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Enhanced Mechanical and Helical Properties with Achiral Calix[4]arene in Co-Assembled Hydrogel which Exhibited the Helical Structure

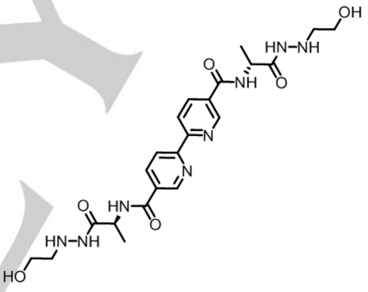
Heekyoung Choi,[a][‡] Hyowon Seo,[a][‡] Misun Go, [a] Shim Sung Lee [a] and Jong Hwa Jung*[a]

Abstract: A mixture of the building blocks **1A** and **2G** having D-alanine moieties and glycine-functionalized calix[4]arene moieties, respectively, formed co-assembled hydrogel. In particular, the remarkable enhancement of helical intensity of co-assembled gel was controlled by achiral calix[4]arene **2G**, which was attributed the bridge effect helically between **1A** and **2G** like helical structure. Furthermore, the improvements of mechanical strength (G´ and G″ values) of co-assembled hydrogel prepared with 1.0 equiv. of **2G** were *ca*. 7000% and *ca*. 4400% as compared to the gel prepared from the 0.25 equiv. of **2G**, respectively. The improved mechanical strength was attributed to the formation of network structure with the H-bonding interaction between **1A** and **2G**. The results indicate that the helical and mechanical strength enhanced of co-assembled gel could be controlled by achiral component **2G**.

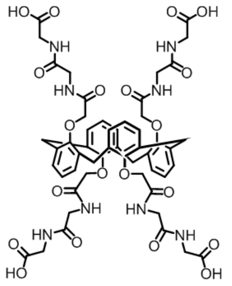
Soft material development has seen a wealth of applications for viscoelastic systems such as organogels and hydrogels, particularly for areas including controlled release[1-3] and soft tissue reconstruction.[4,5] In biomedical applications, gel systems have been implemented in energy capture and storage[6,7] as well as sensing areas[8,9] by the discovery of their exciting responsive properties toward external stimuli such as temperature, pH, light and electric field.[10-12]

In most of cases, gels are formed using one gelator. Mixing different gelators (where each form gels independently) is also interesting.[13-15] Depending on how these gelators assemble, a mixed gel systems could be controlled the various physical properties of the final gels. For instance, Nandi group has been reported the mechanical and electrical properties of co-assembled gels.[16] In contrast, a number of systems have been reported where two components are required to interact to form a gel,[17-20] however, the individual components do not form gels by themselves.

Although several groups have demonstrated the control of the mechanical properties of co-assembled gels,[16,21] the development of co-assembled gels remains important for the improvement and control of the mechanical and helical properties. In particular, the improvement of helicity with achiral components in co-assembled gels is difficult, with relatively few examples reported. Because, generally, upon addition of achiral gelators in co-assembled gels disrupts the helical molecular arrangement. Furthermore, the mechanical property of helical structure gives higher than that linear structure.[22] Therefore, the well-defined helical nanofibrous in gels might be exhibited more strong mechanical property in compared to the linear fiber structures in gels. In this study, we have improved the viscoelastic and the helical properties of co-assembled gel composed of D-alanine appended bipyridine gelator and achiral glycine-appended calix[4]arene gelator. The mechanical property of co-assembled gel was remarkably improved upon addition of achiral component. Upon addition of achiral calix[4]arene in gel system showed remarkable a well-organized helical property. Here we report on remarkable improvement of both the mechanical and the helical properties of co-assembled hydrogel with D-alanine appended bipyridine gelator and achiral glycine-functionalized calix[4]arene gelator as a core building block by bridge effect with the intermolecular hydrogen-bonding interaction.



1A



2G

The bipyridine and 1,3-alternated calix[4]arene moieties were used as a core building block, which enables formation of three-dimensional network and metal ion binding. The peptide moieties were introduced to the control of helicity and the intermolecular hydrogen-bonding interaction in co-assembly system. For instance, compound **1A** possesses bipyridine moiety as metal bind site and peptide moiety to the intermolecular hydrogen-bonding interaction. Compound **2G** consists of each two glycine moieties at upper and lower parts of 1,3-alternated calix[4]arene as a core group, which each two glycine attached at calix[4]arene moiety enable to introduction a well-organized molecular arrangement of **1A** with π-π stacking.

