Gelation Behavior of β-Sheet Peptide RADA16 Coexisting with Synthetic Polymers

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A sequential peptide having hydrophilic and hydrophobic amino acid residues, RADA16 (CH₃CO-(Arg-Ala-Asp-Ala)₄-NH₂), forms a hydrogel in aqueous medium. The formation of the network structure would be based on hierarchical self-assembly; 1) formation of β-sheet structure, 2) construction of amphiphilic fibrous object, and 3) crosslinking between side chain by electrostatic interaction. The synthetic polymers such as polyelectrolyte and hydrophilic polymer mixed into the hydrogel medium gave influence to the physical property of the peptide hydrogel.

Key words: Self-assembly, β-Sheet peptide, Hydrogel

1. INTRODUCTION

Sequential peptide adopting β-sheet conformation is an attractive building block, because of its ability to form fibrous object in nano-meter scale based on intermolecular hydrogen bonding between β-strands.¹⁻³) The fibrous objects would form three-dimensional supramolecular structures depending on its amphiphilicity and interaction between side chains of the composed amino acid residues.⁴⁻⁷) RADA16 composed of 16 residues of amino acids with an alternative sequence of arginine (R), alanine (A), aspartic acid (D), and alanine (A), is one of the most well-known peptide to form a self-assembling hydrogel.⁷) The hydrogel construction is based on the formation of the β-sheet fibrous object and crosslinking with the fibrous objects by electrostatic interaction between side chains (Figure 1). Such peptide hydrogel is expected to have applications due to its high water content (> 99.5%). The peptide RADA16 which is commercially available as PuraMatrix from 3DM Inc. is actually applied as scaffold for cell culture. Recently we have reported such amphiphilic peptide hydrogel are also valuable for capsulation materials and expected for the use of drug delivery systems.⁸⁻¹¹) On the other hand, improvement of the complicated procedure to prepare the hydrogel and control of the physical strength are still essential issues for future use of the hydrogel. From these viewpoints, conjugation of synthetic polymer into the self-assembling systems would be one of the most efficient strategies. In this report, we have investigated the gelation behavior of RADA16, when synthetic polymers such as polyelectrolyte and hydrogen bonding related polymer were added.

Figure 1. Structure of RADA16 and schematic illustration of the hydrogel.

2. EXPERIMENTAL PROCEDURE

Materials The sequential peptide RADA16 was prepared by solid phase method using 9-fluorenlymethoxycarbonyl (Fmoc) strategy. The peptide chain was synthesized on CLEAR-Amide resin
(Peptide Institute, Inc.) by using 3 equivalents of Fmoc-amino acid derivatives. The activation and coupling reactions have been conducted using 3 equivalents of 1-hydroxy-7-benzotriazole (HOAt), and 3 equivalents of 1,3-disopropyl carbodiimide (DIPCDI) in N,N-dimethylformamide (DMF). And 20% of piperidine was used in DMF for removal of Fmoc group. The N-terminus of RADA16 was acetylated by 10 equivalents of acetic anhydride in DMF on the resin. To cleave the peptides from the resin and deprotection of the side chain were carried out with trifluoroacetic acid (TFA)/1,2-ethanedithiol/thioanisole /water (84:8:4:4). The peptides were precipitated and washed by diethyl ether.

All polymers, poly(acrylic acid) (PAAc, Mw 25,000), poly(allyl amine) (PAAm, Mw 17,000), and poly(vinyl alcohol) completely hydrolyzed (PVA, Mw 22,000) were purchased from WAKO and Aldrich, and were used without further purification.

**CD measurement** Circular dichroism (CD) spectra were obtained by CD spectropolarimeter (JASCO J-820K). A quartz cell with 0.1 cm path length was used for the measurement. The peptide solution was diluted to 100 times as much as the hydrogel concentration, since the concentration of the hydrogel was too high for the quantitative interpretation of the results of the CD measurements. The coexisting ratio of the RADA16 and the synthetic polymer was fixed as the ratio in the hydrogel system.

