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SHORT COMMUNICATION

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Microcapsule formation in self-assembly of thermal heterocomplex molecules from amino acids

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Abstract Solid microspheres that were phase-separated in aqueous suspensions of thermal heterocomplex molecules made by heating a mixture of proline and aspartic acid transformed into husk-like microcapsules as pH increased. The transformation from solid microspheres into microcapsules proceeded at two different time scales. Rapid dissolution of thermal heterocomplex molecules from the outer surfaces only during a short period was followed by slow dissolution of those molecules from their inside. The difference of the rates of dissolution led to the formation of microcapsules carrying smaller inner kernels as remnants.

Key words Amino acids – husks – microcapsules – microspheres – self-assembly

Introduction

Molecular self-assembly is a subject matter which has recently experienced a renewed interest from both theoretical and applied viewpoints [1, 2]. The types of self-assembly may be microspherical, microcapsular or microtubular among others [3–5]. In the present article, we report on the formation of husk-like microcapsules from aqueous suspensions of thermal heterocomplex molecules made by heating a mixture of amino acids.

Self-assembly of thermal proteins or proteinoids in their aqueous suspensions, which was first attempted by Fox and his colleagues [6, 7], revealed that microspherical structures could be phase-separated when basic suspensions of the thermal polymers were acidified. In particular, Bergeron et al. [8] recently observed the formation of microcapsules, instead of microspheres, in the solutions of tetrapeptides derived from aspartic acid diketopiperazines when acidified. Yanagawa et al. [9] also observed microcapsules, which they called marisomes, to be formed in the solution mixture of various amino acids and transition metal ions when they were heated to above 100 °C.

Formation of microcapsules from amino acids as their fundamental constituent elements is thus diverse. We examine one more possibility of making microcapsules while starting from solid microspheres of thermal heterocomplex molecules from amino acids.

Experimental

Materials

Thermal heterocomplex molecules made by heating the mixture of two kinds of L-amino acid, proline and aspartic acid, of equal molar weight were prepared [10]. The reaction mixture was placed at 200 °C (EYELA NDO-450N) for 3 h in normal atmospheric conditions. The heated products were solubilized in distilled water at boiling temperature for 20 min and then the solution was immediately put in an ice-bath. The reaction products were subsequently separated and collected by three-fold centrifugation (Kubota KA 1000) at 4000 rpm for 3 min at 20 °C. The collected products of thermal heterocomplex molecules were preserved at 2 °C. All of the specimens

thus prepared were used within 1 month of their preservation.

Molecules constituting the phase-separated microspheres were found to have uniform molecular weights of about 4000 daltons, estimated by gel chromatography with the use of Sephadex G-50 (Pharmacia) under the conditions of 50 mM Na-carbonate-bicarbonate buffer (pH = 9.2). These molecules of molecular weights 4000 which were single-peaked in the chromatography will hereafter be referred to as DP1.

DP1 molecules thus separated and collected were again suspended in distilled water in order to study their accretion into solid microspheres and their subsequent transformation into husk-like microcapsules as pH increased.

Results and discussion

We first prepared DP1 suspensions of 20 mg/ml at boiling temperature and then decreased their temperature down to room temperature (25 °C) through natural cooling. All the subsequent measurements were done at room temperature. Microspherical sediments were separated and collected by centrifugation at 4000 rpm for 3 min, in which we noted that morphological stability of those microspherical sediments at 25 °C was confirmed during the storage over 1 month at pH = 3.0. Their mechanical strength was such that they remained stable during and after centrifugations. Those sediments were then dispersed and stirred in universal buffer (boracic acid–citric acid–potassium phosphate) at pH = 8.0, in which pH was controlled by adjusting the ratio of the amount of the mixture of 0.1 M boracic acid and 0.02 M citric acid to that of 0.1 M potassium phosphate. One hour after their preparation, the suspension was centrifugated. The sediments were then dispersed in universal buffer adjusted at pH = 3.0.

