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Geochimica et Cosmochimica Acta 223 (2018) 216–228

Geochimica et Cosmochimica Acta

www.elsevier.com/locate/gca

Aqueous magnesium as an environmental selection pressure in the evolution of phospholipid membranes on early earth

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Received 25 July 2017; accepted in revised form 30 November 2017; Available online 8 December 2017

Abstract

Early compartmentalization of simple biomolecules by membrane bilayers was, presumably, a critical step in the emergence of the first cell-like entities, protocells. Their membranes were likely composed of single chain amphiphiles (SCAs), but pure SCA membranes especially those with short-chains are highly unstable towards divalent cations, which are ubiquitous in aqueous environments. The prebiotic synthesis of phospholipids (PLs), even in only trace amounts, may also have been possible. PL membranes are much more stable towards divalent cations. Here, we show the transition of fatty acid membranes to mixed fatty acid-PL and, finally, to PL membranes in the presence of Mg^{2+}, which acts as an environmental selection pressure, and we propose different mechanisms for the observed increased Mg^{2+}-immunity. The "fatal" concentration ($[Mg^{2+}]_{fatal}$) at which vesicles are disrupted increased dramatically by an order of magnitude from OA to mixed to POPC vesicles. Two mechanisms for the increasing immunity were determined. The negative charge density of the vesicles decreased with increasing POPC content, so more Mg^{2+} was required for disruption. More interestingly, Mg^{2+} preferentially bound to and abstracted OA from mixed lipid membranes, resulting in relatively POPC-enriched vesicles compared to the initial ratio. The effect was the most dramatic for the largest initial OA-POPC ratio representing the most primitive protocells. Thus, Mg^{2+} acted to evolve the mixed membrane composition towards PL enrichment. To the best of our knowledge, this is the first report of selective lipid abstraction from mixed SCA-PL vesicles. These results may hold implications for accommodating prebiotic Mg^{2+}-promoted processes such as non-enzymatic RNA polymerization on early Earth.

Keywords: Fatty acid; Phospholipid; Magnesium; Fatal concentration; Protocell

1. INTRODUCTION

The most fundamental requirement for the origin of life was the emergence of a self-assembling molecular system capable of both metabolism and replication. The earliest self-assembling life-like unit, the protocell, was likely an enclosed entity that protected genetic and metabolic molecules from degradation by conditions in the external environment. It has been assumed that protocell membranes were composed of single chain ampiphiles (SCAs) such as fatty acids, fatty alcohols, monoalkyl phosphates and monoacyl glycerol phosphate esters, because of their prebiotic availability and ability to self-assemble into bilayer structures (Deamer and Oró, 1980; Oró et al., 1990; Apel et al., 2002; Monnard and Deamer, 2002; Mansy et al., 2008; Budin et al., 2012; Adamala and Szostak, 2013; Albertsen et al., 2014; Dalai et al., 2016; Kee and Monnard, 2016; Fiore et al., 2017). In particular, various SCAs (up to C_{12}) and polyaromatic hydrocarbons have been discovered in carbonaceous chondrites (Yuen and Kvenvolden, 1973; Lawless and Yuen, 1979; Deamer and

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https://doi.org/10.1016/j.gca.2017.11.034
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Pashley, 1989; Komiya et al., 1993; Mautner et al., 1995; Monnard and Deamer, 2002), and fatty acids up to C_{22} were formed via Fisher-Tropsch type (FTT) synthesis under simulated hydrothermal conditions (Nooner and Oró, 1979; McCollom et al., 1999; Rushdi and Simoneit, 2001; Foustoukos, 2004).

Single chain amphiphile membranes are capable of encapsulating and concentrating the building blocks of life, self-division and are thermostable up to ~ 90 °C (Monnard and Deamer, 2002; Mansy and Szostak, 2008). A key step in energy transduction is nutrient uptake and release of waste products across the membrane. Fatty acid membranes are reported to be permeable towards sugars (Gebicki and Hicks, 1976; Hargreaves and Deamer, 1978; Sacerdote and Szostak, 2005), amino acids (Zepik et al., 2007) and nucleic acids (Mansy et al., 2008). However, one of the major limitations of fatty acid membranes is their instability in the presence of salts (Monnard and Deamer, 2002; Monnard et al., 2002). Modern seawater contains ~ 500 mM NaCl and over 50 mM Mg^{2+} among other ions (Berner and Berner, 2012). The dissolution of minerals from komatiite, the rock comprising ancient oceanic crust, and tonalite, representing primitive continental-type crust would have resulted in dissolved salts in Hadean-Archean aqueous environments. Evaporation of post-weathering solutions would have resulted in even higher salt concentrations with estimates of ~ 600 mM NaCl, ~ 0.1 to 10 mM of total dissolved magnesium and sub-millimolar levels of dissolved calcium. Fatty acid vesicles are disrupted at modest ($\leq$1 mM) divalent and moderate (~ 200 mM) monovalent cation concentrations (Monnard et al., 2002; Chen et al., 2005). The instability of pure fatty acid membranes towards divalent cations has posed a significant constraint on studies of model protocellular membranes under plausible prebiotic conditions.

