Trapping a Whole Protein in a Well-Defined Molecular Capsule

Encapsulation of a molecule in a capsular structure is a widely-used phenomenon in natural and artificial systems to achieve unique functions such as storage, emission, and control of the accommodated molecules. Proteins are attractive target molecules but the encapsulation of a protein in a well-defined molecular capsule has not been realized due to the limited size of artificial capsules. In the present study, Professor Makoto Fujita's group in the Department of Applied Chemistry and Professor Koichi Kato at the Okazaki Institute for Integrative Bioscience confined a single ubiquitin molecule within a 7-nm synthetic capsule, whose structure was clearly revealed by NMR, mass spectrometry, and finally synchrotron X-ray crystallography.

In nature, encapsulation of biomolecules, such as proteins or DNA, in huge capsular materials like virus capsids serves to control their structures and bioactivities and to store them. In artificial chemical systems, the encapsulation of small organic molecules within hollow larger capsular molecules, called "host molecules", also functions to control the structures or activities of the encapsulated guest molecules. The encapsulation of biomolecules in artificial host molecules is expected to pave the way for controlling their structures and functions at will, however, huge biomolecules like proteins have never been encapsulated because the sizes of artificial capsular molecules with precise structures are usually limited to up to 2 nm in diameter.

In 2006, we reported that a huge capsular molecule with a diameter of 4.6 nm accommodated several small organic molecules inside the host [1]. The internal chemical environment was modified to be fluorophilic by decorating the internal wall of the host with fluoroalkyl chains, and fluorous guests were extracted from the external solvent into the host. The structure of the host was precisely designed and well defined through a self-assembly process from organic molecules and transition metal ions.

In 2012, the self-assembled huge host was further extended to a diameter of 7.3 nm, and the encapsulation of a whole protein was first achieved within the synthesized capsular molecule (Fig. 1) [2]. An organic molecule tethering a protein, ubiquitin with a molecular weight of 8,600 Da, was synthesized under moderate reaction conditions to maintain the folded protein structure [3]. Then the molecule bearing a protein was mixed in a flask with another organic molecule bearing a sugar chain and palladium (II) ions in the water/acetonitrile solvent. The coordination bonds between the starting materials automatically formed to construct the product including one organic molecule bearing a protein, 23 organic molecules bearing a sugar moiety, and 12 palladium (II) ions with 100% yield. The protein was wrapped in the clustered sugar moieties, and the product showed high stability at room temperature in air.

Solution nuclear magnetic resonance (NMR) spectra showed broad signals, which indicate the formation of a huge structure, and diffusion analysis by 2D DOSY spectra revealed the diffusion coefficient, which showed that the host framework and encapsulated ubiquitin behave as a single molecule through the "host-guest" interaction.

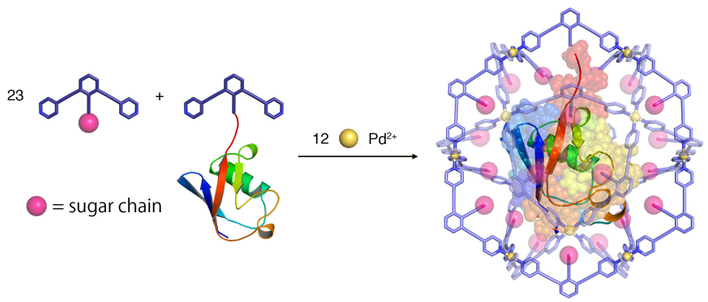

Figure 1: The self-assembly of a huge capsular molecule encapsulating a whole protein is schematically illustrated. The protein was surrounded by clustered hydrophilic sugar moieties.

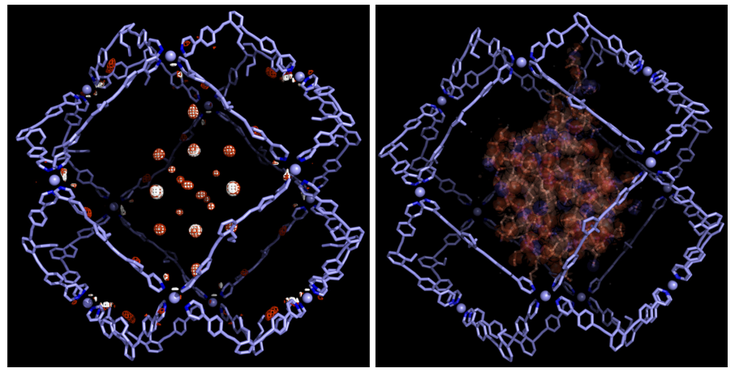

Figure 2: The protein encapsulation was confirmed by crystallographic studies in a crystalline state. (left) The mapping of electron density (red) derived from the trapped ubiquitin in the artificial host (blue). (right) The computationally simulated model structure of the product, where ubiquitin is shown in red and the host in blue.

Determination of the molecular weight was first attempted using cold-spray ionization time-of-flight mass spectrometry (CSI-TOF-MS), but the results were not satisfactory. Next, analytical ultracentrifugation (AUC) in sedimentation velocity measurement mode clearly showed the high monodispersity of the product in solution, and AUC in sedimentation equilibrium measurement mode was used to estimate the molecular weight, which was consistent with the sum of the host framework and one ubiquitin.

The three-dimensional structure of the product could not be accessed by the above-mentioned NMR and ultracentrifugation analyses, and the final structural elucidation relied on single crystal diffraction studies as a solid, crystalline state. Careful selection of the crystallization conditions led to the formation of beautiful single crystals, but a structural analysis of these single crystals could not be carried out successfully by using laboratory diffractometers. This failure could be attributed to the large unit cell of around 60 Å and volume of around 195,900 Å^3 and the severely disordered solvents inside and outside the host. Only strong, sharp synchrotron X-rays generated by the advanced equipment at KEK PF AR-NE3A and BL-17A and SPring-8 BL38B1, BL41XU, BL26B1, and BL26B2 finally elucidated the crystal structure of the host framework with up to 1.8Å resolution. Further analysis by the maximum entropy method (MEM) enabled visualization of the electron density for the encapsulated protein at the center of the host (Fig. 2).

REFERENCES

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BEAMLINES

AR-NE3A and BL-17A

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