Small-Angle X-Ray Scattering from Drug Delivering Nanoparticles in Solutions: Pharmacological Efficiency and Particle-Inner Structures

Drug delivery using nanoparticles containing a pharmaceutical compound can dramatically improve therapeutic effects. This is because many biologically active materials, such as peptide and protein, antibody, vaccine and gene, in general may not be stable in bodies since they might be susceptible to enzymatic degradation or might be cleared by liver and other organs. Some of them cannot be absorbed into the systemic circulation efficiently owing to molecular size or electrostatic repulsion. For this reason, these drugs have to be delivered by exculpatiion with biologically stable nanoparticles. This technology is called a drug delivering system (DDS) and considered that nanotechnology and integrated science, including biology, chemistry, and polymer science, should play an important role. The current trend of drug delivery aims to develop a targeted delivery system, in which the drug is only active or delivered to the target and sustained-released in a controlled manner. We have been studying small-angle X-ray scattering (SAXS) from DNA/cationic lipid [1,2], DNA/polysaccharide [3], and hydrophobic-drug/polymer micelles [4] to correlate their pharmacological efficiency and particle-inner structures.

Complexes made from DNA and cationic lipids, become known as “lipoplexes,” have attracted enormous scientific and technical interest, because lipoplexes could be efficient vehicles for DNA or RNA delivery into a wide variety of eukaryotic cells. The mechanism of transfection is still not well understood, and further investigation is necessary to improve the transfection efficiency for the adaption of lipoplexes to clinical and practical use. SAXS studies of lipoplexes demonstrated that they take highly ordered structures, which are strongly related to the transfection efficiency. One of the proposed structures is a multilamellar phase where DNA is intercalated between lipid bilayers, as presented in Fig. 1(a) (perpendicular interaction). However, thermodynamic studies on the lipoplex formation have been interpreted by the so-called “lateral interaction of the alkyl tails and DNA”: cationic headgroups are localized at DNA phosphates, while hydrophobic tails would lay down on the DNA surface and coat DNA to make the complex hydrophobic (see the bottom of Fig. 1(b)). The resultant complex is presumably captured by the hydrophobic domain of the residual non-binding lipids, as presented in Fig. 1(b). Recent molecular dynamics studies suggest that the two models (lateral and perpendicular interactions) can exist depending on the alkyl chain length and/or the degree of hydrophobic interaction that results in the aggregate ion of alkyl chains.

We reported that aromatic amine and amidine derivatives can be used as transfection reagents with a higher efficiency and a lower toxicity than commercial products. Figure 2 shows the transfection efficiency of our benzyl amine (BA) as a function of composition. The efficiency strongly depends on the composition: the highest one is achieved at BA: DOPE: DLPC=1: 2: 1 (point B) and the lowest ones near the A point (BA: DOPE: DLPC = 1: 0: 1), where DOPE and DLPC are L-a-phosphatidyl ethanolamine dioleoyl and 1,2-dilauroyl-sn-glycero-3-phosphocholine, respectively. The coexisting DOPE and DLPC are considered to make BA to be compatible with water and to be less toxic for cells. However, there has been no information on how these two colipids affect the micellar structure. Figure 3(a) presents how the SAXS profile changes with the addition of DNA at the

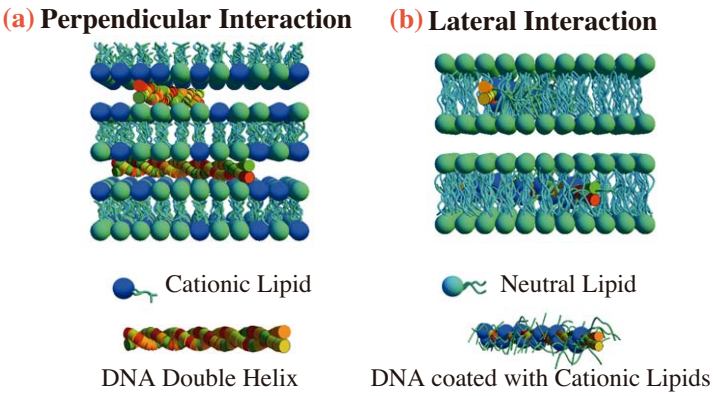

Fig. 1. Schematics of two models for DNA/cationic lipid complexes: **(a)** perpendicular and **(b)** lateral interactions. In the perpendicular interaction, the cationic lipids (blue) maintain the preformed hydrophobic alkyl domain and attach the DNA phosphate, while in the lateral interaction, after binding between the DNA phosphates and the lipid heads, the DNA surface is coated with the alkyl tails to make it hydrophobic and the bond DNA is transferred into the alkyl domains formed by the neutral co-lipids (green).

