Characterizing self-assembled nanoparticles employed in drug delivery

Many biologically active compounds such as peptides and antibiotic molecules are hydrophobic or incompatible with water. To use these compounds as pharmaceutical drugs, they must be dispensed in aqueous solutions. The resultant solutions must be stable for at least a few months and their biological activity should not change at all during that period. Additionally, some of them are not stable in the body since they can be susceptible to enzymatic degradation or removed from the blood by the liver and other organs. Drug delivery using nanoparticles that encapsulate hydrophobic pharmaceutical compounds can dramatically improve their therapeutic effects as their well as stability under biological conditions. This technology is called a drug delivering system (DDS), and it is considered that nanotechnology and the integration of scientific fields, including biology, chemistry, and polymer science, are important in designing and characterizing DDS nanoparticles. The current trend of drug delivery aims at the development of targeted delivery, in which the drug is delivered to the target (such as a specific protein or DNA) with sustained release in a controlled manner. When the target is present inside cells such as in the cytosol or nucleus, the delivering vehicle must accomplish multiple tasks including cellular uptake through receptor recognition, endosomal escape, drug release, and nucleic entrance, as illustrated in Fig. 1.

Using beamline **BL40B2**, we have been studying small-angle X-ray scattering (SAXS) from DDS nanoparticles and the relationship between their structure and biological performance for DNA/polysaccharide [1-3] and hydrophobic-drug/polymer micelles [4,5].

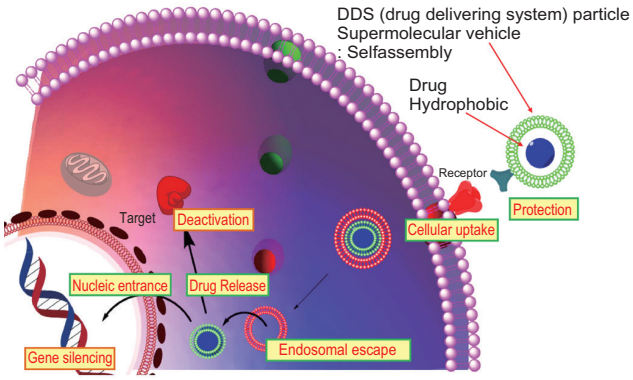

Fig. 1. Major barriers to overcome for cellular targeting delivery.

Polysaccharide/DNA Complexes

We have studied schizophyllan (SPG), a member of the β-1,3-glucans, as a delivery carrier of oligonucleotides, since SPG can complex with nucleotides such as poly(dA) (polyadenine) (Fig. 2(a)). The complex can be recognized by dectin-1 on antigen-presenting cells (APCs) and thus it is expected that β-glucans can specifically deliver the bound oligonucleotides to APCs. In fact, we have found that the complex can induce efficient gene silencing in animal models of fulminant hepatitis and bowel disease. It should be noted that we could observe therapeutic effects even when we applied the complexed antisense oligodeoxynucleotides (AS-ODN) at doses two orders of magnitude less than the reported dose because of the specific targeting. In order to use SPG as a DDS material, it is important to characterize its complex with therapeutic DNA in-site, i.e., in solution [1-3].

Figure 2(b) presents a typical SAXS profile from dA60/SPG (a complex of 60-base polyadenine with SPG), after combining data obtained with two different camera lengths (4.3 and 0.7 m) and extrapolating them to the zero concentration. The data shows the relation of $I(q) \sim q^{-1}$ in the middle range (0.08 nm^{-1} < q < 0.8 nm^{-1}), which is expected for rigid thin rods, and the intensity deviates upward in the low-q region and downward in the high-q region. The former deviation is ascribed to chain flexibility and the latter downward deviation is caused by the finite size of the cross section of the chain. From these deviations, the persistence length (pe) and the diameter of the worm-like cylinder (d) can be determined. By fitting the data with the Norisuye-Nakamura theory, pe and d were determined to be 45 ± 5 nm and 2.6 ± 0.2 nm, respectively.

Amphipathic block-copolymer micelles

Amphipathic block copolymers in aqueous solution undergo microphase separation into hydrophobic and hydrophilic domains [4,5]. When the hydrophilic block is long enough, stable spherical micelles consisting of a hydrophobic core and a hydrophilic shell are obtained. Polymeric micelles have great potential as DDS, because the core can encapsulate hydrophobic drugs and the shell can provide biocompatibility. Knowing how the drugs are distributed inside the core will help us understand the drug-releasing mechanism and increase the drug loading ratio. However, such information is hard to obtain since the core size is normally less than 100 nm and the drug concentration

is normally less than about 10 wt%, which makes it difficult to observe by electron microscopic techniques.

