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Formation of Liquid-Crystal-Like DNA Aggregate under Biological Condition: Phase Diagram, Morphology, and Biological Implication

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It is well known that a large DNA aggregate induced by spermidine, namely macro-aggregate, has a liquid-crystal-like feature in a structural manner. However, nobody finds out that the aggregation condition as well as the pathway to the aggregate under nearly physiological conditions containing Mg^{2+}, ATP, and their mixture. In this work, the formation of liquid-crystal-like DNA macro-aggregate under such conditions has been investigated via measuring the DNA precipitation condition, or phase diagram, and also observing their morphology along the precipitation with polarizing microscopy. These obtained results, such as the DNA phase diagram and morphological variations of the aggregates, suggest not only the effects of the added ions except aggregating agent on DNA aggregation but also biological implications of the present aggregation.

Keywords: DNA aggregation; Spermidine; Mg^{2+}; ATP; Phase diagram; Aggregate morphology

1. INTRODUCTION

DNA inside viruses to higher eukarytes is highly packed like a liquid crystalline state. DNA aggregation in vitro has been studied to understand such a DNA self-assembly and its biological functions. The aggregate is classified into two kinds[1]: 'micro-aggregate' and 'macro-aggregate'. Especially, early researches[1-4] on the latter in a liquid-crystal-like state are insufficient to not only elucidate the formation mechanism but also develop the further bioengineering application to transcription and translation in vitro. In our previous study[5], therefore, we investigated the detail of spermidine-induced DNA macro-aggregation in a simple system of λ DNA-TE buffer, and then found out the following multistage process in the macro-aggregation: the formation of anisotropic long fibers, their parallel bundles, and an cholesteric-like assembly with their multilayers as given in Figures 5a, 5b, and 6c, respectively. Unfortunately, the DNA phase diagram and the morphological variation under a nearly biological condition, such as an aqueous solution of Mg^{2+} and ATP essential for DNA replication, transcription, and translation, still have not been measured. In addition, the quantitative phase transition data form micro- to macro-aggregate have been not available as well.

In this study, first, the precipitation of DNA (mature λ DNA) macro-aggregates by spermidine in the presence of Mg^{2+} were measured with UV absorption spectrometry. Second, the phase transition from micro-aggregate to macro-aggregate was determined by use of the hyperchromic effect due to the micro-aggregate size. These measurements built the DNA phase diagram on both micro- and macro-aggregates in the Mg^{2+}-spermidine-DNA system. Besides of Mg^{2+} ion, the influence of a biologically important substance ATP on the macro-aggregation was also examined. With the aim of analyzing the macro-aggregation pathway, the morphology of the aggregate along the precipitation curve was observed by polarizing microscopy. On basis of these results, the effects of the intracellular ions Mg^{2+} and ATP on DNA aggregation were discussed and also in view of biological implication.

2. EXPERIMENTAL PROCEDURE

2.1. Materials

Mature Bacteriophage λ DNA (48.5kbp) dissolved in the TE solution (10mM Tris, 1mM EDTA, pH7.8) was purchased from Takara Shuzo co., ltd. and diluted in the same TE buffer (Sigma, Sigma-Aldrich Co.) to a concentration of the interest. The spermidine solution to be added into DNA solution was prepared by dissolving spermidine trihydrochloride (Nacalai Tesque, Inc.) into the TE buffer.

2.2. Centrifugation Assay for DNA Aggregation

The addition of the spermidine solution to aliquot of DNA solution resulted in DNA precipitation. The vortexed samples were incubated for a hour at room temperature(about 293 K) and centrifuged for 8 min. at 11000×g by use of a Beckmann GS-15R microcentrifuge. The determination of DNA concentration in the supernatant was performed by directly measuring the absorbance of the supernatant at 260nm with a Beckmann DU700 spectrometer. In the case of a solution of ATP, which owns a large molar absorption coefficient, the supernatants were diluted in TE +5mM $MgCl_2$ to a range measurable by optical density. In this work, the ratio of absorbance of the supernatant at 260 nm to the control was treated as the amount of DNA remaining in the supernatant.

