Prebiotic Cell Membranes

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Prebiotic Cell Membranes that Survive Extreme Environmental Pressure Conditions**

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Abstract: *Attractive candidates for compartmentalizing prebiotic cells are membranes comprised of single-chain fatty acids. It is generally believed that life may have originated in the depth of the protoocean, that is, under high hydrostatic pressure conditions, but the structure and physical–chemical properties of prebiotic membranes under such conditions have not yet been explored. We report the temperature- and pressure-dependent properties of membranes composed of prebiotically highly-plausible lipids and demonstrate that prebiotic membranes could not only withstand extreme temperatures, but also serve as robust models of protocells operating in extreme pressure environments. We show that pressure not only increases the stability of vesicular systems but also limits their flexibility and permeability to solutes, while still keeping the membrane in an overall fluid-like and thus functional state.*

Compartmentalization is an underscoring principle that sets the stage for Darwinian evolution to proceed, as it leads to the first working cellular entity: a concentrated pool of self-replicating informational molecules protected from unfavorable chemical and physical environments.[1] Enticing candidates for cellular compartmentalization are membranes comprised of amphiphiles, that is, surfactants and lipids that display remarkable self-assembling processes, including spontaneous vesicle formation. In the prebiotic era, single-chain amphiphiles or fatty acids are at the focal point for research into the origin of life. They can be abiotically synthesized by Fisher–Tropsch-type reactions under simulated geochemical conditions, such as hydrothermal systems, and have also been detected in carbonaceous meteorites.[2–7] Pioneering work by Deamer et al. and others have documented the ability of these amphiphiles to form membranous structures in solution, the stability of which is strongly dependent on the pH, concentration, ionic strength, and the head–group characteristics of the amphiphiles.[8–11]

Life very likely originated in the depth of the protoocean of the Hadean Earth, that is, under high hydrostatic pressure (HHP) conditions.[12,13] Pressure-related work on prebiotic membrane models is however still terra incognita. The Hadean ocean water would filter harmful radiation and buffer physicochemical variations. In fact, today the greatest portion of our biosphere is also in the realm of environmental extremes, including HHP. Astonishingly, organisms flourish even on the deepest ocean floor (at a depth of about 11 000 m) and in deep-sea sediments where pressures up to about 100 MPa prevail (pH ≈ 7.9), and even higher pressures can be tolerated by particular bacterial strains.[13–15] Pressure-dependent studies have been carried out on present-day phospholipid membranes, only,[12,13,16,17] as well as on rather pressure-stable biomacromolecules, for example, DNA and proteins,[13,18–22] but the structure and physical–chemical properties of prebiotic membranes under such conditions have not yet been explored. What has to some extent been researched though are the more complex and highly evolved membranes of deep-water organisms. Evolution has enabled these organisms to cope with such harsh conditions by producing lipids of particular molecular structures that provide the membranes of extremophiles with appropriate physical–chemical properties to support their biological activity. For example, fatty-acid chains of archaea are based on polyisoprene units (phytanyl chains), where branching that is due to methyl groups primarily serves the same purpose as double bonds in eukaryotic membranes, and keeps the melting transition temperature of the lipids sufficiently low, enabling the organisms to maintain a high fluidity owing to chain disorder even under high pressure conditions and thus maintain their biological activity (homeoviscous adaptation).[23,24,12] Herein, we set out to explore the simultaneous temperature- and pressure-dependent properties and phase behavior of membranes composed of prebiotically highly plausible short-saturated fatty acids. To this end, a series of electron microscopy, spectroscopy, thermodynamics, and

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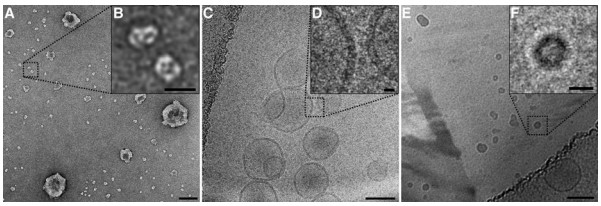

