Microcapsule Formation by Thermal Heterocomplex Molecules from Amino Acids in a Thermal Gradient Microcapillary

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***Abstract*—We report a result of a preliminary experiment on material aggregation in a thermal gradient capillary. We found that packed microspheres consisting of thermal heterocomplex of molecules from amino acids were transformed into microcapsules in a thermal gradient capillary. A hypothesis on the process of microcapsule formation is presented.**

***Keywords*—amino acids, self-assembly, origins of life, thermal gradient, microcapsule**

I. Introduction

Non-equilibrium dissipative structures are intrinsic to working of living systems. Before the emergence of life, pre-existing abiotic dissipative systems would play a critical role on chemical evolution of prebiotic molecules. Among other energy sources available on the primitive earth, thermal gradients are the most abundant one [1].

Submarine hydrothermal environments are one form of geological realizations of thermal gradients, which have been considered as potential sites for chemical evolution towards the emergence of life [2, 3, 4]. Imai et al. [5] showed synthesis of oligoglycine from glycine monomers in a simulated hydrothermal system in which glycine solutions circulated between a high-pressure and high-temperature chamber and a high-pressure and low-temperature chamber. The remarkable point in their experiment is that they had no control on pH and added no salts. In this paper, we will report a result of a preliminary experiment on thermal heterocomplex of molecules from amino acids in a much milder condition with the same minimal human-control spirit.

Recently, micropores within mineral precipitates surrounding submarine hydrothermal vents are of concern [6]. Coupling of convective flow and thermal diffusion induced by a lateral thermal gradient across a vertical micropore can concentrate molecules in bottom region of the pore [1]. Budin et al. [7] showed that accumulated oleate in a microcapillary subject to a lateral thermal gradient formed vesicles.

Complex molecules yielded by thermal dehydration of amino acids have been envisaged as a model of prebiotic material aggregates [8, 9, 10]. Thermal heterocomplex of molecules from amino acids forms phase-separated packed microspheres in aqueous environment. They exhibit versatile behaviors in responses to changes in their environment [11]. For example, the size of microspheres shows thermal hysteresis during cycle between cooling and warming [12]. They can adsorb basic amino acids on their surfaces [13]. They are transformed into microcapsules as pH increases [14, 15]. Here, we will show that thermal heterocomplex of molecules from amino acids forms microcapsules in a thermal gradient microcapillary without pH control and added salts.

II. Materials and Methods

L-aspartic acid and L-proline of equal molar weight were heated at $200^{\circ}\mathrm{C}$ for 3 h under normal atmospheric condition to form thermal heterocomplex molecules. The resultant products were solubilized in distilled water at boiling temperature for 20 min. The solution was immediately cooled down in an ice-bath. The suspension of thermal heterocomplex molecules, which will be hereafter referred as DP-suspension, was preserved at $4^{\circ}\mathrm{C}$ after decantation. Thermal heterocomplex molecules in the DP-suspension will be called DP-molecules. DP-molecules self-aggregated into packed microspheres whose diameters were several μm in the DP-suspension.

The DP-suspension thus obtained was used to study microcapsule formation in a thermal gradient microcapillary.

III. Results and Discussion

A DP-suspension of 15 mg/ml was loaded into a borosilicate microcapillary with a circle section (inner diameter 630 μm, outer diameter 1300 μm, length 6.25 cm). The top and bottom of the capillary were sealed with epoxy. Then it was put

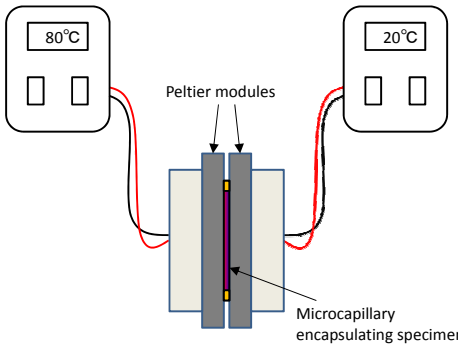

Fig. 1. A schematic diagram of the construction of a thermal gradient microcapillary.

