

Generation of Living Cells from Gas-Exposed Sphingosine Modified Synthetic DNA Crown Cells

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Abstract

Crown cell seeds, which consist of sphingosine, DNA, and adenosine, were multiplied within egg white and developed into DNA crown cells. However, when crown cell seeds (G-crown cell seeds) were prepared with gas exposed-sphingosine (G-Sph), they could be cultivated on agar plates without egg. Thus, G-Sph promotes the spontaneous formation of filamentous seeds that undergo order-dependent self-organization, producing envelope like assemblies with a cell-like morphology. The present study examined whether synthetic DNA crown cells prepared with G-Sph could be cultivated on agar plates. The findings showed that adenosine-monolaurin markedly enhances morphological development and promotes compartmentalization, enabling the assemblies to multiply over several generations. Proliferation is not dependent on biological replication; rather, it arises from autocatalytic self-organization with assemblies recruiting sphingosine, nucleic acid-related molecules, and amphiphilic molecules from the surrounding medium. The results demonstrate a non-biological yet a life-like mode of replication following interface-mediated molecular uptake, suggesting the existence of physicochemical routes toward autonomous structure formation in simple molecular systems.

Keywords: DNA crown cells; Sphingosine-DNA; G-crown cell seeds; G-Sph-crown cells; Gas production; Monolaurin; G-Sph

Introduction

Understanding how autonomous cell-like structures can emerge from simple molecular mixtures is central to exploring the physicochemical origins of biological organization. Lipid-based self-assembly, nucleic-acid templating, and amphiphile-driven compartmentalization have each been proposed as potential routes toward pre-biotic cellularity. However, most known systems require carefully controlled conditions and do not spontaneously proliferate or expand across macroscopic space. The author previously reported that sphingosine exposed to reactive gas environments acquires the unique ability for self-organization [1,2]. Gas-exposed sphingosine (G-Sph) forms filamentous seeds that transform into bundled structures and finally into envelope-like assemblies through an order dependent pathway [4,5,8].

When G-Sph-modified synthetic DNA crown cells are introduced into this system, similar envelope-like structures emerge. These structures exhibit life-like behaviors, including division and expansion. These behaviors are enhanced by adenosine-monolaurin through stabilization interfaces, promoting curvature formation and facilitating compartmentalization. Remarkably, the resulting cell-like assemblies proliferate over multiple generations, and eventually cover the entire culture dish. This observation suggested that the assemblies continuously acquire new molecular materials from the surrounding medium, enabling sustained morphological replication without biological

metabolism. Here, the physicochemical mechanisms underlying this proliferation are described, focusing on interface-mediated molecular uptake and autocatalytic self-organization. Further, it is proposed that gas-activated sphingosine, nucleic acids, and amphiphiles collectively form a dynamic molecular network capable of autonomous structure formation.

Materials and Methods

Materials

Escherichia coli DNA (Sigma-Aldrich, USA), adenosine (Sigma-Aldrich; Wako, Japan), and G-Sph prepared by gas-powder treatment [3] were used. Adenosine and monolaurin solutions were prepared to a final concentration of 0.1 M in distilled water. Agar plates were prepared using agar medium (AS ONE, Japan). A-M compounds were prepared by mixing adenosine and monolaurin [7].

Methods

G-Sph was prepared as described previously [3] and stored in at about 4°C until use. A total of 90 µL of G-Sph (approximately 5 mM) and 45 µL of DNA (1.7 µg/µL) were mixed and 50 µL of A-M solution was added. Then, 15 µL of monolaurin was added and the samples were observed immediately and after 15 min at room temperature. Approximately, 100 µL of the mixture was used to inoculate an agar plate which was incubated at 37°C and observed after 22 and 44 h.

General observations

A drop of culture solution was placed on a glass slide and covered with a cover slip observed under a microscope. Objects on the plates could be observed directly with the naked eye and under a light microscope.

Results and Discussion

Figure 1 shows a microscopic image immediately after mixing G-Sph, DNA, A-M and monolaurin.

Figure 2 shows a microscopic image of objects at approximately 15 min after mixing G-Sph, DNA, A-M and monolaurin.

Figure 3 shows colony and pre-colony formation at 22 h on agar medium after mixing G-Sph, DNA, A-M and monolaurin. Synthetic DNA crown cells prepared from G-Sph, DNA (*E. coli*) and A-M were cultured on agar medium. Multiple colony-like structures appeared after 22 h, indicating the ability of the assembly to undergo growth. In addition, dot-like objects appeared before colony formation.