Recently, we have chosen the hydrophobic compound tetrabromocathecol (TBC) as a drug-equivalent model molecule. The bromine atoms in TBC act as probes in anomalous small-angle X-ray scattering (ASAXS), allowing us to find its localization in the polymeric micelles, the shape and size of which were determined by normal SAXS, as illustrated in Fig. 3. Light scattering measurements coupled with field flow fractionation were also carried out to determine the aggregation number (the number of polymer molecules in each micelle). A core-corona spherical model was used to explain the shape of the micelles, and the distribution of bromine atoms was explained with a rigid sphere model. Interestingly, the radius of the spherical region populated with bromine atoms was larger than that of the sphere corresponding to the hydrophobic core of the micelle. This result suggests that the TBC molecules infiltrate the polyethylene glycol (PEG) hydrophilic domain in the vicinity of the core/shell interface. The results of light scattering and SAXS indicate that the PEG chains in the shell region are densely packed and thus the PEG domain close to the interface has enough hydrophobicity to tolerate the presence of hydrophobic compounds.

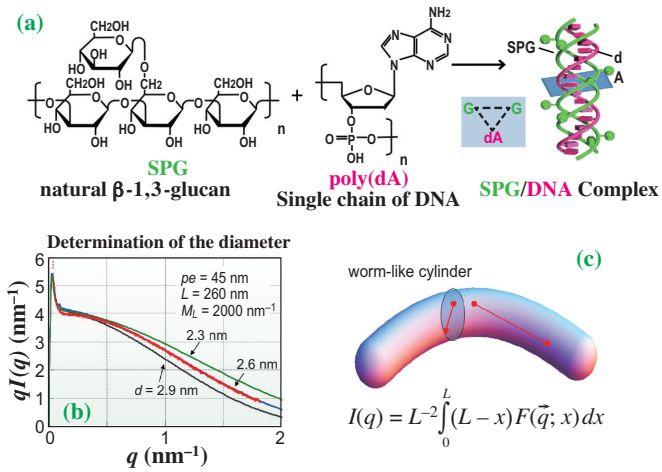

Fig. 2. **(a)** Repeating units of schizophyllan (SPG) and poly(deoxyadenylic acid) (dA_X) and an illustration of their complexation and stoichiometric structures of the triple helix of SPG and the complex, denoted SPG and dA_X/SPG. **(b)** Holtzer plots for the SAXS data of dA60/SPG, compared with results obtained adopting the Norisuye-Nakamura theory with different parameters. **(c)** Scattering function (so-called form factor) of a worm-like cylinder where $F(\vec{q}, x)$ is the Fourier transform of the probability density function that represents the probability that the contour point x is found at a specified point (which can be related to $\vec{q}$), as theoretically proposed by Nakamura and Norisuye.

Our group also carried out an SAXS study of a series of polymeric micelle samples and found that the major factor determining the aggregation number was the hydrophobicity of the chain, and that a high aggregation number induces overcrowding of the tethered PEG chains at the core/shell interface by 2-3 times. Thus, the control of the hydrophobicity may be important for designing more efficient DDS micelles.

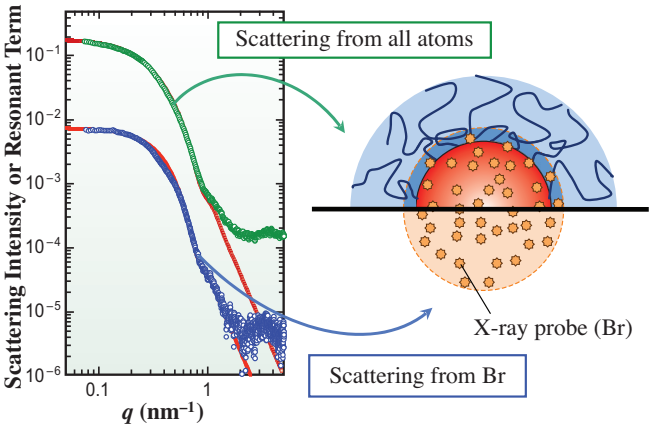

Fig. 3. SAXS and ASAXS profiles from TBC-incorporated polymeric micelles and their interpretation, showing the TBC distribution in the micelle. The red region represents the hydrophobic core, the blue lines represent PEG chains, and the orange polygons represent TBC.

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