2.3. Polarizing Microscopy

DNA macro-aggregate centrifuged with a Beckman GS-15R was deposited between slide and coverslip, which had been cleaned with hydrogen peroxide and then rinsed in Millipore water and ethanol. The coverslip was sealed with DPX (Fulka, Sigma-Aldrich Co.) to prevent dehydration of the sample. Because the aggregate consisted of an ordered phase of DNA molecules, it was observed between crossed polars under a microscope(OLYMPUS BX60).

3. RESULTS AND DISCUSSION

3.1. DNA Aggregate: Micro-aggregate and Macro-aggregate

DNA aggregation is triggered by the addition of spermidine. At the lower DNA concentration than 10 μ g/ml, the resulting DNA micro-aggregate appeared to be invisible. From the early AFM observation [6] and our SEM one (to be submitted elsewhere), the size ranged on the order of submicron. At the DNA concentration above 10 μ g/ml, the DNA aggregate became a visible sediment with a liquid-crystal-like characteristic (Figure 5c). In this paper, we focused on this macro-aggregate.

3.2. Phase Diagram

3.2.1. Mg^{2+} effect on DNA aggregation

DNA precipitations at various DNA concentrations in the presence of 5mM Mg^{2+} are shown in Figure 1, where $C_{thres.}$ and $C_{comp.}$ stand for threshold concentration and completion one for the DNA aggregation, respectively. From other transition data measured at various conditions, the phase diagram of DNA aggregates is illustrated in Figure 2.

As shown in Figures 1 and 2, the addition of Mg^{2+} made not only the spermidine concentration required for the precipitation increase but also the coexistence region extended. Mg^{2+} ions seems to be an inhibitor for the aggregation, since they electrostatically bind negative phosphate groups along double-strand, ion condensation[7,8].

As Mg^{2+} effect on the macro-aggregation, it is interesting that $C_{thres.}$ decrease as increase in DNA concentration, whereas $C_{comp.}$ was independent of DNA concentration. It implies that the possibility that the reaction prior to the midpoint is different from that beyond it. Since the aggregation process can be assumed to be analogous to that in our previous work[5] as described in the 3.3. section, the former and the latter mean fiber formation and fiber assembly, respectively. Therefore, crowding DNA molecules promote the fiber formation. In contrast, Mg^{2+} concentration is the dominant factor for completing the fiber assembly. As a consequence of these effects, the precipitation profiles in the absence of Mg^{2+} were opposite to the present precipitation ones as shown in Figure 1

3.2.2. Phase transition from micro-aggregation to macro-aggregation

In the micro-aggregation, the hyperchromic effect[9] was observed due to the

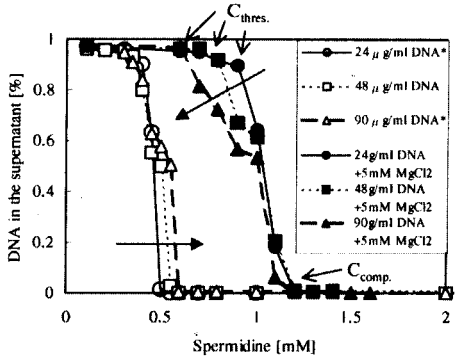

Figure 1. DNA Preciptations by spermindine at various DNA concentrations in the presence of 5mM $MgCl_2$.
Arrows stand for increase of DNA conc. *: our previous work.

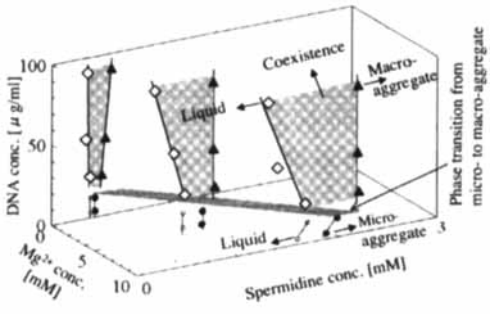

Figure 2. Phase diagram of the spermidine-DNA aggregate in the presence of Mg^{2+}