Figure 4 shows a photograph of the cultures shown in Fig. 3 after 44 h. Colonies possibly derived from dot-like objects appeared.

Figure 5 shows colony formation by objects (2,3,4 and 5 in Figure 4) Some of these objects were cultured on agar medium. Multiple microorganism-like objects appeared after 1 day of incubation, indicating that the objects grew.

Figure 6 shows colony formation on agar medium by objects (1 in Fig.4). Some of these objects were cultured on agar. Multiple colony-like objects appeared after incubation for 18 h, indicating their ability to grow.

Figure 7 shows the microscopic appearance of objects (cannot be observed with the naked eye) within the top frame (Fig.3) and prior to living cell (Fig.4, 3) emergence.

Figure 8 shows the microscopic appearance of dot-like assemblies within the top frame (Fig.3) prior to living cell (Fig.4, 4) emergence.

Figure 9 shows the emergence of living-cell-like structures from assemblies. The microscopic observations of structures transitioning into living cell-like forms showed defined boundaries and increased internal contrast relative to the original assemblies.

Figure 10 shows the growth characteristics of living-like structures on agar medium.

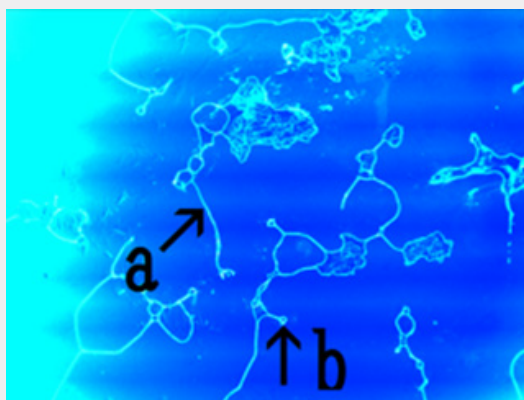


Figure 1: Microscopic image immediately mixing G-Sph DNA, A-M and monolaurin. Long strand-like are observed (Fig.1a). Approximate size of the structure in Fig.1b is 30 μm ..

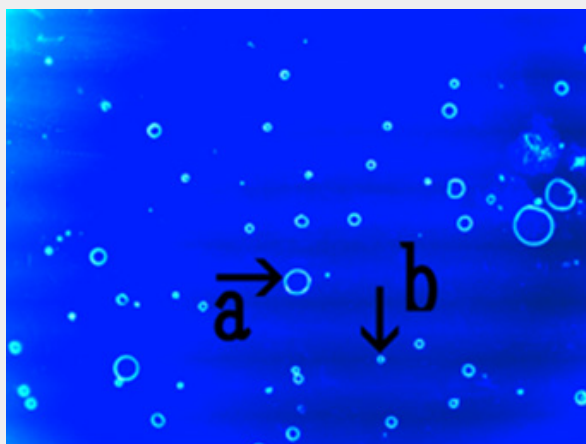


Figure 2: Microscopic image of objects after 15 min at room temperature after mixing G-Sph, DNA, A-M and monolaurin. Round objects of various sizes are observed (Fig. 2b). The approximate size of the structure in Fig.1b is 30 μm ...



Figure 3: Photograph taken at 22 h of culture after mixing Gas-Sph, DNA, A-M and monolaurin on agar. Four large colonies and dots are observed within the frame.

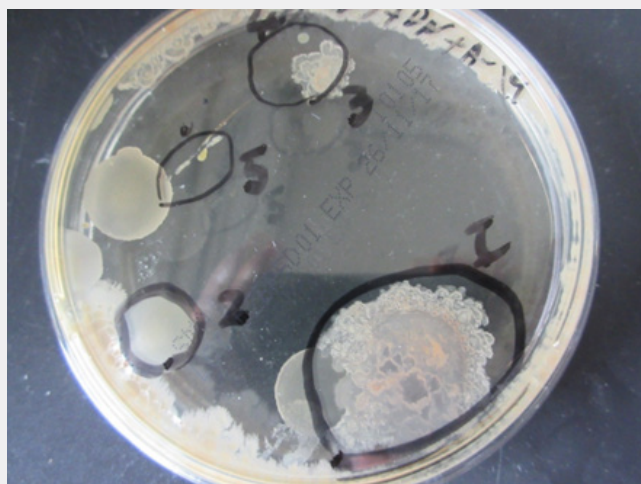


Figure 4: Photograph taken at 44 h of culture after mixing G-Sph, DNA, A-M, and monolaurin on agar. Objects (1, 2,3, 4 and 5) are observed.



