A Supramolecular Chemical Approach to the Construction of Artificial Cells

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Exploring the boundary between living and non-living matter is one of the most challenging problems for contemporary scientists. To understand the cell, which is considered the smallest unit of life, a plausible strategy is to synthesize an artificial cell by using a supramolecular chemical approach, because simple molecular assemblies at one time evolved to create the simple cell on prebiotic earth. As shown in Figure 1, the key elements of a cell are the compartment, information, and a catalyst (*i.e.*, metabolism). We have attempted to construct a chemically based artificial cell endowed with these three elements.

In our laboratory, we attempted to construct two artificial cells by using giant vesicles (GVs) as the compartment. One, developed in collaboration with the Sugawara group (Kanagawa Univ.), is an artificial cell that can proliferate from generation to generation. Now, we have constructed a recursive vesicular artificial cell system with proliferation cycles. By using the vesicular transport system, the second generation GVs, which contain no PCR reagents after self-reproduction, can be replenished by fusing them with conveyer GVs bearing the PCR reagents by changing the pH of the dispersion. After the PCR reagents are replenished, the GV can self-reproduce again. This system could lead to an evolvable artificial cellular system. The other artificial cell is an artificial cell that contains a catalyst-producing system. The GV system can generate catalysts and membrane molecules by transforming their respective precursors, thereby facilitating the proliferation of the GVs with the produced catalyst.

We are now tackling the creation of artificial cells that mimic cellular dynamics, such as cytoskeleton formation in the cell.

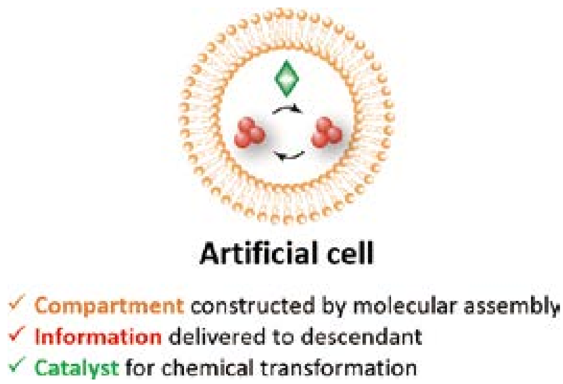

Figure 1. Artificial cell model. The replicating systems of the compartment and the information materials are combined. The reactions in the two replicating systems are accelerated by appropriate catalysts.

Selected Publications

- K. Kurihara, M. Tamura, K-I. Shohda, T. Toyota, K. Suzuki and T. Sugawara, "Self-Reproduction of Supramolecular Giant Vesicles Combined with the Amplification of Encapsulated DNA," *Nat. Chem.* **3**, 775–781 (2011).
- K. Kurihara, Y. Okura, M. Matsuo, T. Toyota, K. Suzuki and T. Sugawara, "A Recursive Vesicle-Based Model Protocell with a Primitive Cell Cycle," *Nat. Commun.* **6**, 8352 (2015).

1. An Artificial Cell Using a Self-Reproducing Oil Droplet as a Scaffold

A cell is a self-organized system that can maintain its state via metabolism. Our previously developed artificial cellular system is robust, but it can self-reproduce only a specific state in the any environments.[1–3] Research on transforming oil droplets into vesicles by use of chemical reactions and self-assembly processes is expected to facilitate our understanding of the origin and definition of life from a chemistry perspective.

The mixing of an aqueous solution of an aldehyde containing an imidazole hydrochloride group with octylaniline led to the spontaneous formation of autocatalytic oil droplets[5] (Figure 2). An aldehyde-bearing quaternary ammonium salt that does not react well with octylaniline was added to this autocatalytic droplet system. As a result, the catalytic molecules that formed within the oil droplets promoted the condensation reaction between the octylaniline and the non-catalytic aldehyde, which ultimately led to the synthesis of vesicular membrane molecules with imine functionality within the molecular aggregates; thus self-reproducible oil droplets were successfully transformed into vesicles upon the addition of the membrane precursor.

