Notes

Atomic Force Microscopy Observation of Poly(A) Grown on the Liquid/Solid Interface from Sodium Bis(2-ethylhexyl) Sulfosuccinate/Isooctane Reversed Micellar Solution

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Introduction

It is generally considered that interface-bound molecules are entropically more favored to condense than those in free solution owing to their reduced translational or rotational freedom.[1] Therefore, it is expected that a polymerization in bounded structures such as within water pools in a reversed micellar solution or in the interior of vesicles proceeds favorably and selectively.

Oparin and co-workers studied the enzymatic polymerization of ADP by polynucleotide phosphorylase (PNPase) and Mg^{2+} ion in coacervates in the pursuit of constructing primitive forms of precellular structures.[2,3] Recently, Luisi and his collaborators[4] have investigated this enzymatic ADP polymerization in sodium bis(2-ethylhexyl) sulfosuccinate (AOT)/isooctane reversed micellar solution, which is thermodynamically more stable than coacervates. This enzymatic polymerization in AOT reversed micellar solution was greatly different from the general aspects of the reversed micellar enzymology. That is, the reaction product poly(adenylic acid), poly(A), precipitated out of the reversed micellar solution in a complex with PNPase, if Wo (=[water]/[surfactant]) was 20. Furthermore, if after the reaction equilibrium was nearly attained at 24 h, the supernatant was removed from the test tube and replaced by a fresh reversed micellar solution containing ADP but no PNPase, poly(A) successively continued to be produced on the bottom of the glass test tube.[4,5] We considered that the precipitated poly(A)−PNPase complex corresponded to functional supramolecular aggregates, self-organized at the interface between the oil phase and the glass. Recently, Ferris et al.[6] observed the synthesis of long prebiotic oligomers in an aqueous solution on mineral surfaces and Kiedrowski[1] proposed that the earliest forms of life may have proliferated by spreading on solid surfaces. Our observation indicates that RNA-like molecules may be enzymatically grown also in an oil phase on the solid surface. Therefore, we observed a nanostructure of the functional aggregates by atomic force microscopy (AFM).

Experimental Section

AOT and PNPase from *Micrococcus luteus*, commercial products from Sigma, were used.

The enzymatic reaction was started by mixing two micellar solutions, one with ADP and the other with PNPase, as described in the previous paper.[4,5] The activities of PNPase were measured by following unreacted ADP.[4,5] After 50−100 μL of the reaction mixture was added to 1 mL of 1 N $HClO_4$, the solution being centrifuged to precipitate poly(A), the unreacted ADP was estimated by measuring the absorbance of a mixture of the supernatant and MeOH (1:1) at 260 nm. The activities of PNPase involved in the precipitate and in the supernatant were measured as follows: After the polymerizations of ADP in 0.2 M AOT/isooctane solution of Wo = 20 (50 mM Tris−HCl, $[ADP]_{wp,o}$ = 10mM, $[MgCl_2]_{wp}$ = 10 mM, and $[PNPase]_{wp}$ = 1 mg/mL: $_{wp}$ and $_{o}$ mean a water pool and an initial time, respectively) proceeded for 2, 6, 12, and 24 h, respective supernatants were transferred to another set of glass test tubes and respective residual precipitates on the bottoms of the reaction tubes were rinsed sufficiently with an AOT/isooctane solution of Wo = 20 (50 mM Tris−HCl, pH 9.5, $[MgCl_2]_{wp}$ = 10 mM) without ADP and PNPase. A fresh AOT/isooctane solution of Wo = 20 containing only ADP solution (50 mM Tris−HCl, pH 9.5, $[ADP]_{wp,o}$ = 10 mM, and $[MgCl_2]_{wp}$ = 10 mM) was added to the precipitate, and the absorbance of unreacted ADP at 260 nm was measured with time. On the other hand, the activities in the corresponding supernatants that were separated after 2, 6, 12, and 24 h, respectively, were measured by adding fresh AOT/isooctane solutions of Wo = 20 containing only ADP but no enzyme (50 mM Tris−HCl, pH 9.5, and $[MgCl_2]_{wp}$ = 10 mM); the initial concentrations of ADP in the water pools were beforehand adjusted to be about 10 mM.

The AFM image of the poly(A) was observed on a Nanoscope from Digital Instruments using a tapping mode. The 0.2 M AOT reaction solution of Wo = 20 (50 mM Tris−HCl, pH 9.5) was as follows; $[ADP]_{wp,o}$ = 20 mM, $[MgCl_2]_{wp}$ = 10 mM, and $[PNPase]_{wp}$ = 2 mg/mL.