Figure 1. Electron microscopy of the prebiotic membrane system showing co-existence of unilamellar vesicles and micelles. Sample comprising of decanoic acid/decanol, 1:1 molar ratio, pH 8.5, was extruded through a polycarbonate membrane with a pore size of 100 nm and imaged. A) Representative micrograph area of negatively stained samples. Scale bar 100 nm. B) Close-up view of small micelles in (A). Scale bar 20 nm. C,E) Representative micrograph areas of vitrified samples. Scale bar 100 nm. D), F) Close-up view of vesicles in (C) and of small micelles in (E), respectively. Scale bar 20 nm. The images show that the system is composed of two types of particles, namely small micelles with a diameter of about 20 nm (B, F) and vesicles of diameters between 80 and 150 nm (C) with a lipid layer thickness of about 20 nm (D). The total amphiphile concentration was 80 mM.

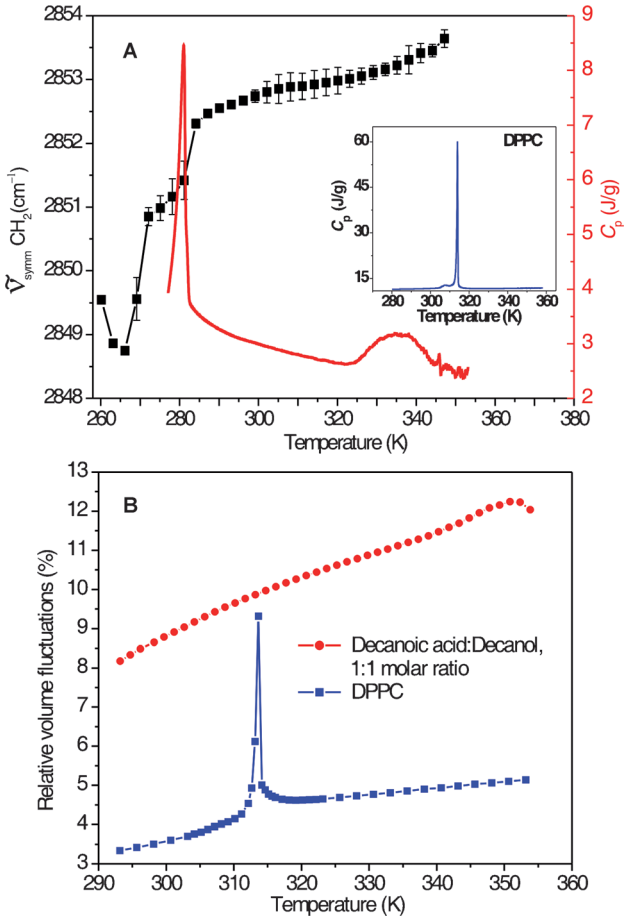

Figure 2. Temperature-induced phase transitions and dynamic characteristics of the prebiotic lipid mixture at ambient pressure. A) Black curve: Temperature dependence of the symmetric CH_2-stretching vibration in the mixture comprising decanoic acid/decanol (1:1 molar ratio, pH 8.5) at ambient pressure. Red curve: DSC scan of the sample at ambient pressure; inset: DSC scan for DPPC unilamellar vesicles. B) Relative volume fluctuations of the fatty-acid lipid mixture and, for comparison, respective data for a model phospholipid bilayer system, DPPC. The total amphiphile concentration was 80 mM.

scattering experiments have been carried out over a wide range of temperatures and pressures.

