Synthetic minimal cell with artificial metabolic pathways

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Abstract

A "synthetic minimal cell" is considered in our work as a cell-like artificial vesicle reproduction system in which an information polymer regulates a chemical and physico-chemical transformation network. In this study, we demonstrate such a minimal cell consisting of three artificial metabolic pathways: energy production unit, information polymer synthesis unit, and vesicle membrane growth unit. Ingredients supplied to vesicles are chemically converted to energy currency molecules that trigger the synthesis of an information polymer. The vesicle membrane plays the role of "template" in synthesizing the information polymer, and the obtained information polymer promotes vesicle membrane growth. By coordinating the vesicle membrane in terms of composition and permeability to osmolytes, the growing vesicles show recursive reproduction over several generations. Our synthetic minimal cell greatly simplifies the scheme of contemporary living systems while keeping their essence. Therefore, the minimal cell's chemical and reproduction pathways are well described by kinetic equations and by applying the membrane elasticity model, respectively. This study provides new insights to understand better the differences and similarities between non-living forms and living forms of matter.

Introduction

Life is the system that reproduces itself, maintained by very complex chemical reaction networks. All living systems we know mainly consist of sophisticated molecules such as biopolymers (DNA, RNA, and proteins) and membrane compartments. These molecules are called “soft matter” in physical science. To elucidate the physical basis for the origin of complex living systems from non-living molecular assemblies, one of the promising approaches is to reconstruct simple autonomous reproduction systems based on chemistry and soft matter physics. To attain such cell-like experimental systems, researchers have focused on the two fundamental components; “vesicle” which is a model cell membrane, and “information polymer” which encodes the property of the vesicle in its structure and encourages reproduction of the vesicles (Szostak, et al. 2001; Imai, et al. 2022).

A traditional approach for the cell-like reproduction system is to reconstruct the metabolic pathways that synthesize lipids with the help of proteins expressed by DNA inside a vesicular compartment so that vesicle reproduction occurs. However, since this approach utilizes the central dogma of molecular biology, we still have a large gap between non-living forms of matter and living systems. An alternative soft matter approach is to synthesize the simplest cell-like systems, also called minimal cells, whereby vesicles reproduce themselves based on instructions encoded in information molecules using non-biological substances.

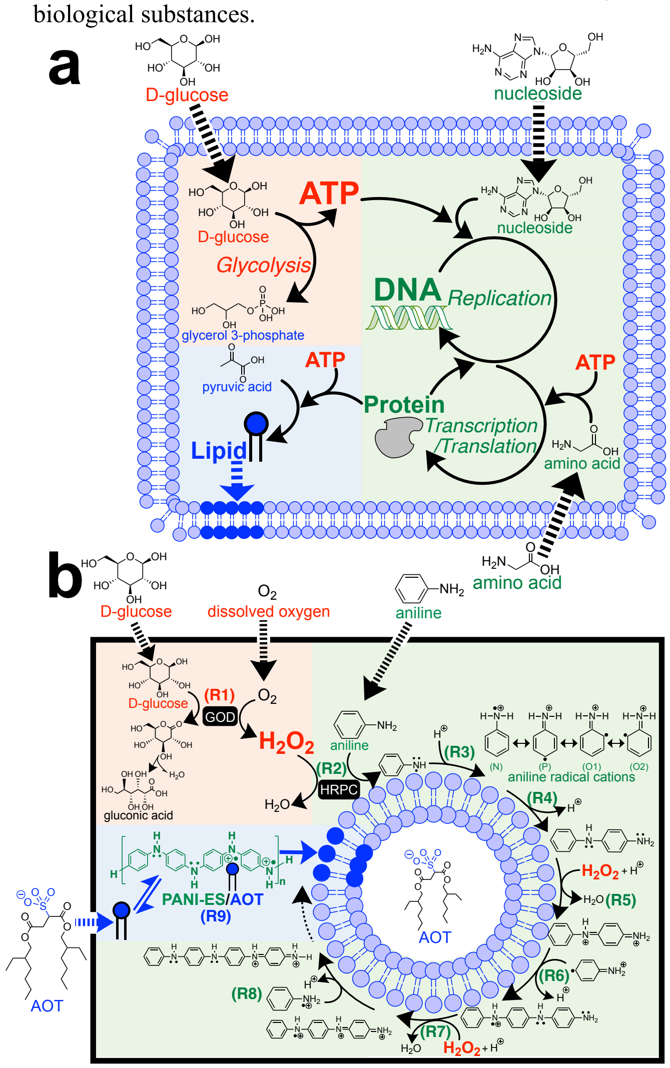

Figure 1: Scheme of reaction pathways highlighting three essential subunits by colors; (a) contemporary cells, (b) our synthetic minimal cell. Bold dashed arrows indicate the supply of ingredients. (Taken from Kurisu, M., et al. *Commun. Chem.* 6, 56 (2023); open access article)

Artificial metabolic pathways

One promising approach to such cell-like reproduction systems is to design artificial reaction pathways that combine a vesicle and an information polymer while keeping the concept of three metabolic subunits in contemporary cells as shown in Figure 1a; (i) the energy production unit (orange) which synthesizes energy currencies (ATP) from ingredients, (ii) the processing of information polymer unit (green) which synthesizes and utilizes specific polymers, e.g., DNA, for the reproduction, and (iii) the membrane growth unit (blue) which grows and divides the membrane compartment, resulting in the proliferation (Gánti, 2003; Xu, et al. 2011).