Figure 1 shows an image of microspherical sediments taken by a differential interference contrasted microscope, in which husk-like microcapsules were found to contain smaller microspherical kernels in their inside. The specimen was suspended in the universal buffer at pH = 3.0 stated in the above. A scanning electron microscope image of those microcapsules in their dried conditions is presented in Fig. 2. Both outer microcapsules and inner solid kernels were identified. In order to get dried samples for the microscope, the products were suspended in 70% ethyl alcohol and collected by centrifugation at 4000 rpm for 3 min. These procedures were successively repeated in 80, 90, 95, 100% (three times) ethyl alcohol and finally 100% *t*-butyl alcohol suspension. Then, *t*-butyl alcohol was subliminated at 4 °C.

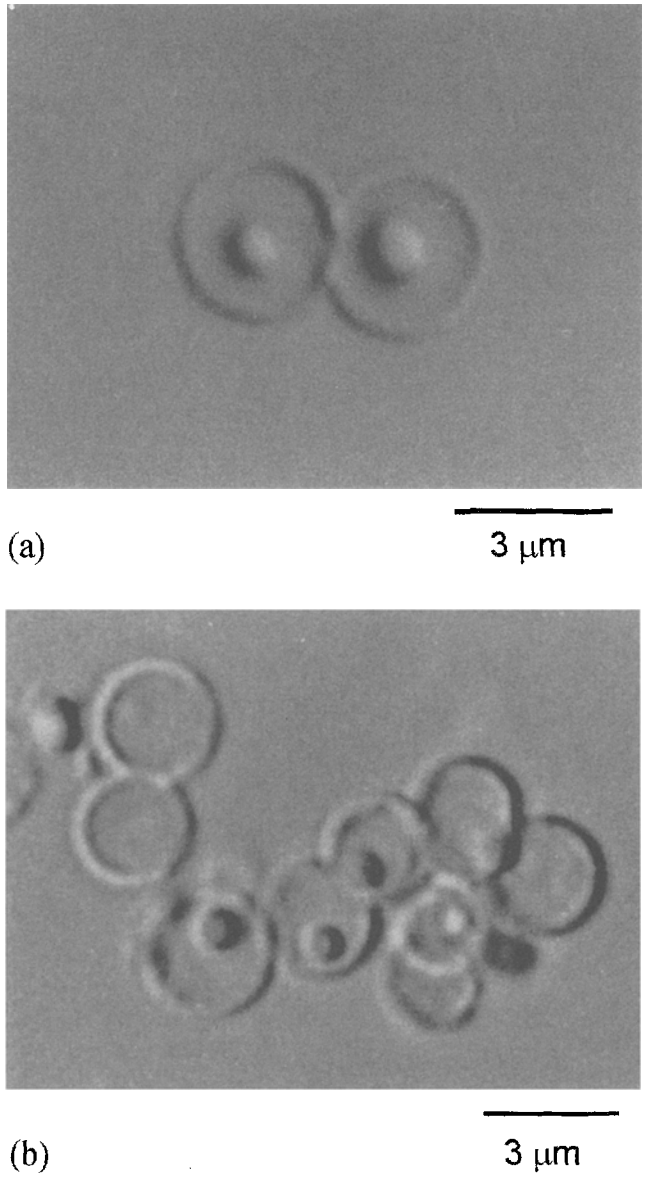


Fig. 1 Image of microcapsules containing inner kernels, taken by a differential interference contrasted microscope

Figure 3 shows how solid microspheres that were made by cooling the boiled DP1 suspension of 5.0 mg/ml down to 25 °C, developed into microcapsules containing inner kernels after the solution was alternated by 2 M tris-HCl buffer at pH = 8.0. The images were taken by a differential interference contrasted microscope. The outer husks were found to remain insoluble while the inner contents were gradually dissolved into the solution as evidenced in the decrease of the diameter of the inner kernel.