As an example, Figure 1 depicts the morphological characteristics of structures formed by decanoic acid and decanol, C10, mixtures revealed by electron microscopy. This system mimics the major product of an abiotic Fischer–Tropsch-type of reaction, which yields a mixture of short-chain saturated amphiphiles.[2] We observe highly heterogeneous lipid structures, including agglomerates of diverse-sized vesicular entities, which might serve as first cell-like compartments (Supporting Information, Figure S1). After extrusion through 100 nm pore-sized filters, essentially unilamellar vesicles were observed (Figure 1 C,D). We also noted the co-existence of significant amounts of micelles in this prebiotic lipid mixture (Figure 1 A–E) with vesicles. In fact, owing to the short-chain lengths compared to present-day dual-chain phospholipid membranes with chain lengths ranging from about 12 to 24 carbon atoms, such phase co-existence can be expected. Single-chain lipid molecules generally form vesicles of lower stability, which is further reduced by decreasing the chain lengths.[16] Fourier-transform IR (FTIR) spectroscopy and differential scanning calorimetry (DSC) were employed to monitor conformational and phase changes of the lipid mixtures. The CH_2 stretching vibrations respond to temperature- or pressure-induced changes in the *trans-gauche* ratio and number of kinks in the lipid chains. A rather sharp phase-transition was detected at about 280 K and assigned to the appearance of disordered, fluid-like chains (Figure 2 A).[25] The wavenumber change observed depicts, at least for a major part of the system, an ordered (gel-like)-to-fluid phase transition. A similar behavior was observed for other decanoic acid-decanol mixtures (for example, molar ratio 1:2) as well as for ternary fatty-acid membranes formed by admixing monoglycerol ester (monocaprin) with decanoic acid–decanol, comprising more complex prebiotic lipid mixtures (Supporting Information, Figure S2). Furthermore, a small broad peak centered around 335 K was observed through DSC, which most likely reflects morphological changes of lipid entities with overall fluid-like chains, such as a shift of the equilibrium between micellar and vesicular structures to larger-sized particles, as confirmed by small-angle X-ray (SAXS) and dynamic light scattering (DLS) data (Supporting Information, text and Figures S5, S6).

Comparison with the main gel-to-fluid phase transition of vesicles composed of diacyl-phosphatidylcholines (for example, dipalmitoylphosphatidylcholine; DPPC) reveals that the widths of the DSC peak of the fatty acid membranes are about four times broader, reflecting a much lower cooperativity of the transition (Figure 2 A, inset). Complementary pressure perturbation calorimetry (PPC) data, providing the temperature dependence of the thermal expansion coefficient of the lipid system and volume changes accompanying lipid phase transitions of the decanoic acid–decanol mixtures,[26] revealed positive volume changes at the thermally induced main transition, which can be explained, in agreement with the FTIR data, by the volume increase of ordered fatty acid

molecules upon chain melting (Supporting Information, Figure S3).

To obtain further insights into the thermomechanical properties of these fatty acid membranes, ultrasound velocimetry was employed to determine the adiabatic compressibility, and in concert with the calorimetric data, the isothermal compressibility. The latter parameter allows the calculation of the volume fluctuations, thus providing information about the overall dynamics of the lipid system.[27] Figure 2B depicts the temperature effect on the relative volume fluctuations of the fluid prebiotic mixture in comparison to DPPC phospholipid bilayers. The volume fluctuations of the prebiotic lipid system are about 2.5 times higher compared to DPPC bilayers.[27,28] Similar high values are only reached at the main phase-transition temperature of DPPC, where density fluctuations increase dramatically. Such high volume fluctuations also imply a significantly higher permeability of solutes across the fatty acid membranes.

To explore the combined temperature–pressure-dependent phase behavior of the prebiotic lipid mixture, Laurdan fluorescence spectroscopy was used. The fluorescent probe Laurdan reports changes in chain packing, membrane fluidity and hence the phase state of the membrane.[29] As an example, Figure 3A shows decreasing general polarization (*GP*) values (see the Supporting Information for details) of the system with temperature at ambient pressure, indicating an increase in the chain fluidity with a broad transition around 280–285 K, in accordance with the DSC and FTIR results. Above 310 K, a plateau value is reached at $GP = -0.7$, demonstrating that an overall fluid-like, disordered state of the lipid system is reached.