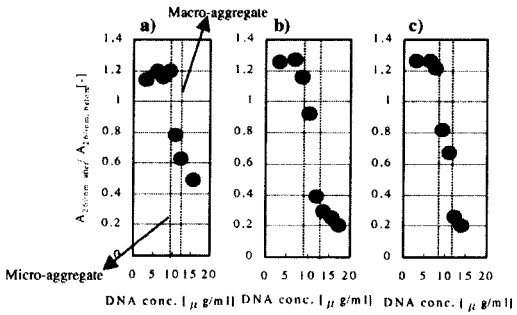

Figure 3. The ratio of the absorbance of DNA solution after and before addition of spermidine as a function of DNA concentration
a:without $MgCl_2$, b:5mM $MgCl_2$, c:10mM $MgCl_2$

light scattering effect. In the macro-aggregation, the absorbance after generating aggregate was depressed by means of its sedimentation. This difference of absorbance between them enables us to determine the transition point.

In Figure 3, the absorbance ratio(A_{after}/A_{before}) of DNA solutions before and after the addition of spermidine able to complete the aggregation was plotted as a function of DNA concentration. Indeed, about 20 % hyperchromic effect was observed until 8-9μg/ml DNA (micro-aggregation region). Above 9μg/ml, the ratio decreased as increase of the DNA concentration. Since it was difficult to determine the transition point precisely, the area(9-12μg/ml) between the dashed lines in Figure 3 was determined as the transition zone. In the phase diagram, the transition zone is drawn as a gray band in Figure 2.

Although Mg^{2+} inhibited spermidine-mediated DNA aggregation, only slight effect of Mg^{2+} on the phase transition was observed. It indicates that the DNA concentration is the predominant factor for the phase transition and also that the formation of millimeter aggregate is attributed to the density of micro-aggregates beyond a critical value. Such a critical concentration of DNA for the phase transition was first quantitatively found out in this work. We suppose that this transition may be demonstrated by the percolation theory.

3.2.3. ATP effect on the macro-aggregation

In this examination, ATP concentration was fixed at 0.5 mM, because the molar absorption coefficient of ATP at 260nm is too large to determine DNA content in the supernatant by UV absorption spectroscopy at higher than 0.5mM ATP. The DNA precipitations in the case of both ATP-spermidine-DNA and ATP-Mg^{2+}-spermidine-DNA systems were plotted in Figure 4. In the former, the spermidine concentration for the aggregation was increased like the case of Mg^{2+}, despite of much smaller amount of ATP than Mg^{2+}. This result suggests that ATP plays a role of stronger inhibitor than Mg^{2+} for DNA aggregation and supports the great affinity between spermidine and ATP in the previous literatures[10,11]. In the latter, the mixing of ATP and Mg^{2+} suppressed the increment of spermidine contents required for DNA aggregation, that is, the increment by the ATP-Mg^{2+} mixture not equal to the sum of one by the individual. The formation of ATP-Mg^{2+} complex[12] is

