Characterizing DDS nanoparticles containing therapeutic DNAs or RNAs

The FDA approved the first therapeutic drug based on RNA interference, Onpattro, in 2018. Loaded siRNA is delivered into the cytoplasm of hepatocytes by means of a lipid nanoparticle (LNP). This LNP–siRNA complex (i.e., lipoplex) consists of its cargo siRNA and several lipids, including cationic ones. The chemical structure of the cationic lipid has been optimized for RNA encapsulation and intracellular delivery. The success of Onpattro has enhanced enthusiasm in related research areas. A large number of nanomedicines have been reported, but only a few of them are clinically approved. The leading cause of the failure may be a lack of preclinical characterization [1]. To attain marketing approval, it is desirable to clarify physicochemical properties such as average particle size, dispersibility, particle shape, surface charge, drug loading and release, and surface coating. It is believed that these physicochemical properties affect the therapeutic effects such as pharmacokinetics, biodistribution, and toxicity.

We have been investigating the relationship between the structural properties of nanomedicines and their biological properties by scattering techniques. We showed, for example, that the structure of drug carriers and the amount of drug loading are closely related [2], and a biocompatible polymer behavior on the surface of drug carriers affects the biocompatibility [3]. In this paper, we introduce the structural characterizations of transfection carriers using SAXS at SPring-8 **BL40B2**.

To achieve efficient transfection, lipid nanoparticles such as cationic liposomes, consisting of cationic lipids, co-lipids, and stealth lipids, are often used. The major roles of cationic lipids are complexation with anionically charged nucleic acids and interaction with the anionic cell. Our group has developed aromatic amine (BA) compounds as gene delivery reagents with a higher efficiency and a lower toxicity than commercial products [4]. We evaluated the transfection efficiency for a BA system containing two phospholipids, L-α-phosphatidyl ethanolamine dioleoyl (DOPE) and 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) and pGL3 cording luciferase. The luciferase gene expression, calculated as a relative light unit (RLU) per mg of total cellular protein, strongly depends on the composition. We compared the supramolecular structures between BA: DOPE: DLPC = 1:0:1 (composition A) with the lowest transfection efficiency (11.6 RLU/ug protein, i.e., almost zero) and 1:2:1 (composition B) with the highest transfection efficiency (2.5×10^5 RLU / ug protein).

The results of SAXS measurement at composition A showed that a spherical micelle was formed before adding DNA, and the structural transition occurred from a micelle to a cylinder at $26.4 > N/P > 4.4$. Here, the N/P ratio generally means the molar ratio of the nitrogen in the lipid to the phosphate in nucleic acids. The fitting analysis of these profiles suggests that once DNA forms an ion pair with BA, the DNA must be included inside the cylinder, and the cylinder surface is covered with the rest of the free lipids. Moreover, we found that further addition of DNA ($N/P > 3.3$) induced the structural transition from cylindrical to lamellar. At composition B, a hexagonally packed cylinder was formed before adding DNA (Fig. 1). The addition of DNA did not induce structural transitions but enhanced the hexagonal ordering and reduced the distance between cylinders from 7.16 nm to 6.85 nm. This indicates that DNA plays a role in adhesion. We conclude that DNA is incorporated into the hydrophobic domain at composition A and the hydrophilic domain at composition B and that the difference in the DNA location in the complex is related to the transfection efficiency.

A recent trend is to use cationic lipids mainly to encapsulate nucleic acids and to cover particle surfaces with a biocompatible polymer, such as PEG or a polysaccharide, because most cationic lipids

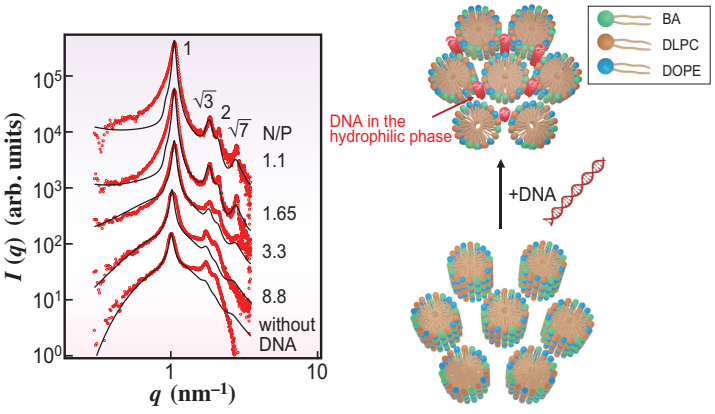

Fig. 1. N/P ratio dependence of SAXS profiles at composition B and schematic illustration of structural transition of the complex by adding DNA. Red dots and black lines show experimental data and theoretical curves, respectively.