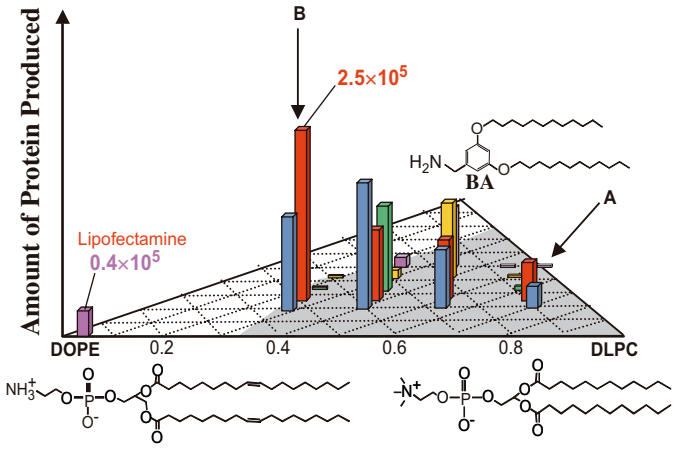

Fig. 2. Composition dependence of luciferase activity for BA/DOPE/DLPC system. N/P ratios were fixed at 3.3 for all samples, where N/P is defined by the molar ratio of the nitrogen of BA to the phosphate of DNA.

composition A (the lowest transfection efficiency). The SAXS profile before adding DNA could be characterized as a multilayered spherical micelle. When DNA was added to the micelle, diffraction peaks appeared and the peak positions satisfied the relation for hexagonally packed cylinders. Further addition of DNA led to the formation of a lamellar structure. Fitting analysis suggested the lateral interaction, as shown in Fig. 1(b). Figure 3(b) shows the changes of the SAXS profile at the composition B (the highest transfection efficiency). Before adding DNA, the profile exhibited peaks that correspond to a hexagonally packed cylinder. With increasing DNA, the first diffraction peak became sharper and more intense and other higher-order peaks became distinct. These features are obtained by the addition of DNA did not induce structural transitions, such as the composition A, but enhanced the hexagonal ordering as well as reduced the amount of the isolated scattering object. This may be interpreted by the intercalation of DNA between the preformed hexagonally packed micellar cylinders. Our results indicate that the location of DNA is related to the transfection efficiency.

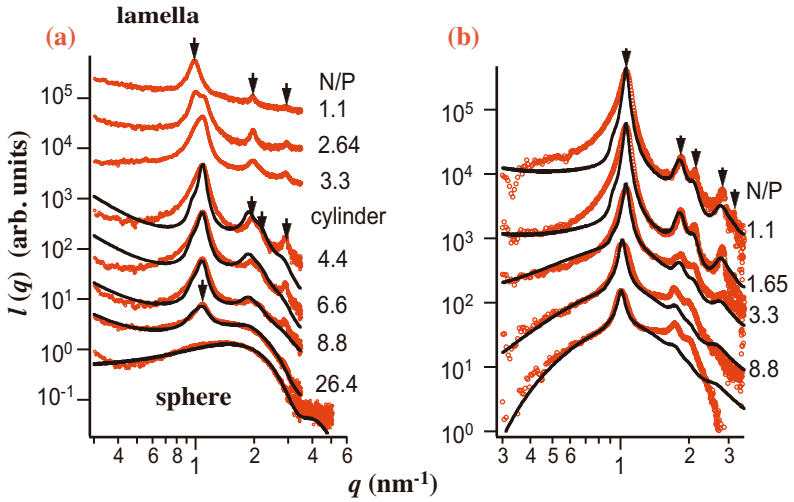

Fig. 3. N/P dependence of SAXS profile at compositions **(a)** and **(b)**.

Kazuo Sakurai

Dept. of Chemistry and Biochemistry,
University of Kitakyushu

E-mail: sakurai@env.kitakyu-u.ac.jp

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