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SHORT COMMUNICATION

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Diffusion controlled formation of husk-like microcapsules

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Abstract Solid microspheres consisting of thermal heterocomplex molecules made from heating a mixture of aspartic acid and proline were transformed into husk-like microcapsules in their aqueous suspensions when pH value increased. The thickness of the outer shell of the husk-like microcapsule decreased as pH increased. Formation of the husk-like microcapsules is discussed to be due to both diffusion of the constituent molecules from the inside of the microspheres and conformational changes of those molecules in the process.

Key words Amino acids – diffusion – husk – microcapsules

Introduction

We have already reported on the formation of husk-like microcapsules from aqueous suspensions of thermal heterocomplex molecules made by heating a mixture of amino acids such as aspartic acid and proline [1]. This is in parallel to another observation of microcapsules in the solution of tetrapeptides derived from aspartic acid diketopiperazines when acidified [2]. These two observations, when combined together, indicate that microcapsules to be formed from complex molecules from amino acids could be sensitive to their solution conditions such as pH values. In fact, the barrier properties of the capsule shells made from the coacervation of gelatin and acacia are found to depend upon the coacervation pH [3]. Stepwise treatments with alkali and acid can also provide multihollow structures from submicron-sized monodispersed polymer particles [4].

In the present article, we examine how the microcapsule formation could be effected by those diffusion flows of constituent molecules from both the outer surface and from the inner kernel of solid microsphere that is made of thermal heterocomplex molecules from aspartic acid and proline. In particular, the thickness of the capsule shell and its outer diameter are examined when the pH value and the viscosity of its suspension are varied in controlled manner.

Experimental

Materials and methods

Thermal heterocomplex molecules made by heating the mixture of two kinds of L-amino acid, aspartic acid and proline, of equal molar weight were prepared, in which we have followed the similar method reported in our previous article [1]. Some of the relevant information is reproduced here. The reaction mixture was placed at 200 °C for 3 h under normal atmospheric conditions. The heated products were solubilized in distilled water at boiling temperature for 20 min and then the solution was immediately cooled in an ice-bath. The reaction products were subsequently separated and collected by threefold centrifugation at 4000 rpm for 3 min at 20 °C. The collected products of thermal heterocomplex molecules were preserved at 2 °C.

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Molecules constituting the phase-separated microspheres were found to have uniform molecular weights of about 4000 Da, estimated by gel chromatography with the use of 50 mM Na-carbonate-bicarbonate buffer (pH = 9.2) [5]. These molecules of molecular weights 4000 which were single-peaked in the chromatography will hereafter be referred to as DP1.

Results and discussion

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. We first dispersed fixed amount of DP1 molecules in Tris–HCl buffer of pH = 8.0, while controlling the molar concentration of the buffer. One hour after the preparation, the suspension was centrifugated. The sediments were then dispersed in universal buffer (boracic acid–citric acid–potassium phosphate) adjusted at pH = 3.0 for the purpose of measuring the structural properties of microcapsules thus formed.

Table 1 shows the average thickness of the outer shells of the formed microcapsules, in which the initial concentration of DP1 molecules and the concentration of Tris–HCl buffer were taken as controlled parameters. Measurement of the thickness was done on the image of scanning electron micrograph [1]. The thickness of the outer shell decreased with the increase of the initial concentration of DP1 molecules. It also decreased with an enhancement of the rate of pH buffering. The thickness of the outer shell was found to decrease as the pH value of Tris–HCl buffer increased as tabulated in Table 2, in which all of the other conditions were maintained the same as those in Table 1.

In order to examine the initial buildup of husk-like microcapsules from solid microspheres, we measured time course of the amount of DP1 molecules that would come to be dissolved into the solution since the pH value of the suspension was fixed at a given level. Figure 1 demonstrates time development of a typical dissolution of DP1 molecules from solid microspheres since these microspheres were placed in Tris–HCl buffer adjusted at pH = 8.0, in which the buffer concentration was maintained at 50 mM. Quantitative measurement of the amount of DP1 molecules freed in the solution, denoted as $DP1_f$, was accomplished by measuring the absorbance of ultra-violet light at wavelength 300 nm [6]. The rate constant of dissolution was found to increase with the increase of the initial concentration of DP1 molecules. A similar time course of the amount of DP1 molecules dissolved into Tris–HCl buffer adjusted at pH = 8.0 when the buffer concentration was parametrized, is presented in Fig. 2 in which the initial concentration of DP1 molecules was fixed at 5 mg/ml. As the rate of buffering increased, the rate constant of DP1 dissolution was found to increase. Dissolution of DP1 molecules for various pH values of Tris–HCl buffer is also presented in Fig. 3, in which both the buffer concentration and the initial concentration of DP1 molecules were fixed. As expected, the dissolution was enhanced as the pH value increased.

Table 1 pH buffering and its effects on the average thickness of the outer shells of the husk-like microcapsules

DP1	Thickness of the outer shell [μm] Tris–HCl (pH 8.0)		
	50 mM	100 mM	500 mM
2 mg/ml	0.333	0.102	–
5 mg/ml	0.249	0.101	0.094
10 mg/ml	0.110	0.097	0.089

Table 2 pH buffering and its effects on the average thickness of the outer shells of the husk-like microcapsules, parametrized in pH value

pH	7.0	8.0	9.0
Thickness of the outer shell [μm]	0.257	0.101	0.063

Note: DP1: 5 mg/ml; buffer: 100 mM Tris–HCl.

