How Does Thermal Gradient Contribute to Microcapsule Formation by Proteinoids?

Taichi Haruna*, Junya Shiozaki*, Sayaka Tanaka*,
* Department of Earth and Planetary Sciences, Graduate School of Science, Kobe University
1-1, Rokkodaicho, Nada, Kobe 657–8501, Japan
Email: tharuna@penguin.kobe-u.ac.jp (T. Haruna)

***Abstract*—Molecular self-assembly is fundamental to biological systems. In this paper, we report an experiment on microcapsule formation by proteinoids in a thermal gradient capillary simulating thermal gradient condition in micropores found around submarine hydrothermal vents. We found that proteinoid microcapsules were formed in a narrow band region between heat source and sink. We present a hypothesis on how thermal gradient contributes to the microcapsule formation and discuss potential implication for chemical evolution toward the emergence of living systems.**

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

I. Introduction

Understanding self-assembling processes is an important issue in both natural and engineering sciences [1]. One of the most fundamental self-assemblies relevant to biological systems is that by molecules. In this paper, we report an experiment on molecular self-assembly by thermal heterocomplex molecules from amino acids (in short, proteinoids) under submillimeter-scale thermal gradient condition.

Proteinoids have been considered as one of prebiotic molecules that play a potential role in chemical evolution toward the emergence of life [2]. They are easily yielded by heating several kinds of amino acids under normal atmospheric condition. They spontaneously form phase-separated microspheres in aqueous environment, whose inside is densely packed by proteinoids [3]. Sakurazawa et al. [4, 5] showed that if a suspension of proteinoids is altered by weakly basic buffer from the original weakly acidic suspension, then microspheres are transformed to microcapsules.

Recently, one of the authors reported that microcapsules are formed in a thermal gradient capillary without pH control [6]. The thermal gradient capillary was constructed to simulate the submillimeter-scale thermal gradient environment found in micropores within mineral precipitates surrounding submarine hydrothermal vents that had been considered as potential geological sites for chemical evolution [7]. A lateral thermal gradient across a vertical micropore concentrates molecules in the pore due to coupling between convective flow and thermal diffusion. Budin et al. [8] showed that this accumulation mechanism can concentrate oleate in a microcapillary subject to a lateral thermal gradient and lead to formation of vesicles.

In contrast to the vesicle formation by accumulated oleate, the mechanism of microcapsule formation by proteinoids in the thermal gradient capillary is still to be uncovered. As a first step to resolve this problem, we performed an experiment to determine the position in the thermal gradient capillary on which microcapsules are formed. In this paper, we report the result of the experiment and present a hypothesis on the mechanism of microcapsule formation based on the experiment.

II. Materials and Methods

L-aspartic acid and L-proline of equal molar weight were heated at $200°\mathrm{C}$ for 3 hours under normal atmospheric condition to form proteinoids. The reaction products were solubilized in distilled water at boiling temperature for 20 min. The solution was immediately cooled down in an ice-bath. DP-molecules self-assembled into microspheres whose diameters were several μm in the suspension. To separate the molecular species called DP1 of molecular weight about 4000 that constitutes microspheres, the suspension was then filtered by polystyrene membrane filter of pore size 0.45μm. The microspherical sediments remaining on the filter were washed several times by distilled water. After reduced-pressure drying for one night, the weight of the dried microspherical sediments was measured to obtain a suspension of proteinoids of 15 mg/ml. The suspension of proteinoids was preserved at $4°\mathrm{C}$.

The resultant suspension was loaded into a borosilicate microcapillary with a square section (inner diameter 500μm, outer diameter 700μm, length 5.0cm). The top and bottom of the capillary were sealed with epoxy. Then it was put on a Peltier device (SPE-UC-100, Sakaguchi) horizontally. Another Peltier device was put upside-down on the microcapillary. The experiment consists of four steps (Fig. 1): (i) the temperature of both upside and downside Peltier devices was kept at $80°\mathrm{C}$ for 3 hours to dissolve the microspheres in the loaded suspension. (ii) The temperature of the upside Peltier device was still kept at $80°\mathrm{C}$ while that of the downside one was set to $20°\mathrm{C}$ and kept for 72 hours. As a result of this step, proteinoids re-aggregated and consequently formed microspheres on the bottom-side inner wall of the microcapillary. (iii) The microcapillary was rotated 90 degrees so that the re-aggregated microspheres were arranged along the thermal gradient. The temperature settings of the Peltier devices are the same as those in (ii). After keeping this setting for 96 hours, (iv) the microcapillary was directly observed by optical microscopy.