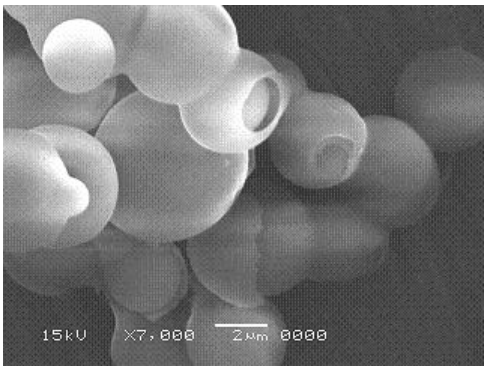

Fig. 2. A scanning electron micrograph of microcapsules formed in a thermal gradient microcapillary. Bar: 2 μm.

vertically between a heat source and sink. The heat source and sink were constructed by two Peltier devices (SPE-UC-100, Sakaguchi). The hot side Peltier module was kept at 80°C, while the cold side was kept at 20°C. The temperature gradient across the inner diameter vertical to the heat source and sink can be calculated by assuming steady state heat conduction across a composite slab. It is given by [7]

$$\Delta T = \frac{ID(T_H - T_C)}{\frac{k_w}{k_g}(OD - ID) + ID}, \quad (1)$$

where T_H and T_C are temperatures of the heat source and sink, OD and ID are the outer and inner diameters of the microcapillary, and k_w and k_g are the thermal conductivities for water and borosilicate glass. By substituting $T_H = 80$°C, $T_C = 20$°C, $OD = 1300$ μm, $ID = 630$ μm, $k_w = 0.6$ W/mK and $k_g = 1.13$ W/mK into (1), we obtain $\Delta T = 38.3$ K. From this, we expect that the temperatures of the end points of the inner diameter vertical to the heat source and sink are 69.2°C and 30.8°C. Fig. 1 shows a schematic diagram of the experimental apparatus.

The specimen was retained in the apparatus for 120 h. We found that microcapsules were formed from packed microspheres consisting of DP-molecules in the thermal gradient microcapillary. A scanning electron micrograph of microcapsules in a dried condition is presented in Fig. 2. We can see that smaller microspherical kernels are contained in the inside of microcapsules.

Microcapsules formed were also identifiable by optical microscopic observations (data not shown). Before introduction to the thermal gradient, we could not find any microcapsule by optical microscopic observations. This suggests that the thermal gradient contributes to microcapsule formation. However, at present experimental setting, microcapsule yield is not so high. Typically, we found only dozens of microcapsules among thousands of packed microspheres by optical microscopic observations. Quantitative analysis of the experimental result is left as future work.

At present, the process of microcapsule formation in the thermal gradient capillary, in particular, the impact of coupling between thermal convection and diffusion on it is unclear. However, to present a hypothesis on the process of microcapsule formation will be valuable for further experimental design. Microspheres located near the side of the microcapillary contact to the heat source would dissolve rapidly. On the other hand, those located around the center of the microcapillary would dissolve rather slowly. Dissolved DP-molecules could diffuse from the hot side to the cold side of the microcapillary by thermal diffusion. In the case of microcapsule formation by pH control, it was suggested that re-aggregated DP-molecules that form microcapsules on the surface of microspheres have different kinetic property of dissolution [15]. If similar change on kinetic property of DP-molecules occur in the thermal gradient microcapillary, then dissolved DP-molecules could re-aggregate on the surface of slowly dissolving microsheres located around the center of the microcapillary due to hypothetical conformational changes in individual molecules in response to the thermal gradient condition. It follows that microcapsules could be formed around the microspheres, while the microspheres still keep dissolving slowly. This hypothesized process of microcapsule formation is in contrast to a “passive” scenario in previous work [7] in which thermally stable molecules (oleate) collectively form metastable structures (vesicles).

Hanczyc et al. [16, 17] reported that fatty acids can self-assemble around mineral particles and form vesicles. It was hypothesized that a layer of positively charged cations around the mineral surface is a major driving force for self-assembly of negatively charged fatty acids. Since DP-molecules are also negatively charged, there might be a shared mechanism between microcapsule formation by DP-molecules in the thermal gradient capillary and fatty acids self-assembly around mineral particles. However, one major difference is that molecules consisting of microcapsules and molecules consisting of the surface of microspheres are the same molecular species in our case.

Microcapsules formed in a thermal gradient capillary may have encapsulated other prebiotic molecules on the primitive earth. They could also have been “physical catalysts”, namely, occurrence of chemical reactions involving prebiotic molecules might have been facilitated on their both inside

and outside surfaces. For example, nucleotide molecules could be synthesized within microcapsules from encapsulated nucleoside and phosphate [18]. The idea that microcapsules consisting of DP-molecules formed in a thermal gradient capillary could be both primitive membrane and catalyst sounds attracting, however, of course, further experimental investigations are expected.

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