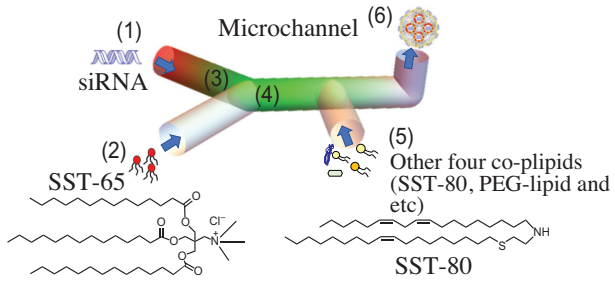

Fig. 2. Schematic illustration of the two-step mixing microchannel and chemical structures of SST-65 and SST-80. The numbers in parentheses show the lipoplex components (and mixing process).

have a high toxicity. Kyowa Kirin Co. designed a new cationic lipid denoted SST-65 with three alkyl tails and a new ionizable cationic one, SST-80 [5]. The cationic lipid/siRNA complex was prepared by two-step mixing using a microfluidic technique (Fig. 2). In the first step, siRNA (1) was mixed with SST-65 in EtOH-rich solution (2) and then mixed with a solution of other lipids (5), followed by solvent exchange with water. We changed the concentration of the co-lipids (5). First, the structure of the siRNA/SST-65 complex prepared in the first step was investigated using SAXS. The result showed that siRNA was coated by cationic SST-65, judging from the increased diameter and length in comparison with siRNA alone [compare scattering profiles (1)–(4) in Fig. 3(a)]. Then, the structures of the final products (6) were evaluated. Cryo-TEM images showed a spherical shape with a 20–50 nm radius packed with some filling (Fig. 3(b)). The inside patterns of the sample without siRNA (Ref. 2) and the complex containing a small amount of siRNA at N/P = 9.5 appeared to be patchy or spotty rather than circular. In contrast, the complex at N/P = 3.5 and 2 showed onion-like patterns (see Fig. 3(b)). These findings indicate the importance of the presence of siRNA. To determine the detailed structures of the complexes, SAXS analysis was carried out by changing the ratio of (5). Figure 3(b) shows the SAXS profiles for samples with different N/P ratios and the sample without siRNA as well as TEM images. The profile of the sample without siRNA showed no peaks but a small shoulder in the range of $q = 1.8–2.5$ nm^{-1}, indicating the thickness of the bilayer structures. The shoulder became obvious at N/P = 9.5, and a diffraction peak appeared at N/P = 6.5. This diffraction peak increased with the concentration of siRNA. According to the analysis using the Caillé theory, the layer spacing is consistent with the length of the adjacent onion patterns in cryo-TEM images, and siRNA leads to more ordering of lamellar structures. Judging from these results, we conclude that the complex forms a core–shell sphere; the densely packed core mainly consists of SST-65 and siRNA, and PEG chains cover the particle surface (Fig. 3(c)).

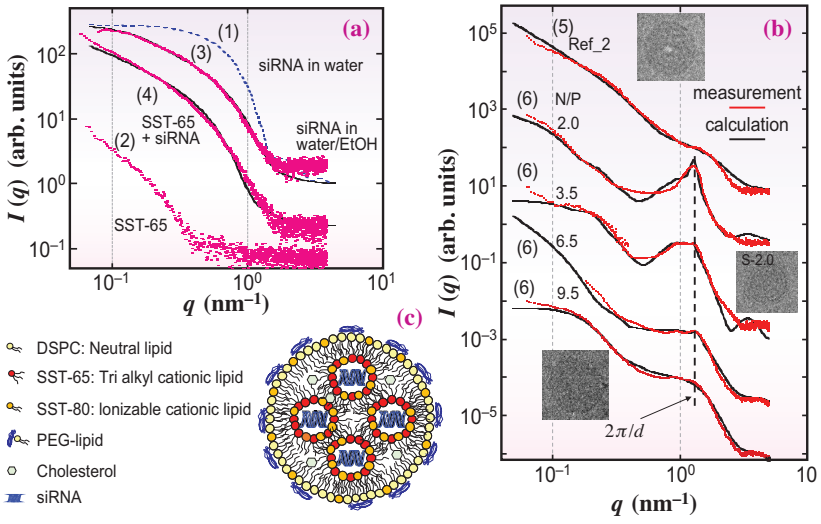

Fig.3. **(a)** SAXS profile changes on adding SST-65 to siRNA, **(b)** SAXS profiles at different N/P samples as well as TEM images. Red dots and black lines show experimental data and theoretical curves, respectively. The numbers in parentheses in (a) and (b) show the corresponding scattering profiles with the lipoplex components shown in Fig. 2. **(c)** Schematic illustration of the lipoplex. [5]

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