Recently, we developed artificial reaction pathways with such three simplified subunits and demonstrated a "synthetic minimal cell" system, which showed recursive production of vesicles instructed by the artificial information polymer (Kurisu, et al. 2023). Figure 1b summarizes the reaction pathways: energy production (R1, orange), synthesis of the information polymer (R2–R8, green), and membrane growth (R9, blue) units. Here the ingredients (D-glucose and O_2) supplied to the vesicle are converted to hydrogen peroxide (H_2O_2), which is an "energy currency" molecule to chemically drive the minimal cells. Then, using in-situ formed H_2O_2 and supplied aniline, a specific form of polyaniline (polyaniline emeraldine salt form, "PANI-ES") is synthesized on the surface of particular vesicles composed of sodium bis-(2-ethylhexyl) sulfosuccinate ("AOT"). The specificity feature of this synthetic minimal cell is the "template" polymerization mechanism between AOT vesicles and PANI-ES through specific hydrogen bonding (Junker, et al. 2012; Kurisu, et al. 2019), where the hydrophilic head structures of vesicle-forming molecules determine whether the surface-confined polymerization of aniline will result in the PANI-ES formation or not. Furthermore, in contrast to the biological information polymers (DNA) that express proteins for synthesizing membrane molecules, the artificial information polymer PANI-ES located on the vesicle surface encourages the growth of the vesicles by incorporating membrane molecules (AOT) from the environment into the vesicles. Again the strong interaction between AOT molecules and PANI-ES is important; the binding of a hydrophilic head of AOT with the vesicle surface-bound PANI-ES decreases molecular hydrophilicity of AOT, which promotes their translocation from aqueous media into hydrophobic vesicle bilayer. Thus, under the supply of ingredients and membrane molecules, the synthetic minimal cells showed vesicle membrane growth assisted by the in-situ production of energy currency molecules and by the information polymer synthesis.

Reproduction of Synthetic Minimal Cells

In addition to the vesicle membrane growth driven by artificial metabolic pathways, the synthetic minimal cell showed vesicle division, i.e., reproduction, by introducing cholesterol (Chol) to the AOT vesicle membrane (Figure 2). This is due to the coupling between Gaussian curvature and the local lipid composition of the membrane (Kurisu, et al. 2019; Jimbo, et al. 2016; Urakami, et al. 2021; Sakuma and Imai, 2011; Chen, et al. 1997). The membrane elasticity theory of vesicles predicts that inverse-cone-shaped lipids having small hydrophilic heads and bulky hydrophobic tails (e.g., Chol) prefer to stay in the inner layer of the vesicle membrane, which induces the deformation to the "limiting shape," i.e., two spherical vesicles connected by a narrow neck. In addition, the coupling between the lipid shapes and the membrane curvature destabilizes the neck structure, which induces the spontaneous division of the vesicle. Therefore, a binary AOT + Chol (9/1) vesicle autonomously achieved deformation and division during the membrane growth. By regulating the supply of ingredients and the osmotic pressure, consequently, the synthetic minimal cells realized a recursive reproduction cycle consisting of the following steps: vesicle membrane growth → vesicle deformation → vesicle division → vesicle inflation (Kurisu, et al. 2023). We confirmed that 22 vesicles out of 50 reached the 3rd generation, and 14 reached the 4th generation (Figure 2).

The advantage of synthetic minimal cell studies is that the physical background of the elementary processes is concise and clear compared to complex biological systems. Based on the chemical schemes in Figure 1b, we constructed a kinetic model of the artificial metabolic pathways, which quantitatively describes the observed polymer synthesis and vesicle growth. By applying the membrane elasticity model, the deformation pathway of synthetic cells in Figure 2 was also described. Understanding such artificial reproduction mechanisms might be helpful for developing Artificial Life and possibly for discovering the reproduction mechanism of primitive, i.e., very early cells on the earth. Synthesizing minimal cells is a promising approach to bridging the gap between non-living and living forms of matter.

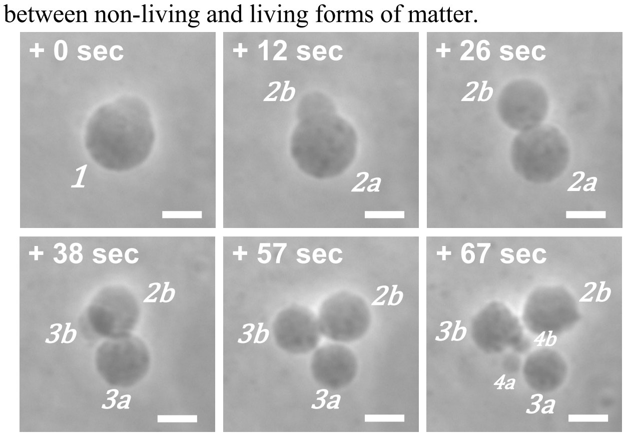

Figure 2: Phase contrast light microscopy images of the synthetic minimal cells. Binary AOT+Chol (9/1) vesicles with the artificial metabolic pathways (Fig. 1b) showed recursive vesicle growth and division. Length of scale bars: 10 µm. (Taken from Kurisu, M., et al. *Commun. Chem.* 6, 56 (2023); open access article)

Methods. AOT+Chol (9/1, mol) vesicles encapsulating 20 mM NaH_2PO_4 and 100 mM D-sucrose (osmolytes) located in 20 mM NaH_2PO_4 solution (pH=4.3) containing 4.0 mM aniline, 0.92 µM horseradish peroxidase (HRPC), 1.0 µM glucose oxidase (GOD), dissolved oxygen, and 100 mM D-fructose (osmolytes). 100 mM D-glucose solutions containing micelles (20 mM AOT and 100 mM SDBS+0.5 mM Chol) were micro-injected toward a target vesicle. Then, D-glucose triggers the reaction network, and AOT and Chol (together with SDBS) are incorporated into the vesicle.

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