We will construct an oil droplet system that synthesizes peptides (simple proteins) and an oil droplet system that forms vesicles. The former is an oil droplet that incorporates amino acids and synthesizes peptides internally. In addition, peptide synthesis inside the vesicles is performed using a water-soluble condensing agent through our developed water-in-oil emulsion centrifugation method.[5)]

2. Vesicular System Containing Peptide Synthesis

In the prebiological era, cooperative interaction of spontaneously polymerizing polymer and self-producing molecular aggregates led to the emergence of primitive cells. Although the membrane potentially provides a reactive field of catalytic reaction, it remains the mystery the cooperation between polymer and molecular aggregates occur without advanced catalyst.

Therefore, we designed and synthesized monomer precursors that spontaneously peptide-polymerized in a reductive water and generated molecular aggregates as the polymerization reaction proceeds. This monomer precursor has disulfide and thioester sites. In the first step of the reaction, the disulfide of the monomer precursor is reduced and a thiol-bearing monomer is produced. Next, benzylmercaptan, which generates the oil droplet, is eliminated by replacing the thiol of the monomer. Finally, the occurrence of S-N acyl transfer forms amide bonds (native chemical ligation).

From the reaction trace by NMR spectrum, the release of benzylmercaptan and subsequent formation of amide bond were detected. Furthermore, as turbidity increased with progress of reaction, the reaction solution formed cell-size oil droplets under microscope observation. These results mean that benzylmercaptan forms oil droplets without layer separation because the generated peptides stabilize the interface. In this system, since spontaneous polymerization of polymer and oil droplet generation are coupled, application of this system to artificial cells is expected.

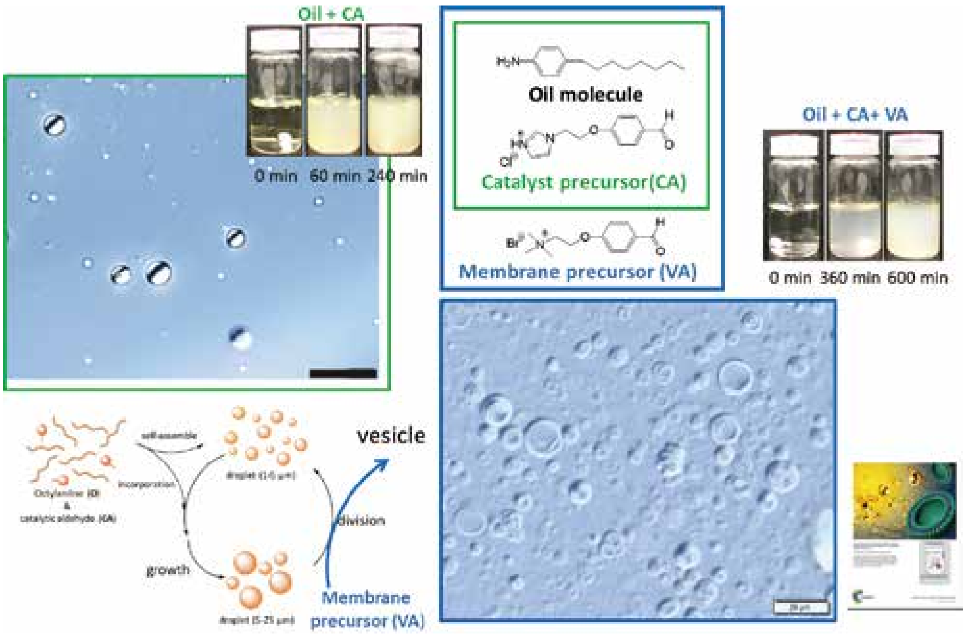

Figure 2. Scheme of the self-reproducing oil droplet (oil-in-water emulsion) and vesicular transformation system.

References

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2) K. Kurihara, Y. Okura, M. Matsuo, T. Toyota, K. Suzuki and T. Sugawara, *Nat. Commun.* **6**, 8352 (2015).
3) L. Sheng and K. Kurihara, *Chem. Lett.* **45**, 598–600 (2016).
4) L. Sheng and K. Kurihara, *Chem. Commun.* **52**, 7786–7789 (2016).
5) Y. Natsume, H. Wen, T. Zhu, K. Itoh, L. Sheng and K. Kurihara, *J. Vis. Exp.* **119**, e55282 (2017).