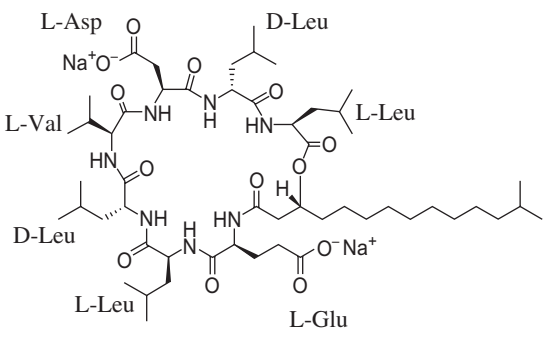
Tubular aggregate with hydrophilic interior formed by one-dimensional assembly of cyclic peptide amphiphile in nonpolar organic media

Peptide amphiphiles consisting of several amino acid residues and a long hydrocarbon chain have attracted much attention in biomedical applications, such as drug vehicles in drug delivery systems and scaffolds in tissue engineering, owing to the biological compatibility and properties endowed by self-assembled structures [1]. The self-assembled structures of peptide amphiphiles are generally determined by the sequences of amino acid residues, the corresponding secondary structures of the peptide chains, and the surrounding environment. In addition, if the molecular structure of a peptide amphiphile has a specific topology, e.g., a ring or branched structure, the self-assembled structure should reflect the molecular topology. Therefore, the unique self-assembled structure and related properties caused by topological features of peptide amphiphiles are of great interest.

Surfactin (SFNa), a cyclic peptide amphiphile produced by *Bacillus subtilis*, consists of a heptapeptide closed by a lactone bond with a β-hydroxy fatty acid (Fig. 1). It has been known that SFNa shows unique properties, e.g., the ability to decrease surface tension at extremely low concentrations of the order of 10^{-2} mM, monodisperse micelle formation, hemolytic activity, protease activity, antibacterial activity, and the ability to facilitate ion transport through lipid bilayer membranes [2]. These properties of SFNa are considered to originate from the cyclic topology of the peptide chain. For example, it has been found that the monodisperse *Platonic* micelle formation of SFNa is caused by the formation of regular polyhedra owing to the flat-on orientation of bulky peptide rings of SFNa at the hydrophobic-hydrophilic interface [3]. Among the unique properties of SFNa, the facilitation of ion transport through lipid bilayer membranes is of particular interest because of its potential application in functional membranes such as biomimetic ion transport membranes. The inner parts of lipid bilayer membranes are regarded as nonpolar hydrophobic media, and the self-assembly of SFNa in a nonpolar organic medium is the key to understanding the molecular mechanism of facilitated ion transport through such membranes. Thus, we investigated the self-assembled structures of SFNa in cyclohexane (CHx), which is a typical nonpolar organic solvent, to clarify the mechanism of this property of SFNa in a nonpolar organic medium.

In nonpolar organic media, the self-assembly of SFNa is driven by hydrogen bonds between peptide groups. Hydrogen bonds in SFNa are formed between different peptide rings owing to the restriction of the peptide rings of SFNa. Inevitably, SFNa assembles into fiber-like aggregates with unimolecular width owing to a one-dimensional arrangement through inter-ring hydrogen bonds. We observed thermo-reversible gelation in CHx solution of SFNa as a result of the formation of nanofibers (Fig. 2). Surprisingly, the SFNa gel and sol can dissolve a certain amount of water, although CHx negligibly dissolves water. This means that SFNa nanofibers include water. Because the ionic groups of SFNa are lipophobic, they are directed toward the inside of SFNa nanofibers to avoid contact with CHx. Therefore, SFNa nanofibers should form water channels. To elucidate the inside structure of the SFNa nanofibers, small-angle X-ray scattering (SAXS) measurements are performed. Figure 3(a) shows SAXS profiles of the SFNa gel and sol obtained at SPring-8 **BL40B2**. $I(q)$ exhibits q^{-1} dependence in the low q region assigned to nanofibers, where q is the magnitude of the scattering vector corresponding to the scattering angle. The solid lines in Fig. 3(a) were calculated using a core-shell cylinder model with the cross-sectional electron density profile shown in Fig. 3(b). Since the electron density inside the SFNa nanofiber is much lower than that of the outer shell, SFNa nanofibers are regarded as nanotubes. As mentioned above, ionic groups on SFNa rings are oriented toward the inside of nanotubes. This means that SFNa nanotubes have hydrophilic interiors. The added water therefore forms a channel through the SFNa nanotube shown in Fig. 3(c). The existence of the water channel in the SFNa nanotube is confirmed



