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Order-Dependent Formation of Self-Organizing Microscopic Objects from Gas-Exposed Sphingosine, DNA and Adenosine

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ABSTRACT

Although microscopic self-organization of simple molecular components can generate structures that exhibit the fundamental characteristics of autonomous behavior, the physicochemical origins of such autonomy remain largely unexplored. It was previously reported that gasses, including dimethyl-sulfide, were produced in a co-culture system of antibiotic crown cells and living cells in the presence of gas-exposed sphingosine-DNA-Adenosine. In this study, using gas-exposed sphingosine, the order-dependent assembly of gas-exposed sphingosine, E. coli DNA, and adenosine were used to clarify how autonomy may develop in non-living chemical systems. Among several approaches examined, adding gas-exposed sphingosine, DNA, and then adenosine produced the most distinct and reproducible behavior. These findings revealed an order-dependent, self-organizing pathway of interactions without genetic replication or metabolism. The method provides a promising model for the emergence of autonomous behavior. Gas-exposed sphingosine was referred to as G-Sph, and crown cell seeds prepared with G-Sph were named G-crown cell seeds. The resulting cells were named G-Sph- coli crown cells, reflecting the DNA used in the preparation of crown cell seeds.

Keywords

DNA crown cells, Sphingosine-DNA, G-crown cell seeds, G-Sph-crown cells, Gas production.

Introduction

Biomolecular self-organization is a fundamental process through which simple chemical components spontaneously form higher-level organic structures. Understanding how such systems acquire dynamic autonomous behaviors that are reminiscent of living cells is an important step towards clarifying the fundamental principles of primordial biochemical organization.

A previous study demonstrated that gasses, including dimethyl disulfide (DMDS) was produced in a culture system containing antibiotic-producing crown cells [1] and living cells were observed to emerge from non-living cells following exposure to this gas [2]. Moreover, living cells developed from gas crown cells seeds prepared with gas-exposed sphingosine (G-Sph) [3].

To clarify how such autonomous objects arise from non-living material, methods using a culture system comprised of Gas crown cells seeds [3] may be suitable, as the system consists of only G-Sph, DNA, and adenosine. Lipid molecules such as sphingosine play central roles in membrane architecture and can undergo marked changes when exposed to external physical or chemical stimuli. Nucleic acids, including DNA, interact with lipids under certain conditions, producing aggregates exhibiting early features of structural organization. Nucleotides, such as adenosine, further, modulate these interactions and may participate in assembly processes that extend beyond simple binding.

This study investigated the spontaneous formation of microscopic objects generated by combining gas exposed sphingosine, *E. coli* DNA, and adenosine. The author observed that the order in which these molecules were mixed played an important role in the nature of the structures that emerged.

When gas-exposed sphingosine and DNA were mixed, numerous small spherical particles, or assemblies, appeared immediately. Subsequent addition of adenosine reorganized these particles, or assemblies, into large envelope-shaped objects, and these enclosed particles exhibited dynamic, autonomous behaviors reminiscent of living cells. Conversely, mixing sphingosine with adenosine first did not produce such spherical particles. These findings revealed the existence of an order-based, self-organizing phenomenon within this lipid-nucleic acid-nucleotide system, providing insights into how autonomous behaviors may have evolved from simple molecular interactions.

Materials and Methods

Materials

Escherichia coli DNA (Sigma-Aldrich, USA), adenosine (Sigma-Aldrich; Wako, Japan), and gas-exposed sphingosine, were prepared by gas-powder treatment [3]. An adenosine solution was prepared to a final concentration of 0.1 M in distilled water, and agar plates (SMA; AS ONE, Japan) were used for culture.

Methods

Gas-exposed sphingosine was prepared from cultures of bovine kaiware seeds ex p, as described previously [3] and stored in freezer at approximately 4°C until use.