A characteristic numerical figure to describe the microcapsule containing its inner kernel is the ratio of the

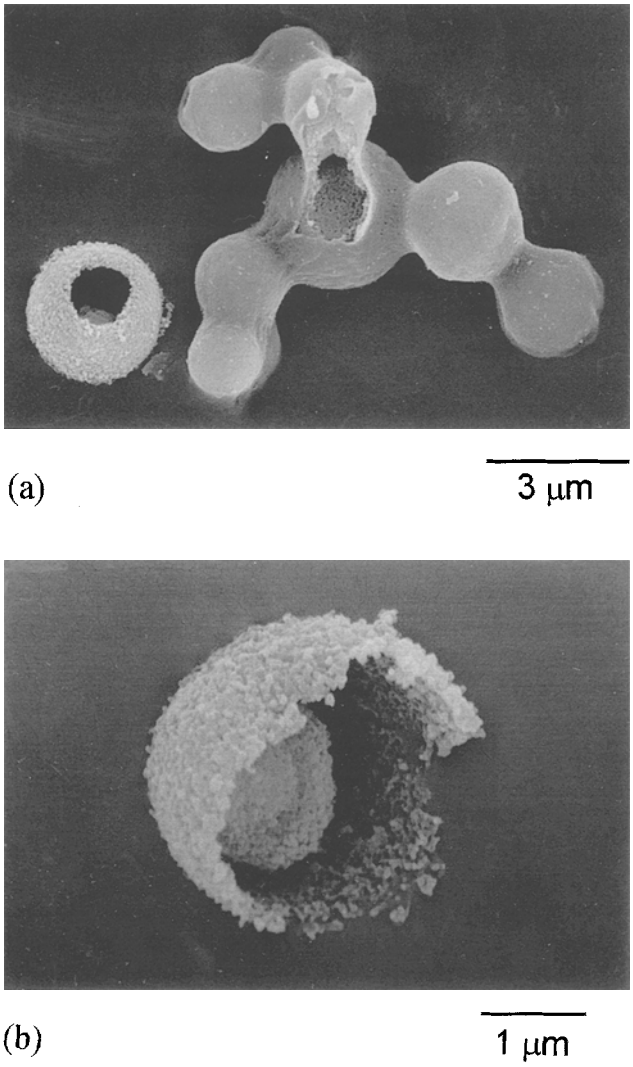


Fig. 2 Scanning electron microscope image of dried microcapsules containing inner kernels

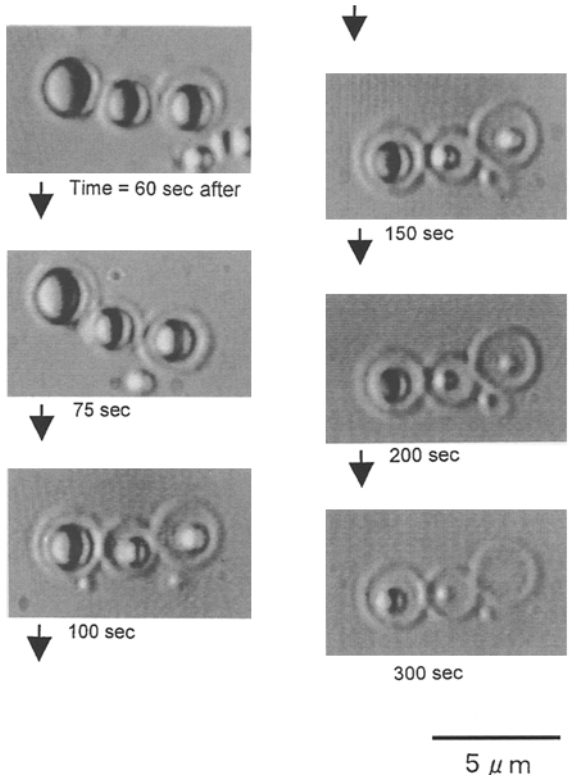
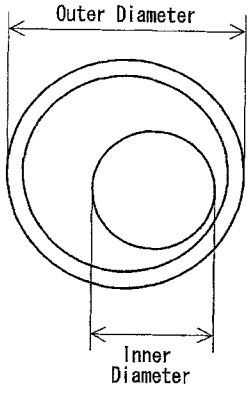