**Preparation of Peptide Hydrogel** The aqueous solution of RADA16 was prepared by dissolving the peptide powder in Milli-Q water and Tris-HCl buffer (pH 7.5). Final concentration of the peptide and pH of the hydrogel were 0.5 wt% and 3.7, respectively. Mixing of the polymers with the hydrogel was carried out by adding the polymer solutions to the hydrogel at a concentration of 0.005 to 0.5 wt%.

**Rheological Characterization** Viscoelastic property was estimated with a rheometer (AR1000, TA Instruments). A 40 mm diameter stainless steel parallel plate was used. Rheological study of samples was conducted by loading 300µL of the prepared sample on the sample platform. The rheological properties of all samples were determined through the following measurement protocol. First, an oscillatory strain amplitude sweep at fixed frequency (ω = 6 rad/s) was performed to establish the linear regime. Having established the maximum strain permitted for linear viscoelastic response, an oscillatory frequency sweep (frequency ω = 0.1-10 rad/s) was performed to measure G'(ω) and G''(ω), the viscoelastic storage and loss modulus, respectively.

### Table 1. Gelation behavior of RADA16 mixing with the polymers

<table>
<thead>
<tr>
<th>Polymer</th>
<th>0.5wt%</th>
<th>0.05wt%</th>
<th>0.01wt%</th>
<th>0.005wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 PAAc</td>
<td>p</td>
<td>p</td>
<td>gel</td>
<td>gel</td>
</tr>
<tr>
<td>2 PAAm</td>
<td>p</td>
<td>p</td>
<td>gel</td>
<td>gel</td>
</tr>
<tr>
<td>3 PVA</td>
<td>gel</td>
<td>gel</td>
<td>gel</td>
<td>gel</td>
</tr>
</tbody>
</table>

| a: Precipitation |

**Figure 2.** Typical images of (a) the formed hydrogel (0.5 wt%), and (b) the precipitates.
structure of the hydrogel. The hydrogel persisted up to the addition of 0.01 wt% of polyelectrolyte.

The secondary structure of RADA16 coexisting with the polyelectrolyte was investigated by CD spectroscopy (Figure 3). Two characteristic peaks, a negative peak at 215 nm and positive peak at 195 nm, are present. This shows that RADA16 adopted β-sheet conformation even coexisting with polyelectrolyte.

The viscoelasticity of the RADA16 hydrogel with polyelectrolyte was investigated by rheological measurement. The results of the frequency measurement were shown in Figure 4. The storage modulus (G') which characterizes the elastic response of the medium was larger than the loss modulus (G''), the viscous response of the medium, over the entire frequency range. The obtained result showed that the typical elastic hydrogel was formed by self-assembly of RADA16.

Values of the G' and G'' decreased, when polyelectrolyte was added into the hydrogel. These results indicated that coexistence of the polyelectrolyte weakened the strength of the hydrogel. The added polyelectrolyte would still interact with the side chain of the β-sheet peptide, and make the hydrogel weak by relatively strong aggregation.

On the other hand, hydrogel was successfully obtained, when PVA was added as much as the peptide amount in the hydrogel (Table 1). CD spectrum showed a negative peak at 215 nm and positive peak at 195 nm. This indicates that the secondary structure of the RADA16 coexisting with PVA was typical β-sheet conformation (Figure 3). In the rheological measurements, increases of the modulus values were observed (Figure 5). For instance, the value of G' at 1 rad/s increased from 96 Pa to 135 Pa, and G'' increased from 6.6 Pa to 10.9 Pa. These significant increases indicate that physical property of the hydrogel was strengthened by adding PVA. PVA having hydroxyl group on its side chain would interact with RADA16 via hydrogen bonding. It was suggested that weak interaction such as hydrogen bonding could keep exquisite balance of the network structure and be effective for control of the physical property of the hydrogel.

4. SUMMARY

The physical properties of the hydrogel constructed by a sequential peptide RADA16 have
changed by adding synthetic polymers. The addition of polyelectrolytes such as PAAc and PAAm has weakened the network structure of the hydrogel based on the electrostatic interaction between side chains. The addition of polymers related with hydrogen bonding such as PVA has strengthened the hydrogel on the basis of hydrogen bonding between side chains. These results indicated that physical strength of the hydrogel would be controlled by the adding amount and designing of the coexisting polymers.

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REFERENCES

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