[A] The sheets of the precipitated samples of Figure 2 were prepared as follows: After mica sheets were soaked in the AOT reaction solutions at 37 °C for 2 and 12 h, respectively, the mica sheets were taken out from the solutions, rinsed with isooctane and with 80% ethanol (v/v) solution to remove AOT, and dried under reduced pressure at room temperature.

[B] The sheets of the supernatant samples of Figure 3 were prepared as follows: After the polymerization in the AOT reaction solution proceeded for 5 min (a), 4 h (b), and 12 h (c) at 37 °C, each supernatant was diluted 50 times with isooctane, in which mica was soaked for a few seconds. The surface of the mica was wiped with soft paper and was dried under reduced pressure at room temperature.

Results and Discussion

Parts a and b of Figure 1 show decreases of ADP concentration in water pools with time due to polymer-

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(1) von Kiedrowski, G. *Nature* **1996**, *381*, 20.

(2) Oparin, A. I.; Serebroskaya, K. B.; Auerman, T. L. *Biokimiya* **1961**, *26*, 499.

(3) Oparin, A. I.; Serebroskaya, K. B.; Pantskhava, S. N. *Biokimiya* **1963**, *28*, 671.

(4) Walde, P.; Goto, A.; Monnard, P.-A.; Wessicken, M.; Luisi, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 7541.

(5) Goto, A.; Hakamata, H.; Kuwahara, Y.; Goto, R.; Walde, P.; Luisi, P. L.; Imae, T. *Prog. Colloid Polym. Sci.* **1997**, *106*, 245.

(6) Ferris, J. P.; Hill, A. R., Jr.; Liu, R.; Orgel, L. E. *Nature* **1996**, *381*, 59.

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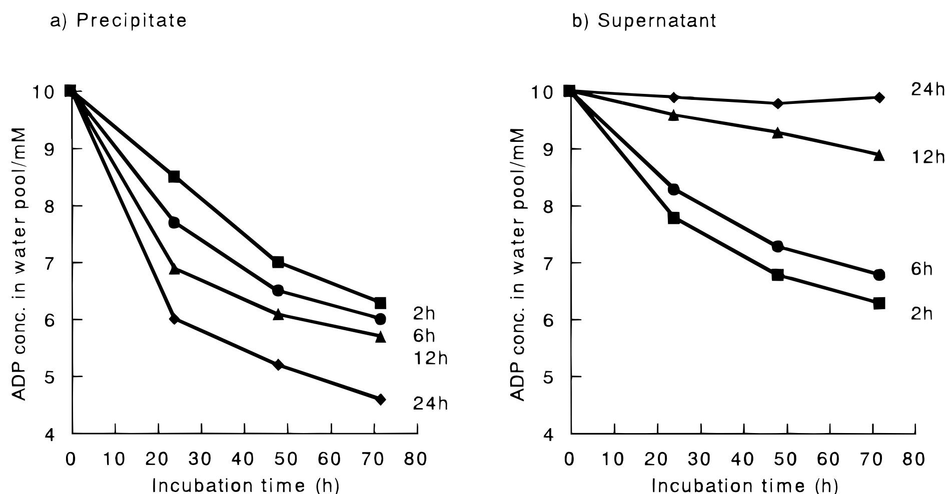

Figure 1. PNPase activities in the precipitate (a) and in the supernatant (b) of 0.2 M AOT/isooctane reversed micelles at 25 °C. After the polymerizations proceeded in four different test tubes for 2 h (■), 6 h (●), 12 h (▲) and 24 h (◆), the PNPase activities involved in the precipitate (a) and in the supernatant (b) were measured.