The temperature gradient across the inner diameter in steps (ii) and (iii) can be calculated by assuming steady state heat

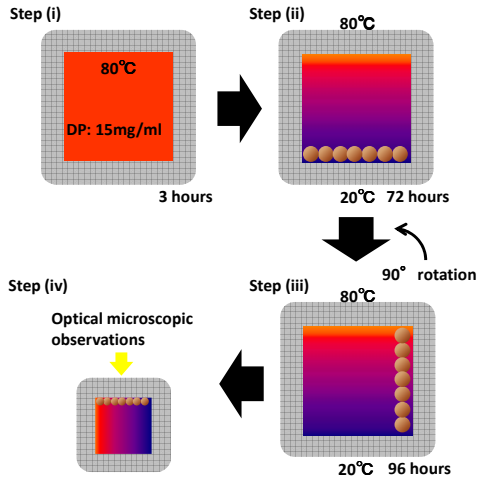

Fig. 1. Schematic diagram of the experimental procedure. In each step, the square section of the microcapillary is shown.

conduction across a composite slab [9]. It is given by [8]

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

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, $OD = 700\mu$m, $ID = 500\mu$m, $k_w = 0.6$W/mK and $k_g = 1.13$W/mK into (1), we obtain $\Delta T = 49.5$ K. From this, we expect that the temperature $T(x)°$C at the position $x\mu$m ($0 \leq x \leq 500$) from the low temperature inner wall is given by

$$T(x) = 0.099x + 25.25. \tag{2}$$

III. Results and Discussion

An optical microscopic image of the microcapillary taken in step (iv) of the experimental procedure is shown in Fig. 2. We found that microcapsules were formed in the region between 185μm and 225μm vertically apart from the cooled-side inner wall of the microcapillary. Microcapsules typically contained inner microspherical kernels. This feature was found and confirmed by scanning electron micrographs in our previous work [6].

In Fig. 3 (a) and (b), we plotted outer and inner diameters (namely, outer diameters of microspherical inner kernels) of 63 microcapsules identified in optical microscopic images against the distance from the cooled-side inner wall of the microcapillary. The averages of the distances, outer diameters and inner diameters are 202.5μm, 6.1μm and 3.1μm, respectively. One can see that the region in which microcapsules were formed is between 185μm and 225μm vertically apart from the cooled-side inner wall of the microcapillary. This is 8 percentages of the whole surface area of the inner wall on which microspheres were formed in step (ii), which explains the small microcapsule yield found in our previous work

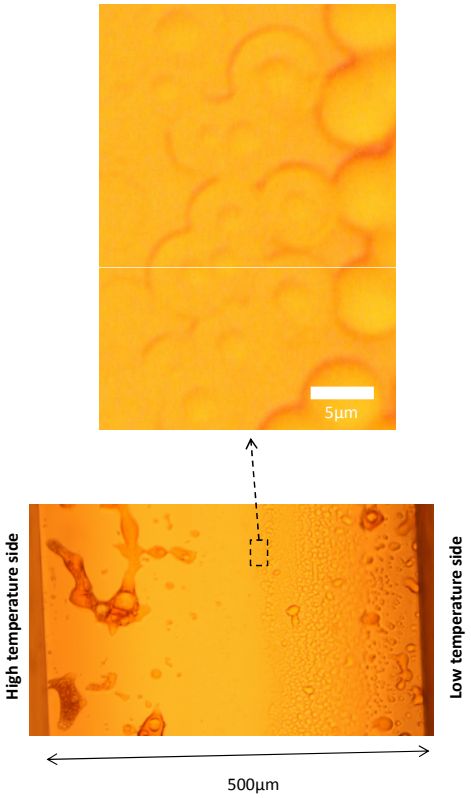

Fig. 2. An optical microscopic image of the microcapillary.

[6]. Both outer and inner diameters of microcapsules have a tendency that they increase as being apart from the cooled-side inner wall of the microcapillary. On the other hand, the ratio between them has no evident dependence on the distance from the cooled-side inner wall (Fig. 3 (c)).

The temperature range based on (2) corresponding to $185 \leq x \leq 225$ is $43.565 \leq T \leq 47.525$. The average temperature of the positions of 63 microcapsules is $45.3°$C. As a control experiment, we performed the same experiment as that described in Section 2 except replacing step (iii) by the following (iii'): (iii') after the microcapillary was rotated 90 degrees, the temperatures of the two Peltier devices were both set to $45°$C and kept for 96 hours. In this control experiment, no microcapsule was found by optical microscopic observations.