M. W. = 1080 g/mol

Fig. 1. Chemical structure of surfactin (SFNa).

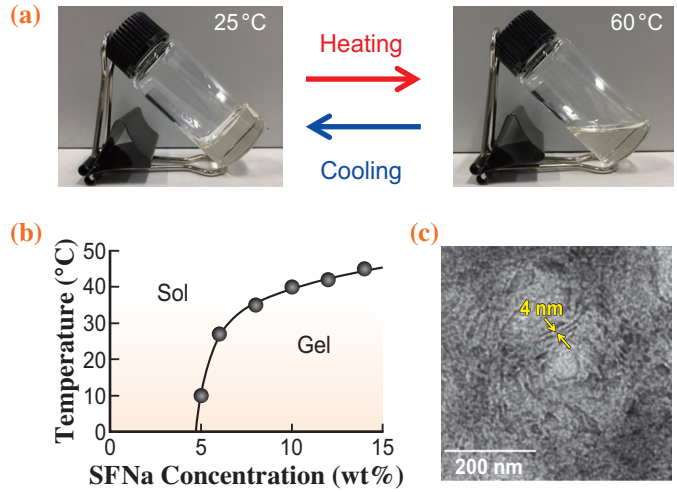


Fig. 2. Thermoreversible organogelation of SFNa in CHx: **(a)** photographs of CHx solution of SFNa at a concentration of 5.0 wt% at 25°C (left) and 60°C (right); **(b)** gel-to-sol transition temperature of SFNa gel; **(c)** TEM image of dried SFNa gel.

by the results of SAXS and small-angle neutron scattering. It has been known that SFNa enhances ion transport through lipid bilayer membranes. The inner parts of the lipid bilayer membranes are regarded as a nonpolar hydrophobic medium. SFNa should therefore form nanotubes with hydrophilic interiors in lipid membranes. The SFNa nanotubes penetrating the lipid membrane should act as ion channels and thus enhance ion transport through the membranes. Such an enhancement of ion transport through the lipid membranes as a result of the formation of ion channels is reminiscent of the relationship of actual biological phenomena. Therefore, we hope that this achievement will lead to the clarification of unsolved biological phenomena and the creation of novel biomimetic materials.

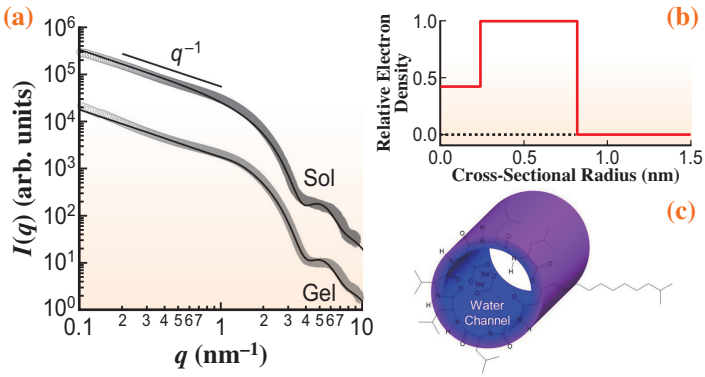


Fig. 3. Structural analysis of SFNa nanofiber: **(a)** SAXS profiles of SFNa sol and gel; **(b)** cross-sectional electron density profile of SFNa nanofiber; **(c)** schematic representation of SFNa nanotube with water channel.

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