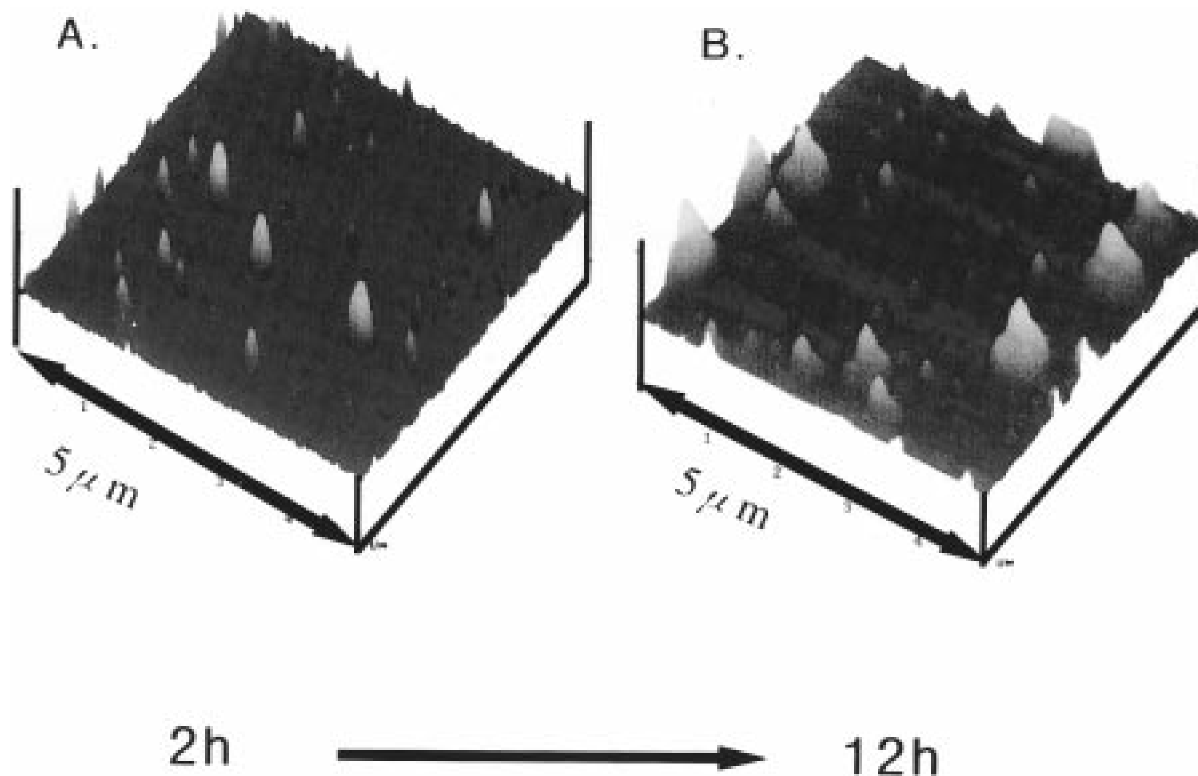

Figure 2. AFM images of the poly(A)−PNPase complex precipitated from AOT reversed micelles after 2 h (A) and 12 h (B[5]).

ization by the PNPases involved in the precipitate (a) and in the supernatant (b), after the reaction proceeded at 25 °C for 2, 6, 12, and 24 h. It was considered that the polymerization during the course of the reaction was catalyzed by both the PNPases in the supernatant and in the precipitate, but the amount of the precipitated PNPase increased with reaction time, while that of the residual PNPase solubilized in the supernatant decreased. Here, the precipitate means an aggregate that falls to the bottom of the tube and is associated with the glass.

It is of importance to confirm the same PNPase acitivity in the reversed micellar media as in the glass-associated precipitate. Therefore, we investigated whether the precipitated PNPase can be resolubilized in the water pools of the reversed micelles or not. After the precipitated PNPase was incubated with 0.2 M AOT reversed micellar solution of Wo = 20 without ADP and without PNPase at 25 °C for 24 h, the activities of PNPase in the precipitate and in the supernatant were measured by the method described above. The PNPase could not be found in the supernatant, while it was observed in the precipitate (data not shown). Therefore, once the PNPase precipitated out of the micellar solution together with poly(A), it could hardly be solubilized again into the water pools. It may be assumed that this polymerization proceeds on the glass surface in a manner akin to the solid phase synthesis of biopolymers.

The enzymatically synthesized poly(A) was investigated by AFM. Figure 2 shows the AFM pictures of the precipitated product obtained after 2 h (A) and 12 h (B), respectively, in which the AFM image after 12 h (B) was shown in the previous paper.[5] The longer the incubation time, the larger the particles observed by AFM. After 12 h, particles with a diameter of ca. 500 nm and a height

Figure 3. AFM images of poly(A)−PNPase aggregates in the supernatants after 5 min (A), 4 h (B), and 20 h (C).

