doi:10.1246/cl.2011.617
Published on the web May 21, 2011

Self-assembled Gels of Amphiphilic Sequential Peptide in Water and Organic Solvents

Rong Cai,[1,2] Ying Zhao,[1] Kempei Ogura,[1] Masayoshi Tanaka,[1] Takatoshi Kinoshita,*[1] and Qing Cai[2]
[1]*Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya, Aichi 466-8555*
[2]*Beijing University of Chemical Technology, Beijing 100029, P. R. China*

(Received February 21, 2011; CL-110143; E-mail: kinoshita.takatoshi@nitech.ac.jp)

An amphiphilic peptide designed with serine as a polar residue showed gelation in both water and organic solvents. This sequential peptide has a strong tendency to adopt β-sheet conformation in these solvents. Such gels of β-sheet peptide based on hierarchical self-assembly consist of nanofiber construction because of the β-sheet structure, and because of crosslinking attributable to hydrogen bonding among hydroxy groups of the serine side chain.

Self-assembled gels are attracting great interest because of their versatile applications in a broad range of biorelated and nanotechnology-related uses.[1,2] Gels constructed in an aqueous medium called hydrogel are the most promising scaffolds for biorelated uses such as drug delivery and tissue engineering.[1–3] Peptides composed of specific amino acids are reliable candidates for use as gel fabrication materials. Especially, β-sheets-forming peptide which have alternating sequences with hydrophilic and hydrophobic residues stabilized by interstrand hydrogen bonding form supramolecular fibrous objects. The β-sheet-based fibrous objects usually polarized into hydrophilic and hydrophobic faces often construct bilayers in aqueous medium by facing the hydrophobic residues to screen from exposure to water. Accordingly, the hydrophilic residues exposed to the outer phase adopt ionic bonds and salt bridges on the side chain, and further self-assemble into hydrogel by interfibrous interaction among amino acid side chains.[4–6] Such sequential peptides which can form hydrogel at 0.5 wt % possess hundreds of times as much aqueous medium as peptide. The self-assembled peptide matrices are expected to be applied as a container for drug delivery and as an extracellular matrix for tissue engineering. Recently, we have found that a peptide scaffold composed of L-amino acids provides recognition for chiral molecules.[5]

Construction of supramolecular network structures which can be obtained in a broad range of solvents may also provide novel applications as enantioselective separation and recognition materials. Self-assembled gels formed in organic solvent also offer great potential for use in optical materials, in oil-recovery, and for drug delivery.[1,2,7] A typical design of such organogel comprises a flexible, solvophilic part and a rigid solvophobic part. Although few examples of amino acid-based designs were reported recently,[8–12] design of the organogelator remains complicated. A guiding principle for construction of organogel is hierarchical formation of the fibrous object and interfibrous interaction, which is common as a strategy for hydrogel construction. Nevertheless, few reports describe a gelator with ambidextrous capabilities to gelate both water and organic solvents.[13–16] In order to design such self-assembled hydrogels, charged amino acids are usually introduced as hydrophilic moieties. Noncharged hydrophilic amino acids have been as of yet scarcely designed in self-assembling sequential peptides, in spite of an expectation that the self-assembled gels may form without any difficulty of charge control. Here we report an amphiphilic sequence designed noncharged hydrophilic amino acid adopting a β-sheet structure in both water and organic solvents capable of gelating solvents of multiple kinds.

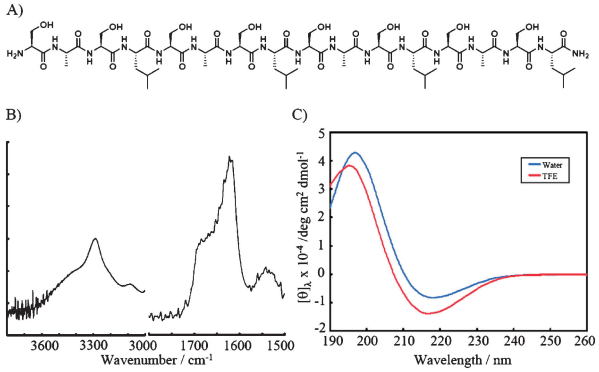

Figure 1. A) Sequence of the amphiphilic peptide, SASL16; B) FT-IR spectrum of peptide SASL16 hydrogel with 0.5 wt % of peptide concentration; C) CD spectra of SASL16 peptide in water and TFE.

