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Photoinitiated Synthesis of Self-Assembled Vesicles

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Supporting Information

ABSTRACT: The aqueous photochemistry of 2-oxooctanoic acid (a single-tailed surfactant) results in the synthesis of a double-tailed surfactant product followed by spontaneous self-assembly into vesicles. The photochemical mechanism is detailed here, and the reaction products are identified using mass spectrometry. Then, the self-assembled vesicles are characterized using dynamic light scattering, fluorescence microscopy, and NMR. Further, their stability over time and in the presence of $MgCl_2$ salt is demonstrated. This work contributes to membrane evolution through the provision of a prebiotic route for the synthesis of plausible membrane components and subsequent self-assembly of a primitive enclosure.

The unique properties of aqueous solutions of aggregated amphiphiles have been a subject of considerable interest in a wide range of fields.[1−4] In addition, enclosures are universally considered necessary for life.[5] The search for the emergence of such primitive cellular constructs in the origin of life on Earth requires the existence and/or synthesis of a prebiotically plausible membrane component (a surfactant), followed by self-assembly into a stable enclosed structure.[6] Although much work has been performed studying the properties of vesicles composed of various surfactants,[7−10] the synthesis of such self-assembled structures and their components under plausible prebiotic conditions has received much less attention. Here we show the photochemical synthesis of a double-tailed surfactant from a single-tailed one followed by spontaneous self-assembly into stable vesicles. The single-tailed surfactant utilized, 2-oxooctanoic acid (2-OOA, an 8-carbon oxo-acid), is a plausible prebiotic molecule: oxo-acids and other molecules of similar chemical functionality have been found in meteoritic samples,[11,12] and the short, 8-carbon chain of 2-OOA is among the most prevalent length synthesized in the common Fischer−Tropsch type synthesis of lipids (C7−C9 are most common).[13,14] With no further perturbation of the reaction system, as the photochemistry proceeds, the products spontaneously self-assemble into stable vesicles. These vesicles were found to be monodisperse (200 nm in diameter), to be temporally (over several months) and thermally (between at least 4 - 22 °C) stable, and to persist in the presence of $MgCl_2$ salt. This work provides a potential photochemical route for the synthesis of membrane components and a stable primitive enclosure under prebiotically relevant conditions and contributes to understanding the evolution of primitive membrane structures.

Figure 1 illustrates the photochemical mechanism resulting in the production of the dimer molecule OOA-OOA (Figure 1A)

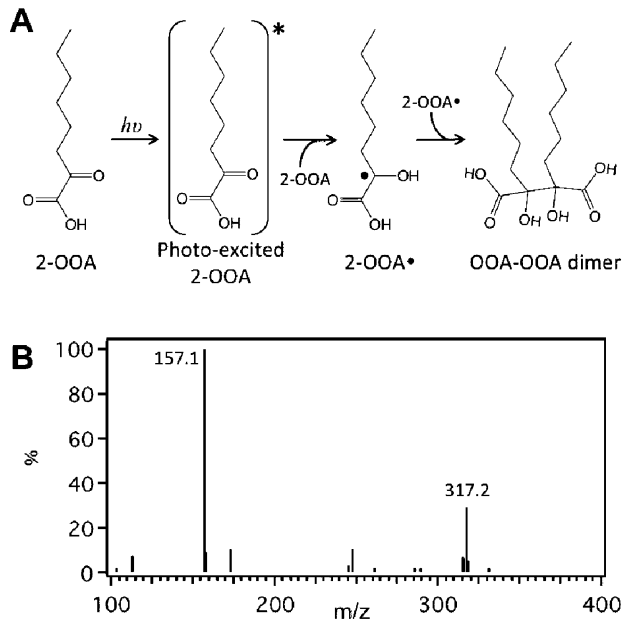

Figure 1. Photochemical synthesis of double-tailed surfactant (OOA-OOA) from single-tailed surfactant (2-OOA). (A) Photochemical reaction mechanism of 2-OOA to produce OOA-OOA dimer. (B) Mass spectrum of photolysis products with peaks due to OOA-OOA dimer (317.2 m/z) and unreacted 2-OOA (157.1 m/z) indicated.

as well as its detection by mass spectrometry (Figure 1B). 2-OOA first absorbs light through its carbonyl chromophore with an absorption maximum of 320 nm (Figure S1), resulting in the production of the dimer molecule OOA-OOA (m/z 317.2 in Figure 1B). Analogous to the well-known photochemistry of pyruvic acid[15−17] (which has the same reactive functionality as 2-OOA), the photoexcited 2-OOA molecule can react with a ground-state 2-OOA molecule to efficiently form the radical intermediate 2-OOA·. Then, two 2-OOA· radicals recombine to form the OOA-OOA dimer. The products of photolysis, when

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extracted into chloroform, were observed to be more surface active than 2-OOA prior to photolysis using Langmuir trough methods (Figure S2), confirming the greater hydrophobicity of the photolysis products. In addition to the double-tailed dimer OOA-OOA, another dimer product was detected at 247.1 m/z in the mass spectrum (see Supporting Information for full characterization and reaction mechanism, Figures S3 and S4). This product is formed through a Norrish Type II photochemical reaction mechanism, but remains as a single-tailed surfactant with similar hydrophobicity to 2-OOA, and is thus not discussed further in detail here.