Since compounds **1A** or **2G** did not form gel in organic solvents and water, co-assembled hydrogel of **1A** (co-hydrogel) was prepared by the addition of **2G**. Gel formation occurred instantly and was characterized by the cessation of flow in the test tube inversion experiments (Figure S1). The co-hydrogel (**1A**-**2G**) was stable for two months at room temperature.

Absorption spectra of **1A**, **2G** and co-hydrogel (**1A**-**2G**) in a water showed in Figure S2. Compound **1A** exhibited two absorption bands at 249 nm and 297 nm, which may be ascribed to be the π-π* and n-π* transition, respectively. In the co-hydrogel

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(**1A**-**2G**), the n-π* transition is slight intense and it is red shifted to 328 nm, suggesting that the formation of co-assembly between **1A** and **2G**. The absorption band at 274 nm gradually increased by the addition of **2G**, which was corresponds to the π-π* transition of **2G**.

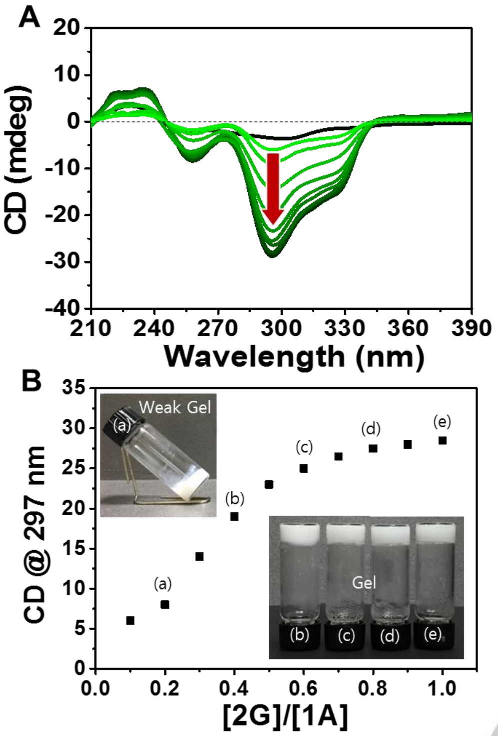

Figure 1. (A) CD spectra of **1A** (black line) and co-hydrogel (**1A-2G**) via concentration of **2G** (0.1-1.0 equiv.). (B) Plot of CD intensity at 297 nm in (A).

To investigate the chiral arrangement between **1A** and **2G**, we observed CD spectra of co-hydrogel upon addition of **2G** (Figure 1A). The CD spectrum of **1A** with 0.2 equiv. of **2G** exhibited a negative signals at 256 nm and 297 nm, respectively, which originated from bipyridine and alanine moieties, respectively. The CD signal at 297 nm was induced CD (ICD). These negative CD signals indicate that **1A** molecules were orientated into the *P*-type (right-handed) helix. The CD intensity of **1A** upon addition of **2G** enhanced at 256 nm and 297 nm, and was reached the equilibrium when *ca*. 1.0 equiv. of **2G** was added to **1A**. These results indicate that two molecules of **1A** was bound to one molecule of **2G** by the intermolecular hydrogen-bonding interaction. The enhancement of CD signal of co-hydrogels upon addition of **2G** is very unique phenomenon, because the glycine moiety of **2G** is a typical achiral compound. **1A** molecules would be well-organized helically by bridge effect with the intermolecular interaction, due to rigid calix[4]arene skeleton of **2G** with bifunctionalized glycine moieties at each upper and lower. Achiral components in co-hydrogels induce the disordered molecular arrangement, resulting in the decrease of CD intensity. Thus, the enhancement of CD intensity was a rare example that the supramolecular helicity of co-hydrogel was controlled by achiral compound **2G**. On the other hand, the CD intensity of sol **1A** was *ca*. 10 folds weak at 297 nm compared to co-hydrogel formed by **1A** and **2G**. This weak CD intensity was due to that **1** molecule existed as monomer species.

Furthermore, the reversibility of CD signals was further observed in the CD spectra of co-hydrogel prepared 1:1 ratio between **1A** and **2G** upon addition of free **1A**. From Figure S3, we can see the decrease in the negative intensity of the CD signal upon addition of free **1A** resulting from a decrease in the *P*-type (right-handed) 1:1 complex. When enough **1A** was added to bring the concentration (**2G**/**1A**) to 0.2 equivalents, the negative CD signal showed the smallest intensity. It is notice that the CD signal induced not only ICD from the bipyridine moiety of **1A**, but also the intermolecular interaction with bifunctionalized glycine moieties at each upper and lower parts of **2G**. Since no significant decrease of CD intensity was observed after several rounds of alternating the addition of **1A** and **2G**, we concluded the CD inversion is fully reversibly as a result of helicity switching in the gel.