Mixing of G-Spa, DNA, and adenosine

1. A total of 90 µL of G-Sph (approximately 5 mM) and 45 µL of DNA (1.7 µg/µL) were combined and observed immediately.
2. Adenosine solution (50 µL) was added to 90 µL of G-Sph and observed immediately.
3. A total of 180 µL of G-Sph was added to 45 µL of DNA and 50 µL of adenosine and mixed. The mixture was observed immediately.

Cultures of G-Sph, DNA-adenosine mixtures (production of G-Sph-coli Crown Cells). Approximately 100 µL of the suspension was poured onto an agar plate and incubated for approximately 18 h at 37°C.

General observations

A drop of the resulting mixtures was placed on a glass slide and covered with a coverslip for microscopic examination.

Objects on the plates could be observed directly with the naked eye and under the microscope.

Results and Discussion

Figure 1 shows a microscopic image immediately after mixing G-Sph and DNA.

Figure 2 shows a microscopic image immediately after mixing G-Sph and adenosine.

Figure 3 shows the microscopic appearance (No. 1) of objects that formed immediately after mixing G-Sph, DNA, and adenosine.

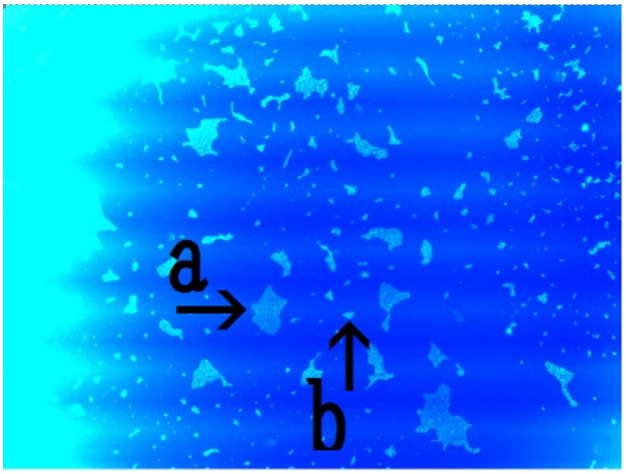

Figure 1: Microscopic image immediately after mixing G-Sph and DNA. An object enclosing an assembly within the frame in Figure 1b is shown (Figure 1a) and spherical objects are shown with the frame (Figure 1c). Approximate size of Figure 1d is 45 µm.

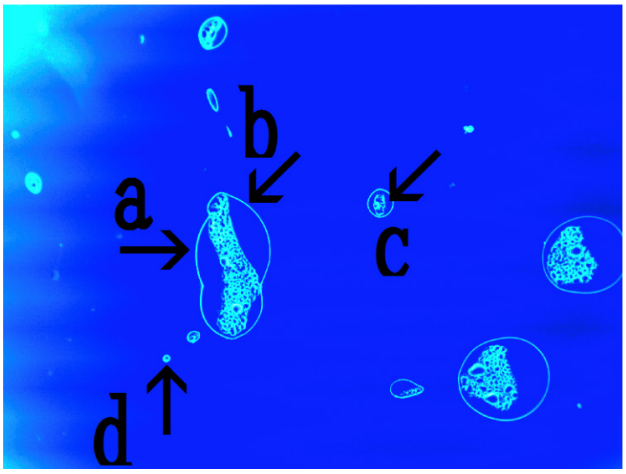

Figure 2: Microscopic image immediately after mixing G-Sph and adenosine. Crystal-like objects are shown (Figure 2a) and an assembly is shown (Figure 2b). Approximate size of Figure 2c is 10 µm.