Mixed vesicles of fatty acids with fatty alcohols, fatty amines, glycerol monodecanoate or polycyclic aromatic hydrocarbons are more tolerant of divalent cations (Hargreaves and Deamer, 1978; Deamer, 1992; Apel et al., 2002; Chen et al., 2005; Mansy et al., 2008; Namani and Deamer, 2008; Groen et al., 2012; Adamala and Szostak, 2013). Recently, it has been reported that nucleobases and sugars prevent the aggregation of decanoic acid (DA) and stabilize it in 300 mM NaCl (Black et al., 2013). Thus, mixed SCA vesicles are more likely to have been stable in salt-rich environments.

In contrast to SCA membranes, the phospholipid (PL) membranes of modern cells are almost impermeable to most solutes and, therefore, need specialized transport machineries for nutrient exchange, and these ion channels or pumps likely evolved later than the emergence of the first protocells. The transition of protocell membranes composed predominantly of fatty acid or mixed SCA membranes to PL membranes would, presumably, have been a necessary step to compensate the limitations associated with both fatty acid and PL systems. The prebiotic synthesis of PLs, even if only in trace amounts, may have been possible (Hargreaves et al., 1977; Rao et al., 1982, 1987; Maheen et al., 2010; Patel et al., 2015), so an intermediate evolutionary stage in which protocell membranes were composed of mixed fatty acid-PL vesicles is envisaged. Following this logic, previous studies examined fatty acid-PL vesicles, but in the absence of divalent cations (*e.g.*, Cheng and Luisi, 2003; Budin and Szostak, 2011). It was shown that mixed vesicles of oleic acid (OA) and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) or of OA and di-oleoyl-phosphatidic acid (DOPA) grow at the expense of pure OA vesicles. However, the resulting vesicles should be relatively more OA-enriched than the starting population and, hence, less stable towards divalent cations, so the evolutionary advantage is not immediately apparent.

Among the divalent ions, Mg^{2+} is one of the most important biologically because it is required at relatively high (50–80 mM) concentrations for both nonenzymatic templated polymerization of RNA nucleotides and nonenzymatic clay-catalyzed nucleotide polymerization (Ferris et al., 1996; Joshi et al., 2009; Adamala and Szostak, 2013). It is important to note, however, that Mg^{2+} is not essential for the formation of short RNA oligomers as long as specifically pre-treated montmorillonite and a high concentration of monovalent alkali cations are present (Joshi and Aldersley, 2013). Also, it has been shown that Fe^{2+} can catalyze single-electron transfer reactions by ribosomal RNA in the absence of oxygen and at mildly acidic pH (Hsiao et al., 2013). Long chain RNA oligomerization, however, does seem to require high Mg^{2+} concentrations and Fe^{2+} may also have a deleterious effect on primitive membranes, so it is critical to understand the stability of protocell membranes and their transition to PL membranes in the presence of divalent cations, especially, high Mg^{2+} concentrations.

Given the key role of Mg^{2+} in protocell evolution, the aim of the present work is to quantitatively investigate the Mg-tolerance of vesicles composed of binary mixtures of OA and POPC as model protocell membranes (Fig. 1) and the mechanisms responsible for the increasing Mg-tolerance as membrane composition changes towards PLs. In so doing, the role of Mg^{2+} in acting as an environmental selection pressure in the transition of fatty acid membranes to mixed fatty acid-PL membranes and, finally, to PL membranes has been revealed. As noted above, membranes composed of DA and decanol (DOH) have been widely examined as the earliest model protocell membranes by some workers (*e.g.*, Apel et al., 2002; Monnard and Deamer, 2002; Sahai et al., 2017). To determine whether the effects of Mg^{2+} obtained here on OA-POPC vesicles are more broadly applicable, we also examined the effects of Mg^{2+} and Ca^{2+} on mixed DA-DOH (1:1 and 2:1) vesicles. To the best of our knowledge, this is the first report on the evolution of fatty acid membranes towards PL membranes driven by an ion.

2. MATERIALS AND METHODS

Oleic acid (C18:1, OA) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (C16:0-C18:1, POPC) were obtained from Avanti® Polar Lipids (Alabaster, AL, USA). Unless otherwise specified, all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) at the highest available purity, and used without further