Since the thermal gradient given in step (iii) was parallel to the gravitational field and decreases from the top to the bottom, convection flow could not occur in our experiment. Thus, molecular transport resulting from the thermal gradient would be dominated by that caused by thermal diffusion. Considering the result of the control experiment, we hypothesize that thermal diffusion flow of dissolved proteinoids at high temperature region in the microcapillary contributes to the microcapsule formation found in our experiment: microspheres located in the region where microcapsules were formed would dissolve slowly because of moderately high temperature in the region. In the case of microcapsule formation by pH control, it was suggested that re-aggregated proteinoids that

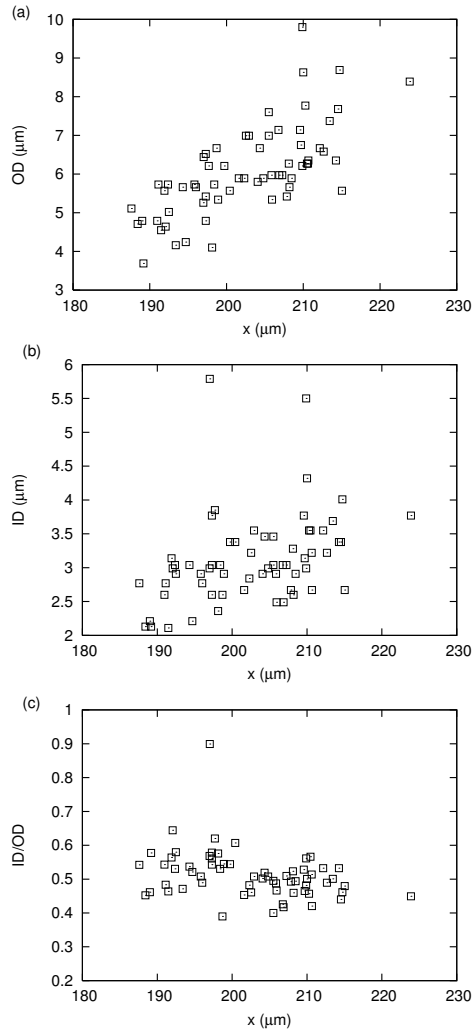

Fig. 3. (a) Outer diameters (OD), (b) inner diameters (ID) and (c) the ratios ID/OD of 63 microcapsules found in optical microscopic images are plotted against the distance x from the cooled-side inner wall of the microcapillary.

form microcapsules near the surface of dissolving microspheres have different kinetic property of dissolution [5]. If similar change on kinetic property of proteinoids occur at high temperature, then dissolved proteinoids carried by the thermal diffusion flow could re-aggregate near the surface of the slowly dissolving microspheres due to hypothetical conformational changes in each proteinoid. Consequently, microcapsules could be formed around the microspheres, while the microspheres would still keep dissolving slowly and thus they are observed as inner kernels.

The surfaces of proteinoid microspheres and microcapsules could be "physical catalysts" on which occurrences of chemical reactions involving prebiotic molecules are facilitated [10]. Moreover, microcapsules could work as compartments that encapsulate other prebiotic molecules on the primitive earth. Thus, our finding suggests that prebiotic material aggregates that could be both primitive compartments and catalysts can spontaneously emerge in a natural geological condition.

ACKNOWLEDGMENT

T. Haruna was supported by JST PRESTO program.

REFERENCES

[1] G. M. Whitesides and B. Grzybowski, "Self-assembly at all scales," *Science*, vol. 295, pp. 2418–2421, 2002.

[2] S. W. Fox and K. Dose, *Molecular Evolution and the Origin of Life*. W. H. Freeman and Company, 1972.

[3] E. Imai, J. Shirasawa, H. Honda, and K. Matsuno, "Contribution of temperature gradient to aggregation of thermal heterocopolymers of amino acids in aqueous milieu," *Origins of Life and Evolution of the Biosphere*, vol. 21, pp. 243–249, 1992.

[4] S. Sakurazawa, E. Imai, H. Honda, and K. Matsuno, "Microcapsule formation in self-assembly of thermal heterocomplex molecules from amino acids," *Colloid & Polymer Science*, vol. 274, pp. 899–903, 1996.

[5] S. Sakurazawa, T. Ishimori, H. Honda, and K. Matsuno, "Diffusion controlled formation of husk-like microcapsules," *Colloid & Polymer Science*, vol. 275, pp. 502–505, 1997.

[6] T. Haruna, I. Kunita, and S. Sakurazawa, "Microcapsule formation by thermal heterocomplex molecules from amino acids in a thermal gradient microcapillary," in *Proceedings of the Second International Conference on Morphological Computation*, R. Pfeifer, H. Sumioka, R. M. Füchslin, H. Hauser, K. Nakajima, and S. Miyashita, Eds., 2011, pp. 60–62.

[7] P. Baaske, F. M. Weinert, S. Duhr, K. H. Lemke, M. J. Russell, and D. Braun, "Extreme accumulation of nucleotides in simulated hydrothermal pore systems," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, pp. 9346–9351, 2007.

[8] I. Budin, R. J. Bruckner, and J. W. Szostak, "Formation of protocell-like vesicles in a thermal diffusion column," *Journal of the American Chemical Society*, vol. 131, pp. 9628–9629, 2009.

[9] A. K. Datta, *Biological and Bioenvironmental Heat and Mass Transfer*. CRC Press, 2002.

[10] M. Kunita, "Thermal heterocomplex molecules as reaction field of chemical evolution," *Viva Origino*, vol. 36, pp. 66–68, 2008.