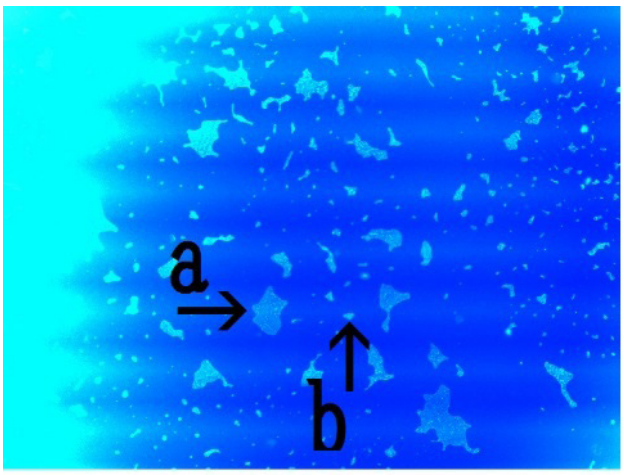

Figure 3: Microscopic image (No. 1) of objects immediately after

mixing G-Sph, DNA, and adenosine. Objects of various sizes are shown. Approximate size of Figure 3b is 50 m.

Figure 4 shows the microscopic appearance (No. 2) of objects immediately after mixing G-Sph, DNA, and adenosine.

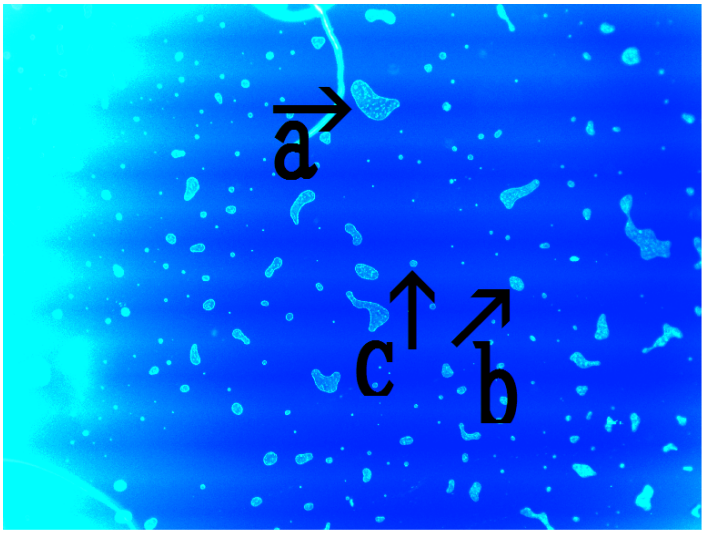

Figure 4: Microscopic appearance (No. 2) of objects immediately after mixing G-Sph, DNA, and adenosine. Objects enclosing several spherical objects are shown (Figure 4a and 4b). The object may have been formed with the envelope-like objects. Approximate size of Figure 4 is 30 µm.

Figure 5 shows culture plate of the G-Sph, DNA, and adenosine mixture. Colony-like objects were observed, suggesting that living emerged cells from the culture of G-Sph, DNA, and adenosine.

Figure 5: Culture plate of the mixture immediately after mixing G-Sph, DNA, and adenosine. Colony-like objects were observed, suggesting that the living cells emerged after mixing G-Sph, DNA, and adenosine.

Figure 6 shows a microscopic image of the colony shown in Figure 5. Numerous G-Sph-coli Crown Cells were observed.