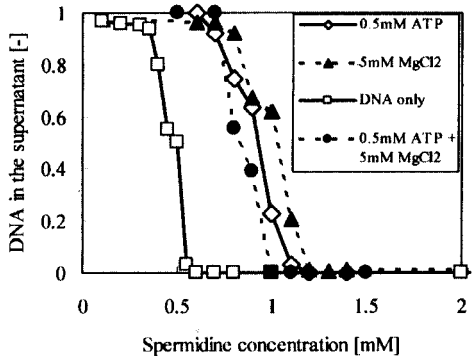

Figure 4. ATP effect on DNA precipitation in the presence and absence of Mg^{2+}

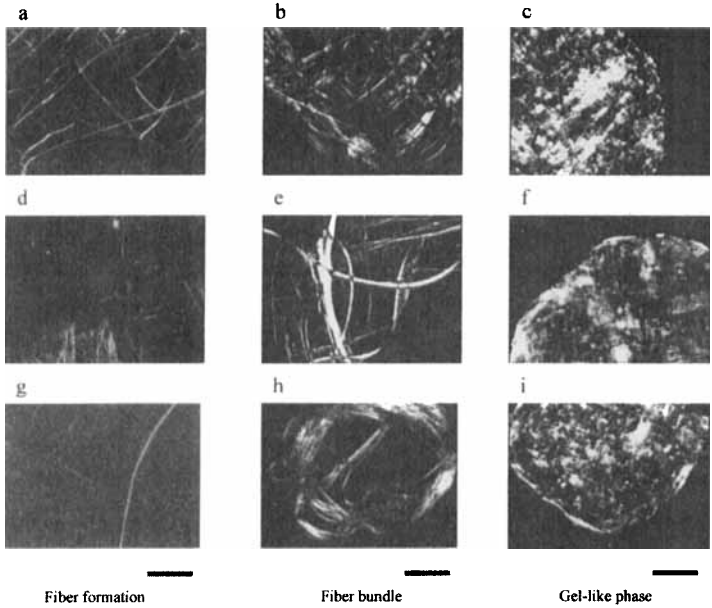

Figure 5. Morphological variations with spermidine concentration
In the absence of Mg^{2+} and ATP: a, b, c; 0.45mM, 0.50mM, 0.55mM, respectively
In the presence of 5mM Mg^{2+}: d, e, f; 0.9mM, 1.0mM, 1.1mM, respectively
In the presence of 0.5mM ATP: g, h, i; 0.8mM, 0.9mM, 1.0mM, respectively
Bar: 200 μ m

expected to result in decrease of free ATP as well as free Mg^{2+} inhibiting DNA aggregation.

3.3. Morphology of Macro-aggregate

Morphology of DNA macro-aggregate at 48μg/ml DNA was examined in the presence of Mg^{2+} and ATP. The aggregates in the coexistence region morphologically varied with spermidine concentration as shown in Figures 5d to 5i. A series of the variations in the presence of Mg^{2+} as well as ATP was described as follows: fiber formation, fiber bundle, and gel-like phase, similarly to Figure 5a to 5c. It is inferred that that Mg^{2+} and ATP does not produce any other aggregation pathway except for the aggregation pathway in the absence of both as shown in Figures 5a to 5c. Furthermore, these polarizing microscope textures show the possibility that the Mg^{2+} and ATP also have little effect on birefringence of DNA fibers. In the other words, there was no markedly difference on liquid-crystal-like phase among these experimental conditions. However, Pelta et al.[2] demonstrated that the ionic species and concentration can change DNA liquid crystal phase mediated by spermidine from their X-ray diffraction analysis. We should try to perform the X-ray diffraction analysis of the obtained aggregate in future.

3.4. Biological Implication

Spermidine concentration can control morphology of the DNA aggregates from a fiber state to a gel-like phase. It is supposed that such morphological variation may be analogous to chromosome deformation to copy DNA sequence at DNA replication or translation. In fact, polyamine contents including spermidine are affected by cell cycle, growth phases, and cell types[13, 14], which result in the induction of morphological variation of chromosome. Also we speculate that the loose fibrous state, which appears to keep an appropriate repulsive force between fibers, is convenient for separation of template DNA and replicated DNA at replication.

Unfortunately, only spermidine is inadequate for the morphology regulation, because the coexistence region, where the morphology dramatically varies, so narrow that a fluctuation of spermidine concentration allows the aggregate morphology to change. The existence of Mg^{2+} and ATP is useful to avoid this problem. They can regulate the threshold and

completion concentrations for the spermidine-induced aggregation as an inhibitor. Moreover, their complexation Mg^{2+}-ATP serves as a suppressor to prevent a morphological change due to increase of both inhibitor concentrations, and consequently enhances robustness of the aggregation. It is noteworthy that this combination of two inhibitors with their complexation in this work generates the regulation using competitive inhibition and the robustness necessary for living organisms.

The final condition, the ATP-Mg^{2+}-spermidine-DNA system, treated in the present work can be approximately considered as a simple transcription in vitro. The transcription in vitro taking account of the morphology of DNA aggregates will make a clear whether the morphological variation becomes a regulation factor.

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