To obtain a proper understanding of the mechanism of co-assembly between **1A** and **2G**, it is important to gain an insight into the structure of the complexes. The FTIR spectrum of pure **2A** exhibited one –C=O stretching peak at 1735 and 1663 cm^{-1} for acid and amide carbonyl groups, respectively (Figure S4). In the spectrum of co-hydrogel, both of these peaks shifted to lower frequencies with band broad (1730 and 1650 cm^{-1}, respectively), which might be due to the formation of H-bonding interactions between **1A** and **2G**. In order to provide a deeper investigation of the nanofiber gel formation, an analytical technique based on nuclear magnetic resonance (NMR) spectroscopy was used to provide qualitative insight on co-hydrogel (**1A**-**2G**). By using variable temperature (VT) NMR, we measured the NMR signal of **1A** (20 mM) when in the presence of **2G** (20 mM) in D_2O (0.6 mL) at increasing temperatures (Figure S5). Upon increasing the temperature, ^1H spectrum of gel **1A-2G** were sharped, and was shifted to low-field. The sharped peaks were due to increase of mobility of **1A** and **2G** by disassembly. The low-field shifts of aromatic protons were due to de-shielding effect, suggesting that there is loss in π-π stacking between **1A** and **1A** by disassembly. Furthermore, we observed ^1H NMR spectra of the different concentrations of gels (20, 40 and 60mM) as shown in Figure S6. The proton peaks were broaden by increasing of concentration of gel, which was attributed to decrease of mobility of **1A** and **2G** by gel formation.

To insight into the morphologies of co-hydrogels formed by **1A** and **2G**, we observed the morphologies of co-hydrogel by FE SEM (Figures 2 and S7). We observed morphologies of the xerogel formed by different concentrations of **2G** (0.25, 0.5, 0.75 and 1.0 equiv.). In the xerogel sample prepared with 0.5 and 1.0 equivalent of **2G**, the SEM image showed well-defined all right-handed helical nanofiber structures with *ca*. 20 nm diameter. This observation was a rare example for the helical structure induced by achiral component in the co-assembly, because upon addition of achiral components induces from the helical structure into non-helical structure. This helicity was well agreement to the CD observation, indicating that the helicity in the molecular level reflected into the macroscopic helicity. Increasing concentration of **2G** induced the increase of branch density of the fibers, which would be caused the network structure by bridge effect between **1A** and **2G**.

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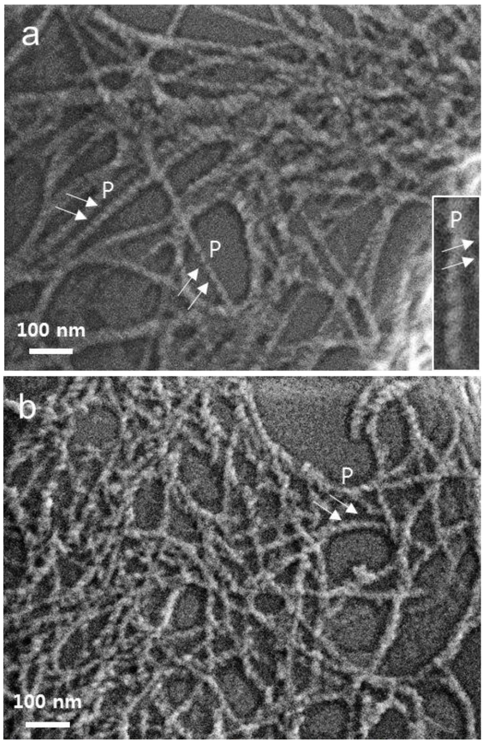

Figure 2. (A) SEM images of helical structure of co-hydrogel (1.0 wt%) prepared by (a) **1A**-**2G** (0.5 equiv.) and (b) **1A**-**2G** (1.0 equiv.).