The amphiphilic peptide H_2N–SASLSASLSASLSASL–$CONH_2$ (SASL16) was designed to have serine (S) as a noncharged hydrophilic amino acid residue (Figure 1A). Alanine (A) and leucine (L) were introduced as hydrophobic residue neighbors on the hydrophilic serine residue. Such alternating hydrophilic and hydrophobic amino acids are promising sequences with a tendency to stabilize the β-sheet conformation.[4,17] The SASL16 was prepared using a solid-phase method with standard Fmoc strategy. It was characterized using MALDI-TOF-MS. The secondary structure of SASL16 in aqueous medium was characterized using Fourier transform infrared (FT-IR) and circular dichroism (CD) spectroscopy. The peptide dissolved in D_2O showed strong amide A absorbance at 3278 cm^{-1}, amide I between 1610 and 1630 cm^{-1}, and amide II at 1537 cm^{-1} in FT-IR spectra (Figure 1B). The presence of the secondary peak at 1688 cm^{-1} indicates that SASL16 adopts an antiparallel β-sheet. The stretching band derived from hydrogen-bonded and non-hydrogen-bonded hydroxy groups appear at around 3169 and 3556 cm^{-1}, respectively.[18] Absorbance of the hydrogen-bonded OH group overlapped with the amide A band. However, no absorbance occurred at around 3500 cm^{-1}, which strongly suggests OH groups of serine residues adopted hydrogen bonding in the self-assembled gel. The CD spectra showed a strong negative peak at 217 nm and a positive peak at 196 nm, which also supports our inference that SASL16 has a typical β-sheet conformation (Figure 1C).

 © 2011 The Chemical Society of Japan

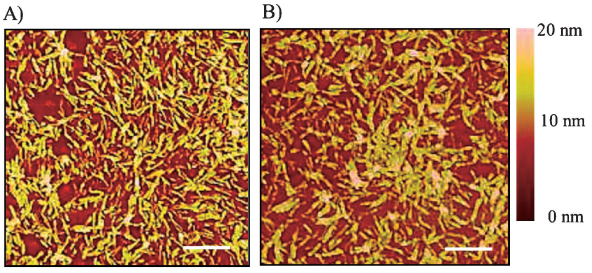

Figure 2. AFM images of SASL peptide nanofibers obtained from the aqueous solution (A) and the TFE solution (B). Scale bars: 200 nm.

The self-assembled structure of SASL16 obtained by drying a drop of the peptide solution on a mica substrate was observed using atomic force microscopy (AFM). Figure 2A shows entanglement of fibrous objects with 6.0 nm width, which agrees with the size of β-strand with 16 residues. Thickness of the fibrous object was estimated to be larger than 2.0 nm. Considering the height of the β-strand is 0.7 nm, the formed fibrous objects are most probably composed of at least bilayer structure. This agrees with previous reports claiming that amphiphilic β-sheet peptides tend to form a bilayer in an aqueous medium.[4–6] The hydroxy group of the serine residues is consequently exposed to the outer phase. Hydrogen bonds among the hydroxy groups would contribute to form entanglement and crosslinking points during the peptide self-assembly to nanofiber networks. Consequently, the observed network architecture of the fibrous object would consist of hierarchical self-assembly: 1) hydrogen bonding among amide groups on the main chain, 2) hydrophobic packing stemming from hydrophobic residues in the aqueous medium and hydrogen bonding among hydroxy group on the side chain. The former interaction contributes to form fibrous objects based on β-sheet structure, and the latter interactions contribute to give multilayer and network architecture by entanglement and crosslinking.