Prior to exposure to light, the 2-OOA solution is clear and devoid of detectable particles (image on left in Figure 2A and

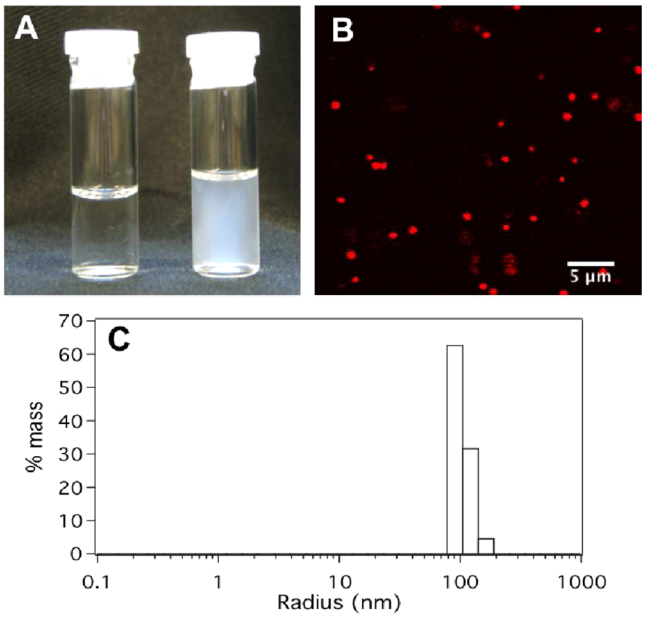

Figure 2. Characterization of self-assembled vesicles. (A) Photograph of clear 2-OOA solution before photolysis (left) and opalescent solution after photolysis (right). (B) Fluorescence microscope image of vesicles stained with Rhodamine 6G. (C) Dynamic light scattering (DLS) of vesicles formed illustrating mean radius of 100 nm.

fluorescence microscope image in Figure S5A). Although the critical bilayer concentration (CBC) of 2-OOA is not known, similar surfactants have CBCs greater than 100 mM,[18] and thus vesicles are not expected, and indeed were not observed (Figure S5), at the low concentration of 6 mM used in this work. Over the course of photolysis, however, the solution becomes opalescent (image on right in Figure 2A) indicating the presence of particulates in solution. These particles were observed microscopically using both phase contrast microscopy (Figure S6) and fluorescence microscopy after staining with the membrane stain Rhodamine 6G (Figure 2B), appearing to be spherical. Further, they were determined to be monodisperse with a narrow size distribution centered around 200 nm in diameter using Dynamic Light Scattering (DLS, Figure 2C). These particles were stable in solution for several months, over the temperature range 4−22 °C and in the presence of $MgCl_2$ salt (Figure S7). At 200 nm in diameter, they are too large to be micelles, and their long-term stability in solution as well as their apparent spherical shape suggests that disordered aggregates of monomers are unlikely.

In addition, the diffusion ordered spectroscopy (DOSY) NMR (Figure 3) spectrum detects these particulates as being

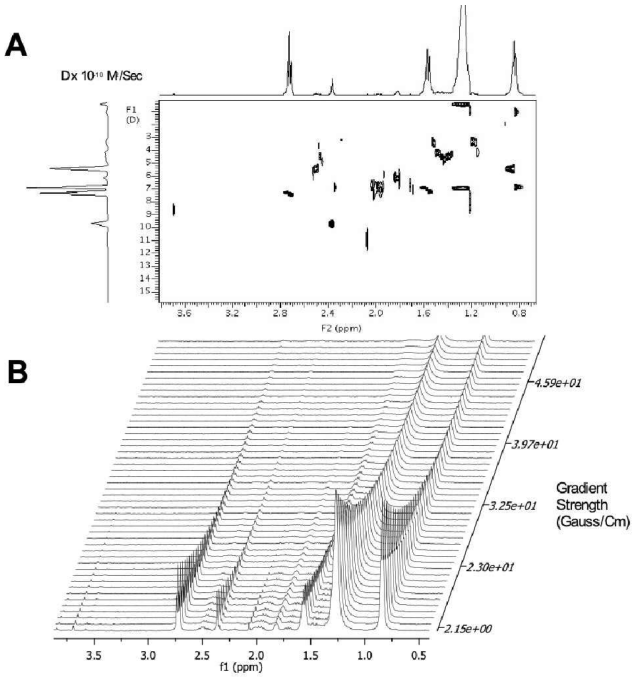

Figure 3. DOSY NMR illustrating the diffusion coefficients observed in the mixture of photolysis products, including the superposition of decay profiles for rapidly and slowly diffusion components, in the 0.5−1.5 ppm range of the NMR spectrum. (A) Full DOSY plot. (B) Decay of NMR signal with increase in gradient strength.