We also obtained information of molecular arrangement of co-hydrogels from an X-ray diffraction pattern of the xerogels. The powder XRD patterns of xerogels were shaped by increasing concentration of **2G** (Figure S8). From 0.4 equivalent of **2G** in co-hydrogel, the diffraction patterns are characterized by several shape refection peaks of 16.55, 9.97, 8.16 and 6.66 Å in the small angle range. These values were obtained constantly between 0.4 equiv. to 1 equiv. of **2G**. Thus, we could assumed that if largest *d* value is 33.1 Å, which was 2 time of 16.55 Å, the relative intensity of which is almost exactly in the ratio of 1:1/2:1/3:1/4:1/5. This means that the xerogels maintain layered structures with the interlayer distance of 33.1 Å. Furthermore, the Bragg reflection pattern exhibited a shape peak at a 2θ value of 22.77 which could indicate a distance of 4.0 Å between the bipyridine groups of **1A** in co-hydrogel. This is clear evidence that the co-hydrogel formed by the intermolecular hydrogen bonding interaction as well as π-π stacking. The findings support the view that the co-hydrogel possesses the well-ordered aggregate structure in the gel state.

We investigated the mechanical properties in detail by first evaluating the storage (G´) and loss (G") moduli by rheometric measurement. Strain sweep, frequency sweep and continuous step strain tests were conducted for each of the co-hydrogels formed with different concentrations of **2G** (Tables 1 and S9). Upon addition of **2G**, the G´ and G" values of co-hydrogels gradually increased. More interestingly, the highest increase in G´ and G" values of co-hydrogels were 7000% and 4400% for 1.0 equiv. of **2G** that of gel prepared with 0.25 equiv. of **2G**, respectively (table 1 and Figure S9A). These remarkable enhancement in the mechanical property of co-hydrogel is due to helical structure as SEM and CD observations, which would be so called “helical effect”. G′ values of co-hydrogels prepared with different concentrations of **2G** at ca. γ= 6% (G"/G´= 1) is almost same to G″ value. The results indicate that collapse of the gel state to a quasi-liquid state did not dependent to the concentration of **2G**. The elastic modulus curves as a function of the oscillation frequency are shown in Figure S9; frequency). The values of G´ for the gels prepared with 0.25, 0.50, 0.75 and 1.0 equiv. of **2G** at 1Hz are *ca*. 0.13 MPa, 1.54 MPa, 6.81 MPa and 9.03 MPa, respectively, which correlate with the amplitude sweep data at the same frequency. G´ value in gels showed a same slope by changing frequency sweep, suggestive no structure changes by frequency sweep. Furthermore, a gel-to-quasi liquid transition is also completely reversible, where G′ and G″ values are recovered rapidly within 30 s when γ is reduced from 100% to 0.1%. This reversibility is thixotropic response.

The elasticity (G´–G″) and stiffness (G´/G″) of the gels, calculated from the rheological data, are presented in Table 1. The elasticity of co-hydrogels increases by increasing the concentration of achiral **2G**, because of attributed to the increase of branch density of the fibers. This improvement in the mechanical properties of co-hydrogel prepared with 1.0 equiv. of **2G** over co-hydrogel prepared with 0.25 equiv. of **2G** may be ascribed to the formation of a co-assembled branched fibrillary structure resulting in stronger cohesive force between the fibrils of the network. In contrast, the co-hydrogels showed no the constant tendency for the stiffness. Conclusively, the remarkable enhancement in mechanical property of co-hydrogel resulted in the mainly increase of elasticity property.

Table 1. Comparison of Rheological Data of Co-Hydrogel vis Concentration of **2G**.

Equivalent of co-hydrogel [**2G**]/[**1A**]	Storage modulus G´ (Pa)	Loss modulus G″ (Pa)	Elasticity (G´-G″)	Stiffness (G´/G″)
0.25	1283	314	969	4.08
0.5	15400	3334	12066	4.61
0.75	68160	11960	55918	5.77
1.0	90320	13960	76360	6.47

In summary, we have demonstrated co-hydrogel formation of both D-alanine attached 1,4-bipyridine derivative and the glycine functionalized 1,3-alternated calix[4]arene as achiral component. In particular, increasing the concentration of **2G** induced the co-hydrogel formation, leading to enhancement of CD intensity of co-hydrogel. The *P*-type helical molecular arrangement of co-hydrogel could be controlled by achiral component **2G**, which was attributed to bridge effect. Furthermore, G′ and G″ values of gels were 7000% and 4400% enhanced upon addition of achiral component **2G**, respectively, due to the formation of network structure with the H-bonding interaction between **1A** and **2G**. In particular, the elasticity of co-hydrogels increases by increasing the concentration of achiral **2G**, due to attributed to the increase of branch density of the fibers. Furthermore, the mechanical property enhanced of co-hydrogel was consistency to the enhancement of CD intensity. A gel-to-quasi liquid transition showed that the reaction was completely reversible, where G′ and G″ values were recovered rapidly within 30s when γ is reduced from 100% to 0.1%. This reversibility was a thixotropic response. Thus, we expect that the potential may exist for extending our in situ gel preparation method to the development of future smart-materials or dual-use functional/structural gels by implementing functional derivatives.

