Contrast variation study of protein/polyelectrolytes complexes with encapsulated drug

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Contrast-matching small-angle neutron scattering (SANS) over an extended Q range to reveal the morphology of complexes formed by proteins and polyelectrolytes (PE) and the effects of encapsulation of the drug in the complexes was carried out.

キーワード Keywords：extended Q-range SANS; polyelectrolytes-protein complexes

１．目的 Objectives

The main objective of the experiment was to use the technique of contrast-matching small-angle neutron scattering (SANS) over an extended Q range to reveal the morphology of complexes formed by proteins and polyelectrolytes (PE) and the effects of encapsulation of the ionic drug in the complexes on their internal structure by matching out the scattering from a polymer, protein, or drug. The morphological transition of complexes formed by a protein (insulin) and block copolymers consisting of a neutral hydrophilic PEO block and a strong or weak PE block (QPDMAEMA, PDMAEMA) was investigated as a function of pH or concentration of the different components.

２．方法 Methods

SANS measurements were performed on the SANS-J-II instrument of JRR-3 on PEO-PDMAEMA/insulin or PEO-QPDMAEMA/insulin complexes with and without the addition of protoporphyrin-IX. Thanks to the combination of pinhole and focusing methods using MgF_3 lenses and a high-resolution position-sensitive detector, the SANS-J-II beamline provided ideal conditions for covering a wide Q range between $2x10^{-4}$ and 1.0 $Å^{-1}$. The solution samples were measured in Banjo-type quartz cuvettes. The raw data were corrected for the contributions of the empty cuvette and dark current and for the sensitivity of the detectors. The corrected data were radially averaged and then calibrated in absolute units and corrected for solvent scattering to be transformed into the scattering cross section of the system under study. Contrast conditions were used that allowed either full contrast (in D_2O), PE block matching (70% D_2O - 30% H_2O for the deuterated QPDMAEMA block), or protein matching (35% D_2O - 65% H_2O). Samples were analyzed under different pH and concentration conditions.

３．結果及び考察 Results and Discussions

Figure 1 shows the scattering patterns of two samples containing the diblock copolymer with a weak (left) or strong (right) PE block at 5 mg/ml and insulin at 20 mg/ml in D_2O for two pH conditions.
Scattering of polyelectrolyte-protein complexes is observed at pH = 7.4, while at pH = 11, superposition of scattering of the two components is observed individually, as no complex is formed at this pH. Data collected on the SANS-J-II in the medium and low Q range are shown in blue or green color, while data measured on the same samples in the medium and high Q range using the TOF-SANS diffractometer TAIKAN of J-PARC are shown in parallel in red or black color. For all experimental conditions investigated, there is very good agreement between the two complementary data sets. The common model analysis of the two scattering data sets collected at SANS-J-II and TAIKAN is in progress. Here we can only briefly report that good agreement is observed between the mid- and low-Q scattering data and the cryo-TEM images with respect to the mid- and large-scale morphology of the polyelectrolyte-protein complexes: one-dimensional flexible morphologies are formed (Q^{-1} power law), which behave on

a larger length scale as a branched morphology resembling that of a solvent-swollen self-avoiding polymer coil ($Q^{-5/3}$ power law). The thickness and segment length can be estimated from the analysis of the cryo-TEM image, while the power law behavior of the scattering patterns at intermediate Q at pH = 7.4 agrees well with this conclusion. At low Q, an upturn in the scattering pattern is observed in both pH conditions, presumably due to the association of the one-dimensional flexible morphologies into a larger network such as a branched morphology or to the morphology of longer PEO blocks that can join together, as previously observed in the case of the morphology formed by the diblock copolymer alone in solution. At high Q, the scattering details observed for the condition pH = 7.4 reveal the structure of the interacting charged components, namely the charged polymer block and the protein, while for the condition pH = 11, the scattering is revealed by the independent components that do not interact.

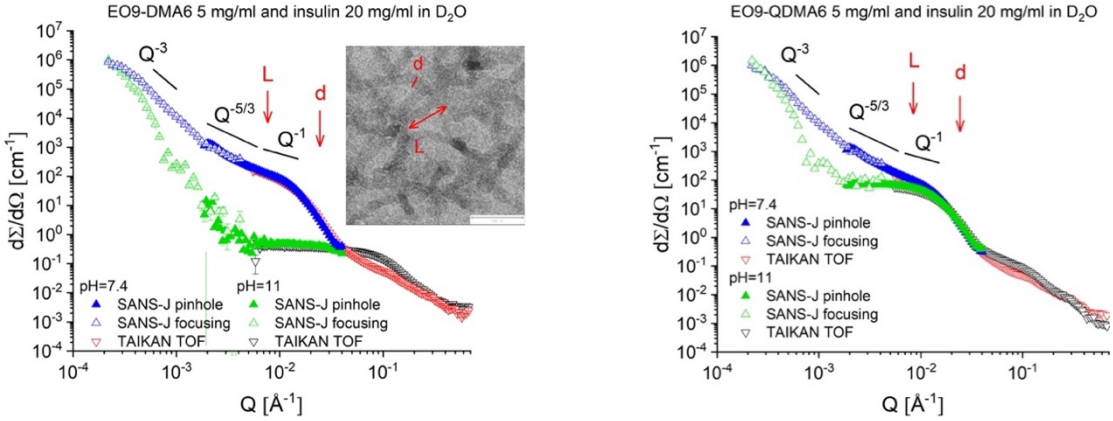

Figure 1 - Scattering patterns from the combination of weakly charged diblock copolymer and protein (left) and strongly charged diblock copolymer and protein (right) at different pH conditions; the cryo-TEM image is shown as an inset in the left graph; the power law behavior of the scattering patterns in different Q ranges and the estimated thickness and length of the complex one-dimensional morphology are indicated.

The contrast matching results (Figure 2) for the QPDMAEMA block (full symbols) or insulin (empty symbols) confirm the morphology formation scenario described above: a $Q^{-5/3}$ power law behavior is observed over an extended Q range when the deuterated charged block is used in the diblock copolymer, while in the high Q range a broad peak-like scattering feature occurs in both contrast conditions, though with different intensity. The power law behavior suggests a self-avoiding walk or one-dimensional branching behavior of the polymer-protein complexes, while the broad peak could indicate a correlation effect between the interacting charged components in the complex system, namely the QPDMAEMA block and insulin, which are made visible or invisible in the scattering experiment depending on the contrast condition. A detailed analysis of the scattering data in terms of structural models is in progress.

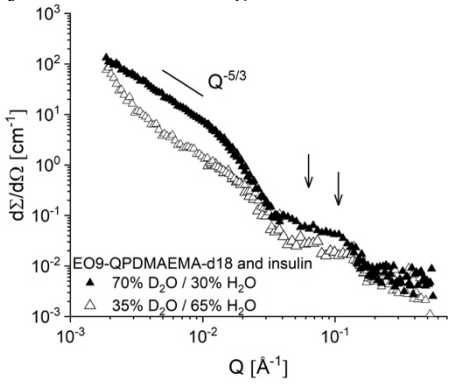

Figure 2 - Scattering patterns of the complex formed from the diblock copolymer with deuterated strongly charged block (5 mg/ml) and protein (20 mg/ml) at pH = 7.4 under different contrast conditions. Arrows indicate the scattering features representing correlation effects between the charged components of the complex.