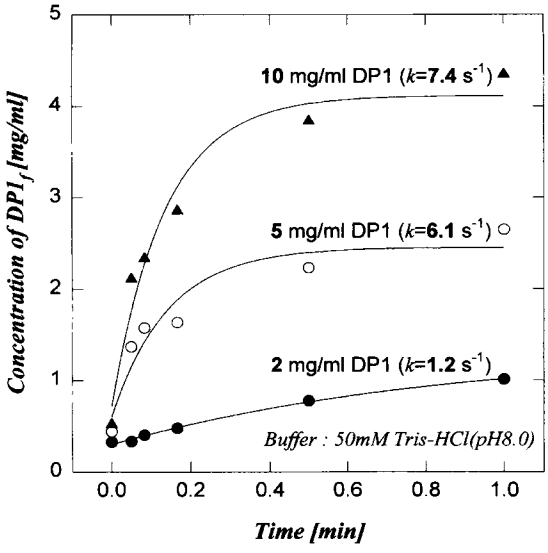

Fig. 1 Concentration of dissolved and free DP1 versus the rate constant k following the formula $[DP1_f] \propto (b - \exp(-kt))$, where t is time and $b \sim 0.791 \pm 0.0036$

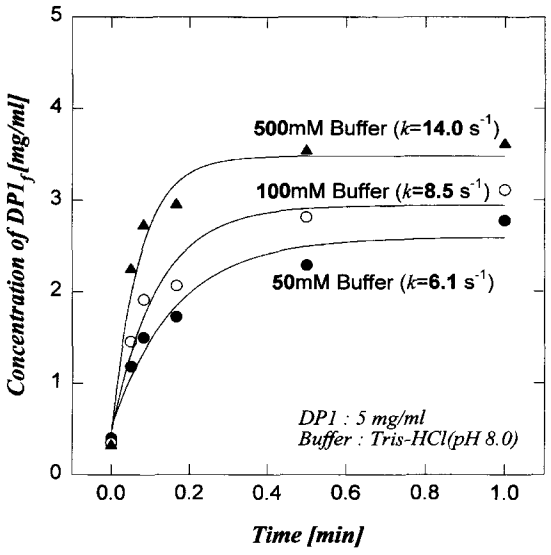

Fig. 2 Concentration of dissolved and freed DP1 parametrized in buffer concentration versus the rate constant k following the formula $[DP1_f] \propto (b - \exp(-kt))$, where t is time and $b \sim 0.840 \pm 0.042$

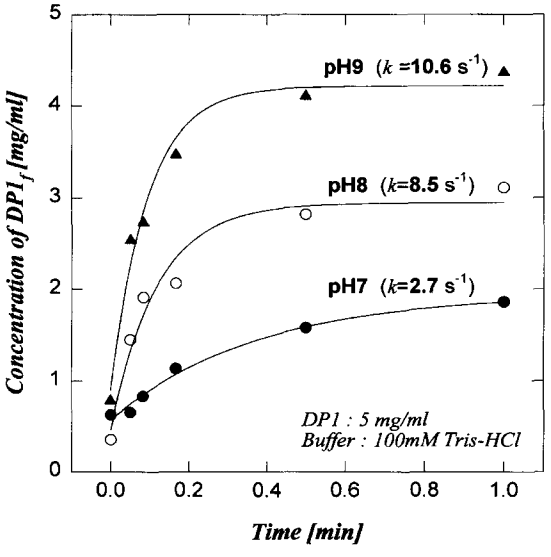

Fig. 3 Concentration of dissolved and freed DP1 parametrized in pH value versus the rate constant k following the formula $[DP1_f] \propto (b - \exp(-kt))$, where t is time and $b \sim 0.817 \pm 0.027$

These observations suggest that the formation of husk-like microcapsules from solid microspheres is due to diffusion of DP1 molecules from various parts of the microspheres not necessarily only from the outer surfaces. In order to confirm the occurrence of diffusion flows, we again measured the thickness of the outer shell in the experimental conditions similar to the ones for Table 1 with the only exception that 0.5 M saccharose was further added just for the purpose of increasing the viscosity of the solution. The result is tabulated in Table 3. Increase in the viscosity of the solution certainly suppressed the dissolution of those molecules comprising the microspheres.

Table 3 pH buffering and its effects on the average thickness of the outer shells of the husk-like microcapsules when saccharose added

DP1	Thickness of the outer shell [μm] Tris–HCl (pH 8.0), 0.5 M saccharose added		
	50 mM	100 mM	500 mM
2 mg/ml	0.509	0.228	0.183
5 mg/ml	0.430	0.175	0.172
10 mg/ml	0.183	0.167	0.157

One of the intriguing findings in the present measurement is dissolution of DP1 molecules from the inside of the microspheres into the solution while leaving the outer shells relatively stable. In addition, as the concentration of DP1 molecules dissolved into the solution increased, the dissolution was further enhanced as both Table 1 and Fig. 1 indicated. Nonetheless, the outer shells could remain relatively stable while becoming thinner. The demonstrated contrast between enhancement of DP1 dissolution with the dissolution and relative stability of the outer shell suggests a possibility that the outer shell may differ from the inner kernal in its kinetic property of dissolution into the solution. Rather, it may be due to some conformational changes of DP1 molecules constituting the outer shell. An indirect evidence for the possibility of such conformational changes can be seen from an enormous enhancement of stability of the outer shell against pH increase. Conversely, it may suggest that those DP1 molecules undergoing conformational changes subjected to high pH can then form the outer shell that could become almost insoluble in the high pH solution.

Conclusion

Experimental evidence that conformational changes of DP1 molecules may be necessary for the formation of the husk-like microcapsules has been indirect in our present study. However, we have demonstrated that thermal heterocomplex molecules from aspartic acid and proline exhibit a transformation of their property during their dissolution and re-aggregation depending upon their solution conditions. Our model systems provide a new addition to a likely mechanism of making and forming husk-like microcapsules.

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