles of tape structures several hundred nanometers width (Figure 1d and inset). The AFM height profiles indicated the tapes possessed uniform thicknesses (approximately 4.2 nm), comparable to the single fiber thickness of pure gel from **1** (4.5 nm based on AFM, Figures S2).<sup>16</sup> The aggregation of the single fibers to form tapes seemed to induce the gel–floc transition.

The following experiments suggested that the formed tapes, prepared from **1**/HEC mixture, were composed of almost pure **1**. The tapes were separable from the mixed solution by centrifuge, which afforded analysis of both the sediment tapes and residual fluid separately. IR spectra indicated that the tapes consisted of **1** and showed no peak corresponding to HEC (Figure S5).<sup>16</sup> In addition, labeling of HEC by fluorescent tetramethylrhodamine molecules (TMR-HEC) and subsequent analysis supported the results (Figure S4).<sup>16</sup> In microscopic observation, the tapes obtained from a mixture of a **1**/TMR-HEC (2 wt %/2 wt % in pure water) gave almost no fluorescent image of the tapes by excitation of the rhodamine moiety. The UV–vis measurements of initial TMR-HEC solution and the supernatant fluid from the mixture revealed that only 7 wt % of TMR-HEC adsorbed onto the tapes, and the other was left in the solution as uncomplexed.

All of these results indicated that the flocculation upon HEC addition is induced by osmotic pressure of nonadsorbing polymer, i.e., “depletion flocculation.” Other possible mechanisms such as bridging flocculation of polymer chains adsorbed onto the fiber surfaces was ruled out, since the above characterization of separated tape and solution by IR measurements and labeling experiment showed that HEC did not adsorb onto the subsequent tapes.<sup>17,18</sup>

Molecular weight dependence of the flocculation behavior described below further supported the depletion flocculation mechanism. In general, a polymer with a larger radius of gyration (a larger  $R_g$ ) induces stronger depletion force than that with a smaller  $R_g$ , at the same polymer weight fraction.<sup>19</sup> Therefore, the dependence on HEC molecular weight was examined (Figure 2). The critical flocculation concentration of **1** (CFC) was measured by turbidity at 400 nm at 20 °C. The gel–floc transition concentration ( $C_{\text{gel-floc}}$ ) was also estimated by ball sink test as well. As shown in Figure 2, both measurements showed the flocculation of fiber started at concentration of 0.3 and 0.8 wt % for  $1300 \times 10^3$  and  $90 \times 10^3$  HEC, respectively, indicating a similar dependence on the molecular weight; the HEC with a higher molecular weight induces the flocculation the more efficiently. A decrease in the turbidity of the **1**/HEC above 1.4 wt % of  $1300 \times 10^3$  HEC probably stemmed from strong phase separation by the enhanced depletion flocculation.<sup>18</sup>



**Figure 2.** Turbidity of aqueous **1**/HEC mixtures (**1**: 0.5 wt %) dependence on HEC concentration at 20 °C monitored at 400 nm. Red and black dotted lines indicated  $C_{\text{gel-floc}}$  of each **1**/HEC (MW:  $1300 \times 10^3$  and  $90 \times 10^3$ , respectively) estimated from ball sink test.

Taken together, we revealed the polymers induced the gel–floc transition of the supramolecular hydrogels by depletion effect. This property is applicable to controlled release of drugs from the gel. In fact, a mixing of the hydrogel of **1** with thermosensitive poly-NIPAM induced flocculation of the hydrogel below 32 °C corresponding to lower critical solution temperature of the poly-NIPAM (Figure S6).<sup>16</sup>

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