Mass spectrometric analysis of proteinoids in spontaneously self-assembled microspheres

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Abstract

Introduction

Proteinoids are prebiotically synthesized polyamino acids. In previous research, it has been reported that proteinoids dissolved in hot water spontaneously form molecular assemblies called proteinoid microspheres during cooling. However, with the formation mechanism of molecular assemblies, there are many unsolved questions. We conjectured that there was a selection process of the proteinoids during the formation process of the proteinoid assemblies.

In this research, we aimed to study the relationship between the terminal structure of proteinoids and the formation of molecular assemblies by mass spectrometry.

Experiments

The proteinoids have poor solubility in water, acetonitrile, and various alcohols; however, they show better solubility in *N, N*-dimethylformamide (DMF). Therefore, DMF was used as the solvent for sample preparation in this study. But DMF deteriorated the flow path of the MS analyzer and the signal intensities of proteinoids were low.

Accordingly, we surveyed the solvent for the MS analysis. We found that a solution of ACN: H2O: DMSO, 45:45:10 (v/v/v) was a good solvent for the MS analysis of proteinoids by electron splay ionization mode. Various ions of proteinoids were detected on the mass spectra and the elemental compositions of the each proteinoids were estimated using by high-resolution mass spectrometry.

Proteinoids (a) were synthesized from the monoammmonium malate salt by thermal polycondensation at 180 for 72h. The synthesized proteinoids were refluxed in water to disolve the proteinoids. Thereafter, they were filtered under heated condition to separate into the non-soluble proteinoids (b) and the hot water-soluble proteinoids. The hot solution containing water-soluble proteinoids were cooled to allow the formation of the proteinoid microspheres, which were then fractionated into precipitated microspheres and a supernatant liquid by a centrifuge.

Thus, the initially formed proteinoids (a) were divided into three of proteinoids: non-soluble proteinoids (b), proteinoids in the microsphere (c) and proteinoids in the supernatant (d). The proteinoids of (a), (b) and (c) were dissolved in ACN: H2O: DMSO, 45:45:10 (v/v/v). The proteinoid of (d) was dissolved in water. Those proteinoids were analyzed by high resolution mass spectrometry.

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Results and Discussion

We found several homologues of proteinoids, those being unhydropolyaspartic acids with different terminal structures.

Most of the proteinoids of (a) and (b) were similar, but some homologues might be altered during the reflux. Proteinoids of (c) and (d) were almost the same. And they were portions of proteinoid (b), but some of these were different. It indicates that different homologues were separated in the process of filtration due to hydrophobicity.
This result suggests that proteinoids microspheres were formed from the hot water soluble proteinoids depending on the solubility of the proteinoids into water and that some proteinoids having specific a terminal structure spontaneously formed microspheres.