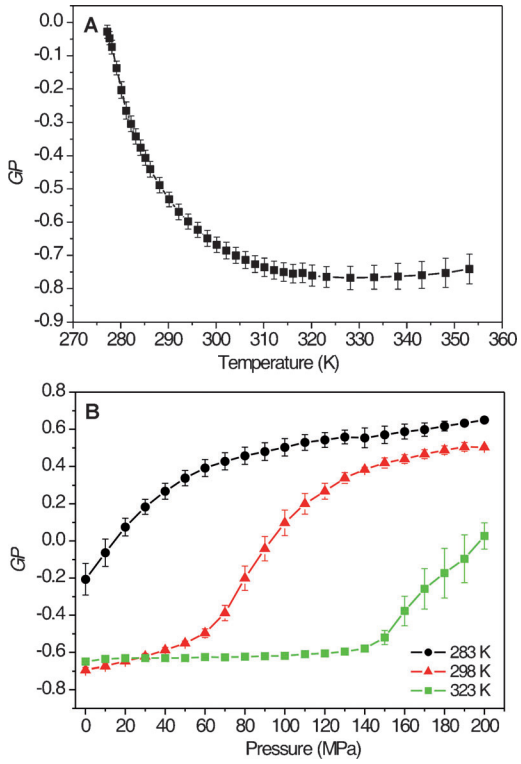

Figure 3. Temperature- and pressure-induced changes in the fluidity and conformational order of the prebiotic lipid mixture. A) Temperature- and B) pressure-dependence of Laurdan *GP* values for the prebiotic lipid mixture comprising of decanoic acid/decanol (1:1 molar ratio, pH 8.5). The total amphiphile concentration was 80 mM.

The effect of hydrostatic pressure on the *GP* data of the Laurdan-labeled prebiotic membrane mixture at some selected temperatures is depicted in Figure 3B. Upon pressurization at 283 K, the chain order increases steadily and approaches a plateau value above 100 MPa ($GP = 0.5$), marking that an ordered, gel-like state is reached in this pressure region.[29] At 298 K, the overall conformational order gradually increases, with a sigmoidal-like shape in the pressure interval between 60 and 130 MPa. In this pressure region, fluid and ordered gel phases co-exist. A plateau *GP* value is reached above 130 MPa ($GP = 0.45$), as the system enters a fully ordered state, and is unaffected by further compression. At 323 K, the onset of the fluid-to-ordered transition takes place at 140 MPa, that is, the phase-transition temperature to the ordered phase increases with increasing pressure, at a rate of about 25 K/100 MPa.

To explore the structural properties of the lipid system under high-pressure conditions in more detail, SAXS was employed (Supporting Information, text and Figures S5, S6). The analysis of the SAXS data reveals the presence of not only spherical particles with radii of 20 nm and more, but also of a significant population of small-sized particles (that is, micelles), pointing again to the co-existence of vesicles and micelles in the prebiotic lipid mixture. Upon pressurization, the scattering intensity at small angles increases, indicating formation of more and larger vesicles at the expense of small-sized micelles (Supporting Information, Figure S5B, inset). Therefore, we can conclude that pressurization leads to a redistribution of the population of micelles (M) and vesicles (V); that is, a shift of the M–V equilibrium, favoring vesicular particles under pressure. This would in fact be expected: Pressure generally leads to chain ordering, accompanied by a decrease of the partial lipid volume. Such chain ordering is energetically highly unfavorable for micellar systems owing to their high intrinsic curvature. The system can relax by formation of larger vesicular structures that can accommodate lipid molecules of cylindrical shape and can hence pack more densely.

Taken together, the results presented permit the construction of a temperature-pressure stability diagram of this prebiotic lipid mixture, which is depicted in Figure 4. At temperatures above 283 K, an all-fluid lipid phase consisting of vesicles, larger aggregates and micelles prevails. Generally, at least a partially fluid-like membrane characteristics is considered prerequisite for the function of membrane-associated processes, such as fusion, fission, and transfer of solutes. Between about 270 and 283 K, a two-phase region of coexisting fluid and ordered lipid phases is observed. The two-phase region is shifted to higher temperatures with increasing pressure, at a rate of about 25 K/100 MPa. With increasing pressure, an all-ordered, gel-like phase state is finally observed. This physiologically inapt state is reached at about 120 MPa at ambient temperature (298 K), and at about 40 MPa for a temperature as low as 278 K, the average temperature in the deep ocean. It is important to note here