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Keywords: Chirality • Co-assembly • Helical structure • Mechanical properties • Supramolecular chemistry

[1] H.-J. Kim, K. Zhang, L. Moore and D. Ho, *ACS Nano* **2014**, *8*, 2998.
[2] I. Tokarev, S. Minko, *Adv. Mater.* **2010**, *22*, 3446.
[3] J. H. Lee, C. Kim and J. H. Jung, *Chem. Commun.* **2015**, *51*, 15184.
[4] S. Rose, A. Prevoteau, P. Elziere, D. Hourdet, A. Marcellan and L. Leibler, *Nature* **2014**, *505*, 382.
[5] J. Malda, J. Visser, F. P. Melchels, T. Jüngst, W. E. Hennink, W. J. A. Dhert, J. Groll and D. W. Hutmacher, *Adv. Mater.* **2013**, *25*, 5011.
[6] J. Germain, J. Hradil, J. M. J. Fréchet and F. Svec, *Chem. Mater.* **2006**, *18*, 4430.
[7] Y. Shi, L. Peng and G. Yu, *Nanoscale* **2015**, *7*, 12796.
[8] J. H. Lee, S. Kang, J. Y. Lee, J. Jaworski and J. H. Jung, *Chem. Eur. J.* **2013**, *19*, 16665.
[9] A. Döring, W. Birnbaum and D. Kuckling, *Chem. Soc. Rev.* **2013**, *42*, 7391.
[10] M. A. C. Stuart, W. T. S. Huck, J. Genzer, M. Muller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov and S. Minko, *Nat. Mater.* **2010**, *9*, 101.
[11] E. A. Appel, J. D. Barrio, X. J. Loh and O. A. Scherman, *Chem. Soc. Rev.* **2012**, *41*, 6195.
[12] H. Qian and I. Aprahamian, *Chem. Commun.* **2015**, *51*, 11158.
[13] M. Tena-Solsona, S. Alonso-de Castro, J. F. Miravet, B. Escuder, *J. Mater. Chem. B* **2014**, *2*, 6192.
[14] J. Raeburn, A. Zamith Cardoso, D. J. Adams, *Chem. Soc. Rev.* **2013**, *42*, 5143.
[15] E. R. Draper, E. G. B. Eden, T. O. McDonald, D. J. Adams, *Nat. chem.* **2015**, *7*, 848.
[16] P. Chakraborty, P. Bairi, B. Roy, A. K. Nandi, *ACS Appl. Mater. Interfaces* **2014**, *6*, 3615.
[17] J. Li, X. Du, S. Hashim, A. Shy, B. Xu, *J. Am. Chem. Soc.* **2017**, *139*, 71.
[18] M. Deng, L. Zhang, Y. Jiang, M. Liu, *Angew. Chem. Int. Ed.* **2016**, *55*, 15062.
[19] P. Xing, P. Li, H. Chen, A. Hao, Y. Zhao, *ACS Nano* **2017**, *11*, 4206.
[20] C. Colquhoun, E. R. Draper, E. G. B. Eden, B. N. Cattoz, K. L. Morris, L. Chen, T. O. McDonald, A. E. Terry, P. C. Griffiths, L. C. Serpell, D. J. Adams, *Nanoscale* **2014**, *6*, 13719.
[21] W. Edwards, D. K. Smith, *J. Am. Chem. Soc.* **2013**, *135*, 5911.
[22] H. Choi, J. Ahn, S. Kim, H. Kim, J. H. Jung, *Chem. Commun.* **2016**, *52*, 7600.

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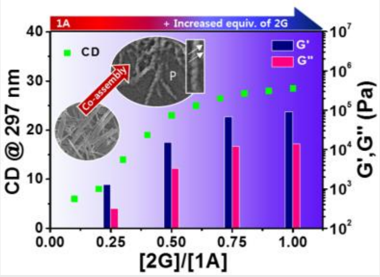
***P*-type helical structure**: co-assembled hydrogel composed of **1A** and **2G** induced the remarkable enhancement of helical intensity and mechanical properties.



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