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Analysis of Self-assembled Structure of Synthetic Polypeptide Hydrogel

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1 Introduction

Animal-derived materials such as collagen and Engelbreath-Holm-Swarm (EHS) gels are widely used as scaffolds for tissue engineering because of their general compatibility with living tissues. However, such animal-derived materials can cause allergic reactions and carry dangerous pathogens including prions that cause a variety of neurodegenerative diseases in humans and animals. Therefore, alternative sources of animal-derived scaffolds are required.

Self-assembling peptides are one of the candidate materials to solve these problems. The complete sequence of a self-assembling peptide was originally found in a region of alternating hydrophobic and hydrophilic residues in zuotin, which is characterized by a stable β-sheet conformation that undergoes self-assembly into nanofibers. The nanofibers form interwoven matrices that further form a hydrogel scaffold. Self-assembling peptides are a complete chemically synthesized material. Therefore, the use self-assembling peptide hydrogels can minimize the risk of biological contamination.

Here, we will report the structure analysis of the hydrogels which forms in water of pH ~ 7 by small/wade angle X-ray scattering (SWAXS).

2 Experiment

The sequence of the synthesized peptide was [CH_3CO]-RLDLRLALRLDLR-[NH_2] (SPG-178, R = arginine, L = leucine, D = aspartic acid, A = alanine). The isoelectric point of the SPG-178 peptide was designed to be 11.5 by employing four cationic arginine and two anionic aspartic acid residues. Furthermore, leucine residues were employed to increase the hydrophobic interaction among the SPG-178 peptides, which was the main driving force of the self-assembly, and stabilize the hydrogel formation.

The fiber structure of the hydrogel containing SPG-178 of 1.5 wt% was analyzed by SWAXS.

SWAXS detector was the Dectris PILATUS300K and 100K for SAXS and WAXS, respectively.

3 Results and Discussion

SAXS profile of the hydrogel (SPG-178) of 1.5wt% was shown in Figure 1a. The solid line was calculated SAXS profile as assuming that self-assembled peptide formed nanofibers with diameter of 5 nm. The theoretical scattering profiles fitted well with experimental one. The size of 5 nm was consistent with the lateral size of the fiber observed TEM observation and the self-assembling peptide model illustration (the chain length of one SPG-178 molecule) in Figure 2b. WAXS profile was simultaneously measured with SAXS. The WAXS profile of the SPG-178 hydrogel was shown in Figure 2. In the WAXS profile, the sharp diffraction peak was observed. This peak can be attributed to the formation of β-sheet structure of the peptide assembled. The formation of β-sheet in the hydrogel was also confirmed by FT-IR.

The nanofiber in the hydrogel was easily destroyed to be shorter one under irradiation of ultrasonic wave. The peptide can be reconstructed after stopping the irradiation. We are investigating the real time formation behavior of the gel.

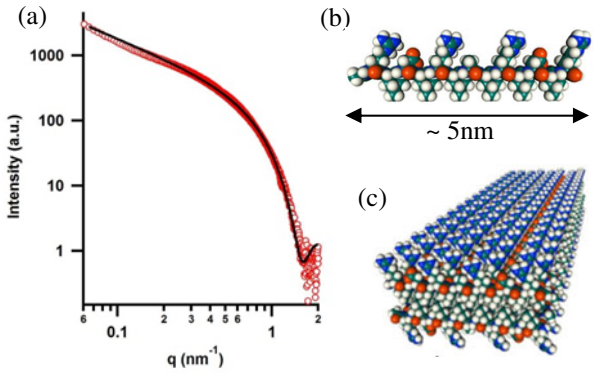

Figure 1. SAXS profile (a) of SPG-178 hydrogel (1.5wt%) and calculated scattering intensity (line). Molecular model of single SPG-178 (b) and self-assembled peptide fiber (c).

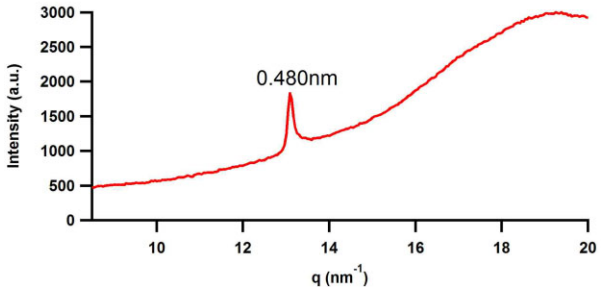

Figure 2. WAXS profile of the self-assembled SPG-178.

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