Fig. 3 Transformation in time from solid microspheres to microcapsules due to dissolution of the constituent DP1 molecules



$$\text{I/O Ratio} = \frac{[\ \text{Inner Diameter}\ ]}{[\ \text{Outer Diameter}\ ]}$$

Fig. 4 A schematic definition of I/O ratio

diameter of the inner kernel to that of the outer husk, that is, I/O ratio of the microcapsule. Our definition of I/O ratio is depicted in Fig. 4. Figure 5 presents a time development of I/O ratios under various pH conditions, in which universal buffer was used for controlling pH. The origin of the time axis was chosen to be the time point at which cooled DP1 suspensions of 20 mg/ml were alternated by the solutions of designated pH values. Below pH = 5, I/O ratio was found to be almost unity, implying that solid microspheres remained insoluble. From these observations, it can be said that the shell is permeable for material transport as the outer diameter of the shell remains unaffected during the dissolution process of the inner kernels.

In order to see how DP1 molecules constituting solid microspheres dissolved into the solution, we measured time-course of the ratio of the amount of DP1 molecules freed in the solution to that of the total DP1 molecules in the suspension. The origin of the time axis was chosen to be the time point when 41.4 ml of cooled DP1 suspensions of 5.56 mg/ml were dispersed into 4.6 ml of 2 M tris-HCl buffer. The pH value was 7.65. Measurement of the amount of DP1 freed in the solution was done by eliminating

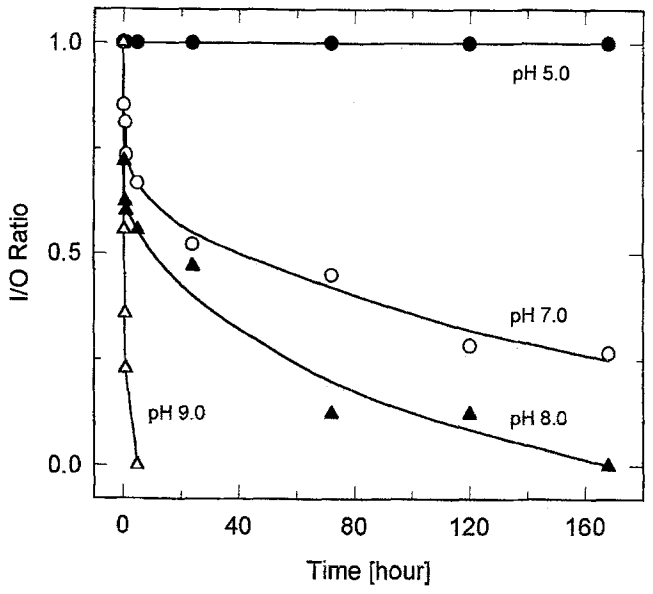


Fig. 5 Time development of the ratio of the diameter of an inner kernel to that of the outer husk, denoted as I/O ratio