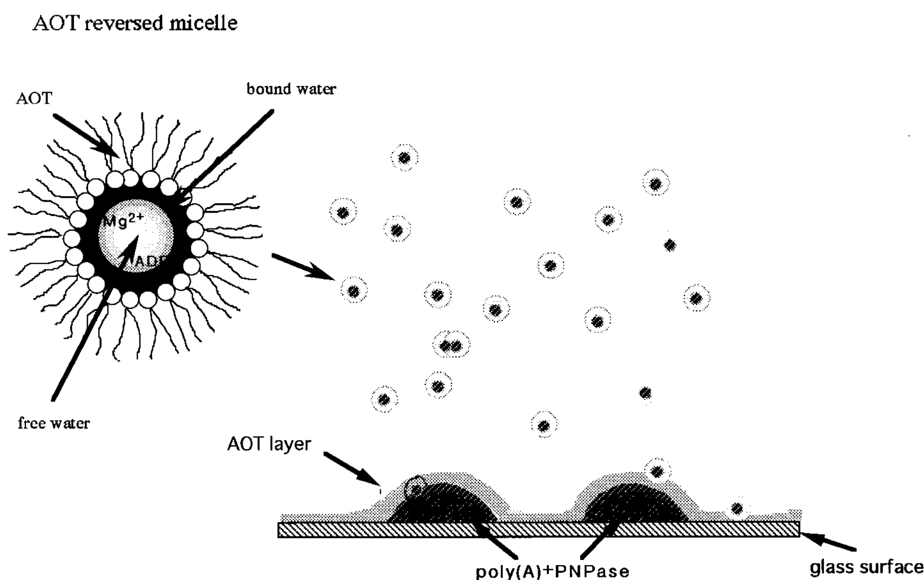

Figure 4. Schematic model of the nanostructures of functional aggregates self-organized on the interface between AOT reversed micellar solution and solid glass.

of 100−200 nm could be visualized. To confirm that the particles were the poly(A) product, the precipitate was dissolved in a Tris buffer of pH 7.0 and analyzed by HPLC, which indicated the presence of polydisperse poly(A). This dissolved poly(A) was also investigated by AFM, and it was shown that the particles were similar to those in Figure 2, although their size was smaller (data not shown). Therefore, it can be concluded that the particles detected in Figure 2 are those of the poly(A)−PNPase complex. However, poly(A) and PNPase could not be discriminated by AFM, because the size of PNPase is below ca. 20 nm, as shown in the previous paper.[5] The precipitated particles seem to be just aggregates composed of PNPase, poly(A), and water in various molar fractions and to grow on the solid surface due to both proliferation and coalescence.

Figure 3 shows AFM pictures of the supernatants. After the polymerization proceeded for 5 min, 4 h, and 20 h, respectively, mica sheets were soaked in the respective supernatants for 5 s. After 5 min, small particles were observed, and after 4 h the size of the particles considerably increased, while after 12 h the number of the particles decreased, although significantly more grown particles were visualized. These observations suggest that the reaction progresses first in the water pools of the reversed micelles, and the polymerized products precipitate after a relatively short time under the conditions used. The particles in Figure 2 tended to be larger than those in the supernatant (Figure 3). Therefore, it is assumed that the particles (Figure 2) consist of not only the directly precipitated poly(A) but also the poly(A) further grown on the solid surface. This AFM image of the polymerization process is at least in qualitative agreement with the result shown in Figure 1a,b.

Since the AOT micellar solution is very dynamic,[7] the reactant monomers in the water pools might be hence successively and easily supplied to the enzyme in the aggregates on the glass surface and the polymerization might be carried out in a manner akin to the solid phase synthesis of biopolymers.[6,8,9] When the precipitate was rinsed with 80% ethanol (v/v) to remove the AOT layers wrapping the poly(A) and PNPase aggregates, almost all the activity of PNPase was lost. However, after the precipitate was rinsed with AOT reversed micelle solution of Wo = 20, the activity was maintained, as described above. This indicates that the AOT layers wrapping the products on the glass surface could play an important role in making the poly(A) particles grow on the solid surface. The model is schematically given in Figure 4.

In the present work, we focused on the poly(A) production in AOT reversed micelles, but the model might not be restricted to the polymerization of ADP. Ferris and his collaborators[6] proposed a plausible model of prebiotic polymerization in which the polymers of life were more likely to have been proliferated on a solid surface instead of in free solution. Although in our work an enzyme catalysis is involved, the results presented in this paper indicate that solid surface polymerization may be a more general principle; in the present case involving not an aqueous phase in contact with the solid surface but an oily reversed micellar solution.

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(7) Luisi, P. L.; Giomini, M.; Pileni, M. P.; Robinson, B. H. *Biochim. Biophys. Acta* **1988**, *947*, 209.
(8) Ferris, J. P.; Ertem, G. *J. Am. Chem. Soc.* **1993**, *115*, 12270.
(9) Kawamura, K.; Ferris, J. P. *J. Am. Chem. Soc.* **1994**, *116*, 7564.