Figure 5: Photograph in culture of objects 2, 3, 4 and 5 shown in Fig. 4. Objects similar to microorganisms are observed..



Figure 6: Photograph in culture of object 1 shown in Fig. 4. Objects similar to microorganisms are observed..

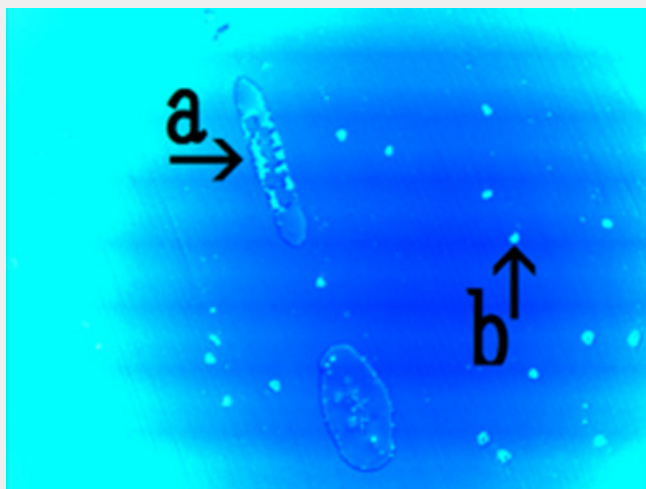


Figure 7: Micrograph of objects within the top frame (cannot observe with the naked eye) at 22 h of culture (Fig. 3) and before objects (Fig. 4.3) emergence. Elongated objects with small round objects are observed (Fig. 7a). The approximate size of the structure 5 μm -8 μm .

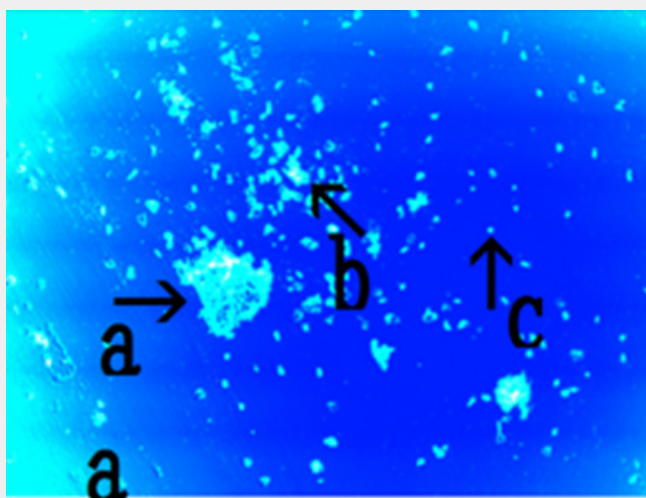


Figure 8: Micrograph of dot-like objects within the top frame at 22 h of culture (Fig. 3) and before objects (Fig. 4, 4) emergence. Large assemblies are observed (Fig. 8a). Also, numerous small objects are observed (Fig. 8b). The approximate size of the structure shown in Fig. 8c is 2-3 μm .

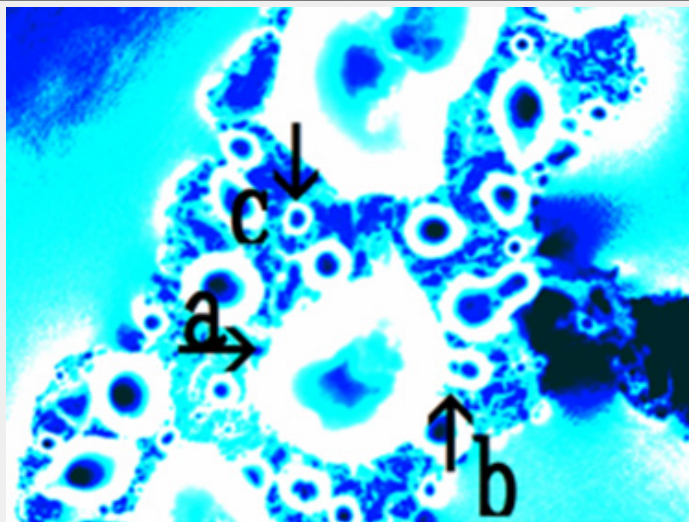


Figure 9: Microscopic appearance (No.1) of objects (1) shown in Fig. 6. Large objects are observed (Fig. 9 a), and round objects derived from objects (Fig. 9a) are observed (Fig. 9b). The approximate size of the structure shown in Fig. 9c is 70 μm .