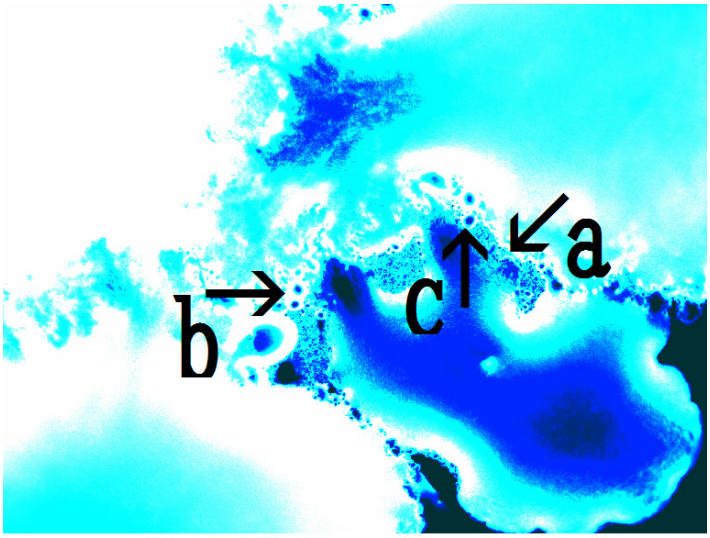

Figure 6: Microscopic image of the colony shown in Figure 5. Numerous small, round objects are shown Figure 6a, as well as objects with a rounded structure in the center (Figure 6b). Approximate size is 60 µm (Figure 6c).

The findings of the present study revealed the existence of an order-dependent pathway through which gas-exposed sphingosine, DNA, and adenosine, spontaneously assembled into structures that exhibit the core characteristics of autonomous behavior. This process provides insight into how autonomy may arise from non-living chemical systems. A key finding is that adding sphingosine and DNA first results in them combining to form thin filamentous complexes that subsequently thicken upon adenosine addition and ultimately reorganize into cell-like, envelope-shaped structures [4-6]. These cell-like envelopes formed under specific conditions may correspond to the "nest" described in a previous study [7].

This filament-to-bundle-to-cell transition provides new conceptual framework for understanding how autonomy may emerge from simple molecular systems.

Gas-exposure plays a central role in this process. When sphingosine, DNA, and adenosine are directly exposed to gas, living-cell-like objects form immediately [2]. Likewise, using gas-exposed sphingosine is also sufficient for reproducing the same autonomous structures [3]. These observations indicate that gas acts primarily on sphingosine, partially cleaving the S-S bond in DMDS to generate reactive sulfur species that interact with sphingosine's amino group and hydrophilic chain. These changes enable sphingosine to bind to DNA in a manner not observed in untreated conditions. The finding that DNA also shows altered behavior suggests that gas may influence both components, promoting filament formation.

As shown in Figure 1, these interactions generate small spherical seeds, or assemblies of objects, composed of G-Sph-DMDS-DNA complexes.

Mixing sphingosine with adenosine first did not result in the

production of these spherical seeds and instead produced crystal-like or assembly-like objects (Figure 2).

The formation of these seeds does not require enzymatic activity or biological replication; rather, it emerges spontaneously from a physicochemical association among the components.

Upon addition of adenosine, the seed objects undergo striking reorganization. Adenosine mediates bridging interactions among the seeds promoting aggregation into large assemblies (Figures 3 and 4). These assemblies transform into envelope-like structures that resemble primitive cellular compartments that exhibit dynamic behavior, including directional movement, deformation, and responsiveness to external stimuli. When the fluids contained in these envelope-like objects were cultured, colony formation and cell proliferation were observed (Figures 5 and 6), suggesting that living cells emerged from these envelope-like objects that were created with G-Sph, DNA and adenosine. These envelope-like objects may therefore represent the origin of life. Interestingly, the process by which these envelop-like objects developed occurred without heating or cooling of DNA, suggesting that the system possesses an intrinsic capacity for self-directed organization and environmental interactions. Importantly, increases in the number and size of these envelope-like objects does not indicate biological replication of DNA, as no DNA polymerase, ribosomes, or metabolic activity is present in this systems. The observed growth arises solely from reorganization and redistribution of existing molecular components. The structures observed here indicate that the autonomous activity is physicochemical rather than biological, yet it captures essential features of living systems such as boundary formation, internal polarization, and adaptive motion.

Together, these findings support a model in which gas-exposed lipids, nucleic acids, and nucleotides can engage in a sequence of actions that result in hierarchical self-organization and emergent autonomy. This seed-to-envelope-like transition followed by autonomous behavior provides a conceptual framework for understanding how life-like properties may originate from simple chemical mixtures without genetic replication and metabolic activity.

Gas-exposed sphingosine thus represents a promising platform for exploring the earliest steps in the emergence of autonomous organization.

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