When SASL16 was dissolved in pure water at 1.0 wt %, a viscous liquid was obtained (Figure 2B). The viscous liquid was pH 3.7, which is lower than the pK_a of the N-terminus amine. Therefore, the cationic species were yielded at the N-terminus of the peptide. The cationic moieties facilitate the solubility of the peptide into water and induce an ionic repulsion, in which the crosslinked network structure would be weakened. Generally, such ionic repulsion between the charged moieties would be reduced by the addition of salts. SASL16 dissolved in the solution of NaCl (0.02 M) at 1.0 wt % immediately transformed into a transparent hydrogel at room temperature. The added salts, which screen the cationic charge derived from N-terminus amino group moderated the energetic barrier to give an appropriate condition to form the self-assembled hydrogel. Acetylation of the N-terminus was also effective to screen the ionic repulsion. A transparent hydrogel was actually obtained by dissolving acetylated peptide (Ac-SASL16) in pure water. However, the acetylation of N-terminus simultaneously induced decrease of solubility.

The viscoelasticity of the SASL16 hydrogel was investigated by rheological measurement. The results of the frequency sweep measurement are shown in Figure 3. The obtained result showed that typical elastic hydrogel was formed by self-

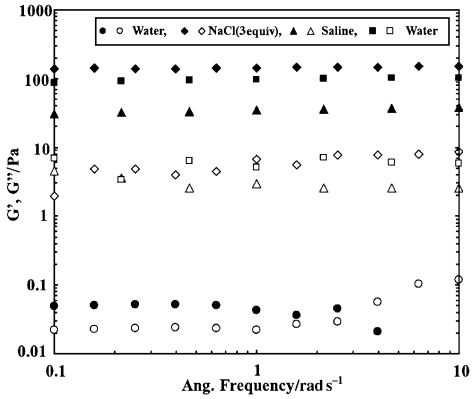

Figure 3. Rheological measurement of the network architectures obtained from SASL16 peptide nanofibers in aqueous solution. Storage modulus (G', solid) and loss modulus (G'', open) as frequency sweep.

assembly of the peptide due to the storage modulus (G') values exceeding the loss modulus (G'') over the entire frequency range. The values of G' and G'' almost agreed with the values of the hydrogels obtained from the amphiphilic peptide which are applied as an extracellular matrix.[4] It is noteworthy that the peptide also gelates 0.15 M NaCl solution, which is known as physiological saline solution. The gelation system is expected to be applicable as a scaffold for medical and therapeutic use.

Figure 1C shows that SASL16 also presented specific CD peaks at 217 and 196 nm in 2,2,2-trifluoroethanol (TFE). Results indicated that the peptide formed β-sheet conformation in TFE, although TFE is known as a helix-inducing solvent.[19,20] A similar network structure was observed from the dropped film of TFE solution (Figure 2B). Each fibrous object agreed with the calculated value of the peptide width with β-strand, and fibril entanglement was observed. In polar solvents such as TFE, apolar alanine and leucine residues might face one another by solvophobic effect. Therefore, SASL16, which adopted a β-sheet structure in TFE, is explainable as the mechanism of network construction by the hierarchical self-assembly described above. The SASL16 turned into gel immediately after dissolving in TFE at room temperature, which suggests that the fibrous object formed with β-sheet conformation steadily formed hydrogen-bond-linking side chains of serine residues. The gelation of SASL16 in other organic solvents is presented in Table 1. The SASL16 gelates polar solvents such as DMF, DMSO, and NMP. In the rheological measurements of the organogels, the G' values exceeded G'' (Table 1). The viscoelastic properties were dependent on the solvents. High viscoelastic gel which compares with the properties of previously reported hydrogels was formed in TFE and DMF.

In summary, results show that the amphiphilic peptide designed with serine as the polar residue gelated both water and some polar organic solvents. The sequence has strong propensity to adopt a β-sheet conformation and form hydrogen bonds among hydroxy groups on the serine side chains. Such a simple design concept and the ease of construction of the ambidextrous gels based on the sequential peptide present new possibilities for their use as functional gel materials for biorelated applications and nanotechnologies.