much larger than any monomers in solution and to be very slowly diffusing (Figure 3A), yielding a diffusion coefficient of 4×10^{-8} cm^2/s, consistent with the value of 3×10^{-8} cm^2/s determined by DLS. This is in contrast to the measured diffusion coefficient of the aqueous free monomer (2-OOA), measured by DOSY NMR to be 7×10^{-6} cm^2/s. When looking at the decay of the NMR signal with gradient strength (Figure 3B), it is also apparent that the signals due to the hydrocarbon chain protons specifically near the ends of the tails exhibit a two-component decay profile: one short decay with sharp signals sitting atop broadened signals with a very long decay. This is indicative of two types of environments present in solution, one of monomers and one of similar molecules with confined motion. Additional information about the confined molecules can be obtained by noting the absence in signal from the protons closest to the polar head groups at large gradient strength. This is common in lipid vesicles,[19] where the NMR signals from the protons on the rigid head groups are broadened due to dipolar relaxation, while the signals from the more flexible tails exhibit less dipolar broadening and are thusly easier to detect. Therefore, taken together with the size, monodispersity, and stability, these data indicate that the particulates detected in solution are ordered structures consistent with self-assembled vesicles.

This work illustrates the advantageous environment provided by the water surface region. 2-OOA, as a soluble surfactant, partitions significantly to the water surface (Figure S8), resulting in an inhomogeneous distribution of 2-OOA molecules in solution. The surface excess concentration of 2-OOA allows for a local concentration that is greater than the

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nominal bulk concentration of 6 mM. This increases the probability of contact between reactive species, thereby increasing the reaction yield. In addition, it allows for orientation of the hydrocarbon tail of 2-OOA, resulting in a greater propensity to undergo the photochemical reaction mechanism illustrated in Figure 1A forming the OOA-OOA dimer, rather than the Norrish type II reaction (Figure S4) resulting in a single-tailed dimer. The Norrish type II reaction necessitates more motion of the hydrocarbon tail, allowing it to fold over on itself, a situation requiring a more dilute solute environment (less likely in the surface region). Water surfaces, such as those found on oceans, lakes, and atmospheric aerosol particles, have been implicated as unique prebiotic reaction environments previously.[20−27] Here, we add an additional example of the advantageous environment provided by the water surface for chemistry.

Thus far, simple vesicles (enclosures) composed of fatty acids have been used as a model system for primitive cellular constructs; they have the ability to mimic many functions of modern life, and their monomers (fatty acids) are plausible components on early Earth.[5−8,10,13,28,29] However, such protocells are unlikely to have been the first enclosures due to the very specific environmental constraints put on their synthesis and stability (requisite pH, high concentration, intolerance of vesicles to salts).[30,31] Modern cells are enclosed in a membrane composed of primarily double-tailed surfactants having none of the constraints put on fatty acid vesicles; however, their prebiotic synthesis is often thought to be too difficult due to their chemical complexity.[18] Here we provide an alternative, sunlight-driven route to primitive enclosures in addition to what has been traditionally considered. Beginning with a dilute solution of a short single-chained soluble surfactant, well below its CBC, simulated sunlight prompts synthesis of a double-tailed surfactant followed by self-assembly without external perturbation. No complicated synthetic steps or preparations or high concentrations of surfactant are required. In addition, the resultant vesicles are stable over time and persist in the presence of Mg^{2+} ions, a likely component of the early ocean.[32] In contrast, the more common models of fatty acid vesicles are unstable in the presence of divalent cations.[31]

Regardless as to whether or not the first enclosures were composed of single-tailed surfactants, a transition is needed at some point in molecular evolutionary history to membrane constituents with a double-tailed structure, as they are prevalent throughout modern biology. Once mixed membranes composed of both single- and double-tailed surfactants are facilitated, even with minimal double-tailed surfactant, unique competition and subsequent evolution have been seen to emerge.[33] The present study illustrates a simple, prebiotically plausible transition from single-tailed to double-tailed surfactants using only the energy provided by the early sun. Therefore, the work presented here provides a possible route to the prebiotic synthesis of a simple enclosure as well as a potential contribution to primitive membrane evolution.

■ ASSOCIATED CONTENT

Supporting Information

Experimental methods as well as supporting data and reaction mechanisms are presented in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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