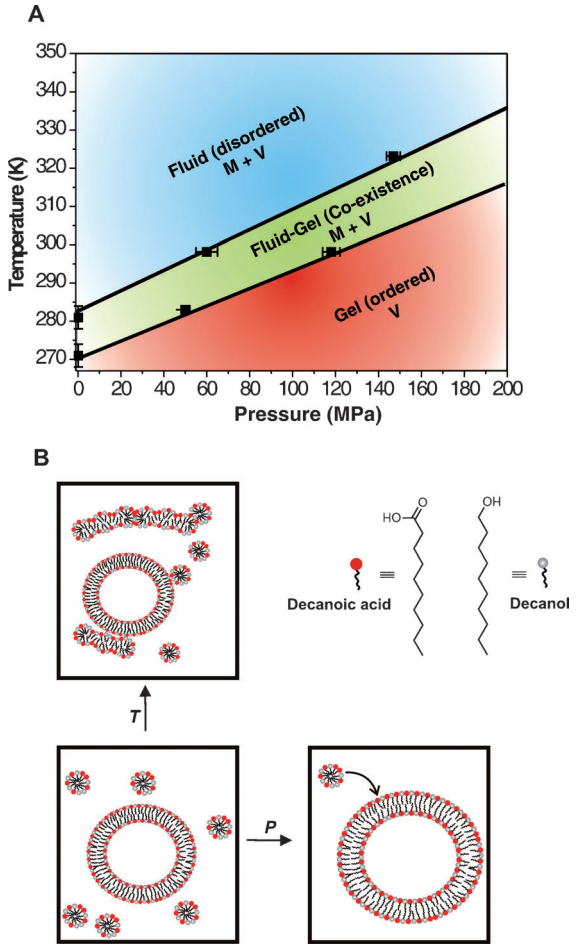

Figure 4. Structural insights into the prebiotic fatty-acid membrane in the temperature-pressure phase space. A) Pressure–temperature phase diagram for the prebiotic lipid mixture comprising of decanoic acid/decanol (molar ratio 1:1, pH 8.5, total amphiphile concentration 80 mM) as revealed by FTIR and Laurdan-fluorescence spectroscopic, calorimetric, and scattering data (M micelles, V vesicles). B) Structural characteristics of the system under ambient conditions upon increasing temperature and pressure. The chemical structures of the two amphiphiles used in the study are also depicted. A similar phase behavior is foreseen for the other prebiotic mixtures tested in this study.

that as the temperature-induced changes in the solution pH are small (for bicine: $d(pK_a)/dT \approx -0.018$),[30] and, moreover, the decanoic acid/decanol prebiotic mixture is stable over a broad pH range (Supporting Information, Figure S7 A), the phase behavior is expected to be essentially unaltered by minor changes in pH conditions. Furthermore, there is ample experimental evidence that the extrusion process does not affect the temperature- and pressure-dependent phase behavior of the system (Supporting Information, text and Figures S7 B, S8, S9).

In summary, our results propose that, from the perspective of membrane biophysical chemistry, the most plausible planetary environments for the origin of life on Earth would in fact include HHP conditions. Hydrostatic pressure increases the amount of vesicular structures of short-length fatty acid membranes, which can serve as prebiotic membrane envelopes. Lacking the complex, highly evolved protein machinery of present-day biomembranes, the dynamic nature of fluid fatty acid membranes seems particularly well-suited for growth, division, and nutrient uptake. Our studies also show that pressures in the range encountered in the deep sea could serve as a multifunctional toggle: limiting their flexibility and permeability (and thus leakage rate) to solutes, while still keeping the membrane in a fluid-like state, which is required for function. Therefore, fatty-acid-based vesicles are not only highly temperature-resistant, but they are also pressure-stable up to the high pressures reached in the deep sea and sub-seafloor crust. Our observations extend the range of tolerable environments for early cell membranes and may add another layer of complexity to developing laboratory models of primitive cell membranes. Such approach to explore laboratory models of primitive cell membranes under extreme environmental conditions aids in better understanding of the evolutionary pathway that led to the first forms of boundary membrane that might potentially isolate a primitive catalytic replicating system from the nutrients required for growth.[31]

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