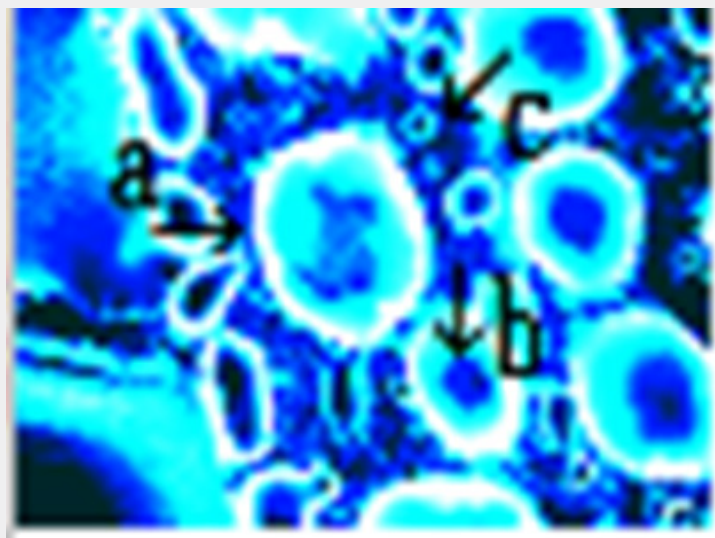


Figure 10: Microscopic appearance (No.2) of the objects (1) shown in Fig. 6. Objects of various sizes are observed (Fig. 10a). The approximate size of Fig.10c) is 50 μm .

Living cell-like entities derived from G-Sph, DNA, adenosine-monolaurin exhibited limited but detectable proliferation on agar medium. The image clearly shows both early and later stages of growth. The present study therefore demonstrates that living cell-like assemblies (Fig. 4, colony) can be generated from synthetic DNA crown cells (assemblies), as shown in Fig. 3, 7, and 8). These synthetic DNA crown cells were formed with G-Sph, DNA, and adenosine-monolaurin. Cell-like assemblies were observed to proliferate; however, this proliferation is not biological and physicochemical; rather, the assemblies act as molecular attractors that recruit amphiphiles, nucleic acid fragments, and other organic molecules from the culture medium. The medium therefore functions as a large molecular reservoir, supplying the materials required for continued growth.

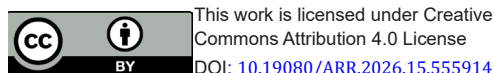
A key mechanism in this process appears to be interface-mediated molecular uptake. The envelope-like structures formed by G-Sph, nucleic acids, and adenosine-monolaurin (Figs. 1 and 2) possess mixed hydrophobic, π -stacking, and hydrogen-bonding domains that strongly bind molecules in the medium. This adsorption increases local concentrations by orders of magnitude, enabling reorganization of the adsorbed molecules into new envelope structures. This process constitutes an autocatalytic loop, with assemblies generating new assemblies by reorganizing molecular driven from environmental.

Thus, the G-Sph substituted synthetic DNA crown cells are capable of growing on agar medium. Although numerous assemblies were generated, only a very small fraction developed into living cells. Assemblies that possess envelopes are likely

capable of growth, but this relationship has not yet been clearly established. Adenosine-monolaurin plays a central role in stabilizing curvature, enhancing compartmentalization, and reducing structural noise. This process allows the assemblies to maintain consistent morphology across generations, producing well-defined, replicating, cell-like structures (Figure 9 & 10). The resulting system thus exhibits morphological replication, a new-biological analogue of culture proliferation. These findings suggest that autonomous, proliferating structures can arise from simple molecular mixtures in the presence of reactive lipids, nucleic acids, and amphiphiles under gas-exposed conditions. The system provides a physicochemical model for pre-biotic cellularity, demonstrating how life-like organization may emerge without genetic replication or metabolism. Further investigations into molecular uptake dynamics and the role of gas-induced reactivity in the spontaneous development of autonomous structures in molecular systems need to be conducted in the future. Such studies could clarify the formation of self-replicating artificial cells.

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