 © 2011 The Chemical Society of Japan

Table 1. Gelation properties of SASL16

Solvent	State[a]	MGC /wt %[b]	G'/Pa[c]	G''/Pa[c]
Water	V	—	—	—
Water (NaCl)	G	0.5	140	6
Saline	G	0.5	40	3
TFE	G	0.5	270	16
DMF	G	0.5	120	6
DMSO	G	1.0	10	1
NMP[d]	G	1.0	39	2
Methanol	I	—	—	—
Ethanol	I	—	—	—
Chloroform	I	—	—	—
Acetonitrile	I	—	—	—
HFIP[e]	S	—	—	—
THF	I	—	—	—
Ethyl acetate	I	—	—	—

[a]G, Gel; S, Solution; V, Viscous liquid; I, Insoluble. [b]Minimum Gelation Concentration. [c]Average value from 0.1 to 10 rad s^{-1} of the gel at 1.0 wt %. [d]1-Methyl-2-pyrrolidinone. [e]Hexafluoroisopropyl alcohol.

References

1 A. R. Hirst, B. Escuder, J. F. Miravet, D. K. Smith, *Angew. Chem., Int. Ed.* **2008**, *47*, 8002.
2 M.-O. M. Piepenbrock, G. O. Lloyd, N. Clarke, J. W. Steed, *Chem. Rev.* **2010**, *110*, 1960.
3 C. Tsitsilianis, *Soft Matter* **2010**, *6*, 2372.
4 H. Yokoi, T. Kinoshita, S. Zhang, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8414.
5 Y. Zhao, M. Tanaka, T. Kinoshita, M. Higuchi, T. Tan, *J. Controlled Release* **2010**, *147*, 392.
6 H. Rapaport, H. Grisaru, T. Silberstein, *Adv. Funct. Mater.* **2008**, *18*, 2889.
7 M. George, R. G. Weiss, *Acc. Chem. Res.* **2006**, *39*, 489.
8 P. Zhu, X. Yan, Y. Su, Y. Yang, J. Li, *Chem.—Eur. J.* **2010**, *16*, 3176.
9 M. I. Gibson, N. R. Cameron, *Angew. Chem., Int. Ed.* **2008**, *47*, 5160.
10 M. Suzuki, S. Owa, M. Kimura, A. Kurose, H. Shirai, K. Hanabusa, *Tetrahedron Lett.* **2005**, *46*, 303.
11 M. Suzuki, K. Hanabusa, *Chem. Soc. Rev.* **2009**, *38*, 967.
12 T. Nishide, J. P. Gong, H. Yasunaga, N. Nishi, Y. Osada, *Macromolecules* **1994**, *27*, 7877.
13 Y. Imura, K. Matsue, H. Sugimoto, R. Ito, T. Kondo, T. Kawai, *Chem. Lett.* **2009**, *38*, 778.
14 P. Duan, M. Liu, *Langmuir* **2009**, *25*, 8706.
15 R. Orbach, L. Adler-Abramovich, S. Zigerson, I. Mironi-Harpaz, D. Seliktar, E. Gazit, *Biomacromolecules* **2009**, *10*, 2646.
16 T. Kar, S. Debnath, D. Das, A. Shome, P. K. Das, *Langmuir* **2009**, *25*, 8639.
17 H. Rapaport, K. Kjaer, T. R. Jensen, L. Leiserowitz, D. A. Tirrell, *J. Am. Chem. Soc.* **2000**, *122*, 12523.
18 B. Lambie, R. Ramaekers, G. Maes, *J. Phys. Chem. A* **2004**, *108*, 10426.
19 S. Collino, J. S. Evans, *Biomacromolecules* **2008**, *9*, 1909.
20 F. D. Sonnichsen, J. E. Van Eyk, R. S. Hodges, B. D. Sykes, *Biochemistry* **1992**, *31*, 8790.

 © 2011 The Chemical Society of Japan