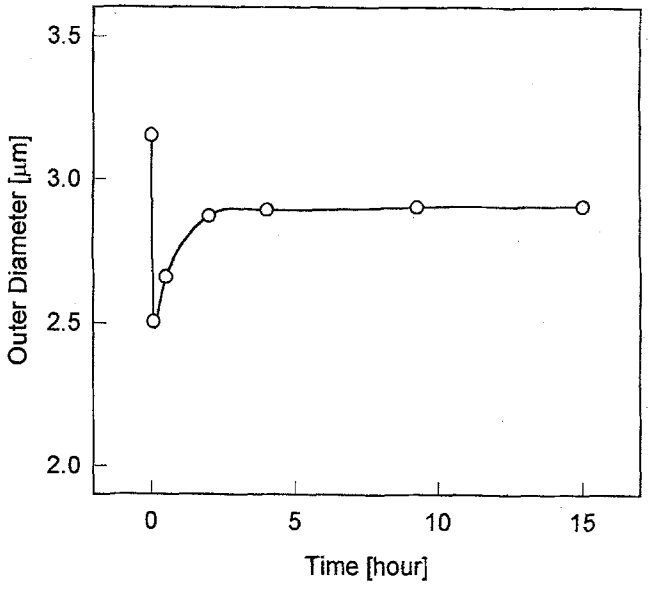


Fig. 7 Time development of the outer diameter of microcapsule

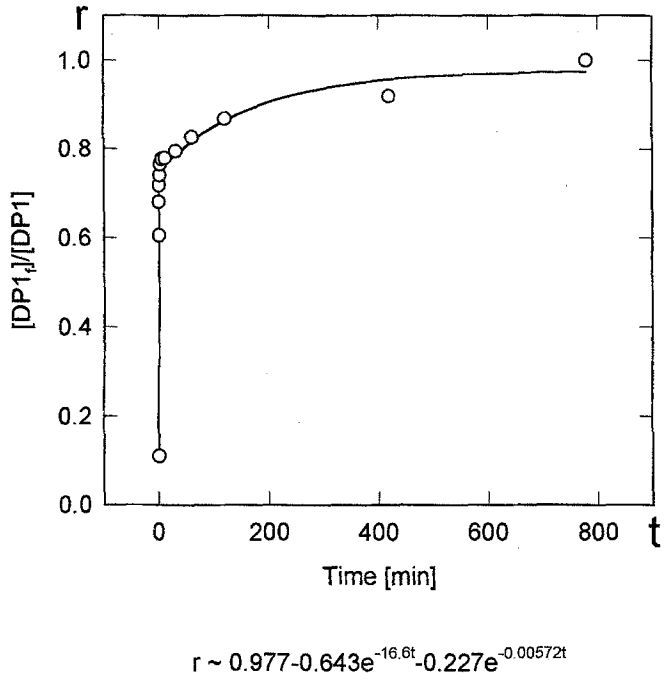


Fig. 6 Time development of the ratio of the amount of DP1 molecules freed in the solution to that of the total DP1 molecules in the suspension. The ratio was found to develop as following a simple expression presented therein

those constituting microcapsules and their inner kernels with use of membrane filter of pore size 0.45 μm. Quantitative measurement of the amount of DP1 molecules was accomplished by consulting the absorbance of ultra-violet light at wavelength 300 nm [11]. The result is shown in Fig. 6, in which the ratio of the amount of DP1 molecules freed in the solution to the total amount in the suspension is presented. We thus find that the dissolution of DP1 molecules into the solution proceeded at two different rates, fast and slow. The characteristic time for the fast process was a few seconds, while that for the slow process was several hours.

Further structural details on forming microcapsules containing smaller inner kernels were identified by directly measuring time-course of the diameter of the outer husk of microcapsule. Figure 7 demonstrates such a time-course of the diameter of microcapsule, in which cooled DP1 suspensions of 20 mg/ml were alternated by universal buffer of pH = 8.0 and the origin of the time axis was chosen to be the time point of the solution alternation.

These results suggest that the initial rapid dissolution of DP1 molecules from the outer surfaces of solid microspheres was gradually alternated by a slow accretion process, while those molecules constituting the inner kernels were constantly kept dissolving into the solution at the rate depending upon the pH value of the solution.

Solid microspheres phase-separated in the solution of thermal heterocomplex molecules from amino acids exhibit versatile structural changes of their own depending upon their environmental conditions. Formation of microcapsules containing smaller inner kernels, which we observed in this report, certainly manifests a structural and functional capability latent in thermal heterocomplex molecules from amino acids and microspheres made thereof.

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