Chemical Autopoiesis: Self-Replicating Micelles and Vesicles

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Abstract

Micelles, reverse micelles and vesicles (liposomes) are examples of geometrically bounded molecular structures. Conditions have been found under which micelles and reverse micelles can be brought to self-replication by a simple chemical reaction which takes place at the boundary of the micellar system, at the interface between the micelles' interior and the exterior bulk solvent. During this reaction, the molecules of the boundary are produced which leads to the formation of more micelles as time progresses. The examples are micelles and reverse micelles build by octanoate molecules, and the reactions which lead to self-replication are ester hydrolysis or alcohol oxidations.

In another approach, the reproducing micelles are first created chemically by a simple hydrolysis reaction in a two-phase system made of an alkaline aqueous phase and an organic fatty acid ester phase. In an extension of this concept, as upper organic phase octanoic anhydride was used. The initial conditions of the alkaline aqueous phase were so that the pH drop during the reaction led to the formation of vesicles which were able to take up anhydride molecules and to act as a catalyst for the hydrolysis of the remaining anhydride molecules.

The investigation of fatty acid micelles and vesicles is related to the possible role of bounded molecular structures during the early prebiotic evolution on Earth. Because self-replicating micelles and vesicles can be considered as examples for the chemical version of an autopoietic unit, the requirements of the minimal and universal definition of the living are fulfilled. (According to the definition by Maturana and Varela, an autopoietic unit is a structure which is self-generating and self-perpetuating as a consequence of its own activities within a boundary of its own making).

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Introduction

Micelles (aqueous or reverse) and vesicles are geometrically bounded three-dimensional structures in which the boundaries are either a monolayer of surfactant molecules (micelles) or a closed bilayer (vesicles), Fig. 1. In some cases, aqueous micelles can be regarded as 'precursors' of vesicular structures which are currently considered as very simple model systems for the lipidic shell matrix of contemporary biological cells. For this reason, it is argued that simple surfactant systems, such as micelles - and in particular vesicles - may have played an important role in the primordial time on the primitive Earth, more than about $3x10^9$ years ago. Therefore, vesicles have recently been proposed as most relevant geometrically closed aggregates to fulfill the minimal requirements for a protocellular structure (Deamer and Oró, 1980; Morowitz et al., 1988).

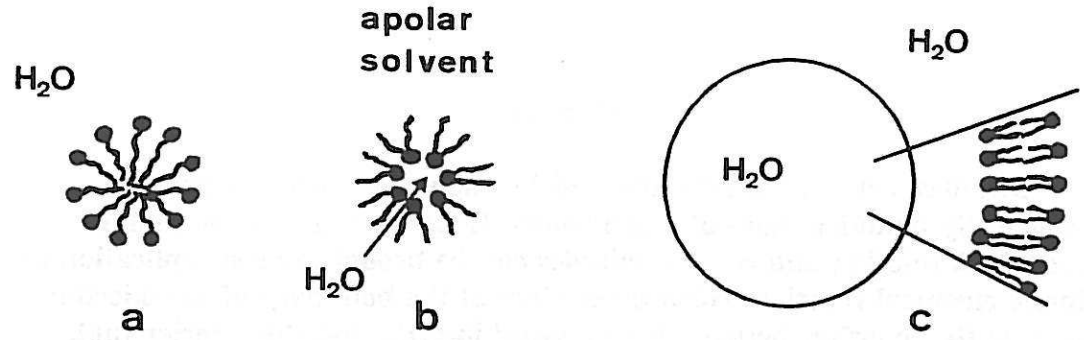

Fig. 1: Schematic representation of different geometrically bounded three-dimensional structures: aqueous micelle (a), reverse micelle (b) and unilamellar vesicle (c).

With the term "autopoiesis", two biologists - Humberto R. Maturana and Francisco J. Varela - have introduced and discussed the principles of the self-generating organization of the living: autopoiesis identifies the living as a system which arises as a consequence of its own activities within a boundary of its own making (Varela et al., 1974; Varela, 1979; Fleischaker, 1988, 1990).

We have undertaken a first attempt to 'create' experimentally minimal chemical systems which satisfy the criteria of autopoiesis. The principle is the following: a micelle (or vesicle) formed by a surfactant C hosts a reaction which leads to C. In this way, the system is self-producing owing to a reaction taking place within its own boundary (the minimal requirement for autopoiesis), Fig. 2.

Self-replicating aqueous and reverse micelles

Different micellar (reverse and aqueous) systems have been studied so far (Bachmann et al., 1990, 1991a, 1991b, 1992). One of the micellar system is illustrated schematically in Fig. 3 (Bachmann et al., 1991a).

The system is composed of sodium octanoate micelles in isooctane/octanol

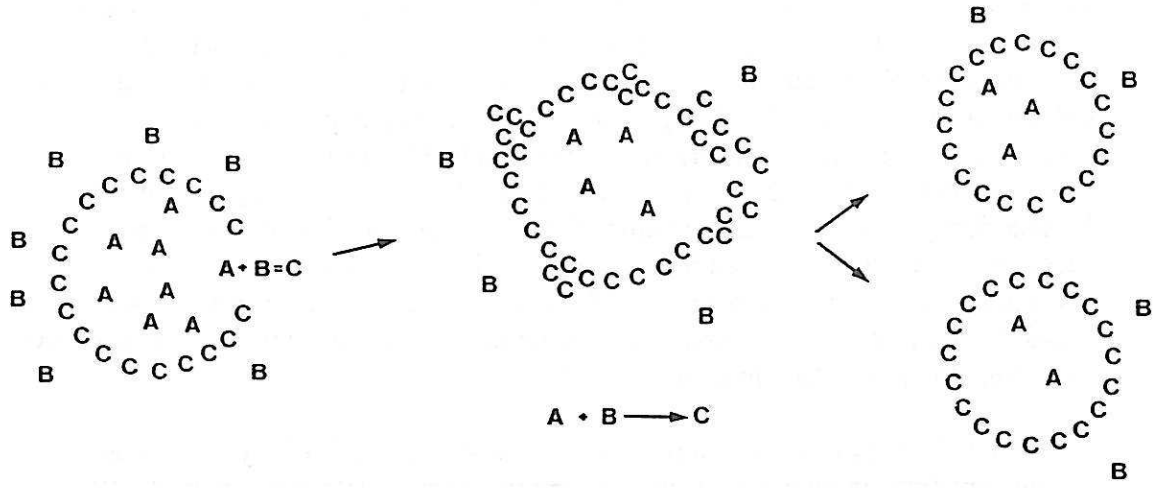

Fig. 2: Schematic general representation of self-replicating micelles. The micelles host a reaction which takes place at the boundary of the micelles leading to the formation of C, the component of the boundary (Luisi and Varela, 1989).

(85:15, v/v) in the presence of a small amount of water (w_o=[H_2O]/[octanoate]=30). Octanol is cosolvent as well as cosurfactant; and therefore, octanoate and octanol are the molecules of the boundary of the reverse micelles, separating the aqueous interior of the micelles from the bulk solvent. If now sodium permanganate is solubilized in the polar pool of the micelles, octanol will slowly be oxidized to octanoate at the interface of the micelles as time progresses; and as a consequence more micelles will be formed. In this way - and with initially 50 mM octanoate and 97 mM permanganate - the concentration of reverse micelles increases by a factor of 9.3 from initially 0.132 mM to 1.24 mM after completion of the reaction, (Bachmann et al., 1991a).

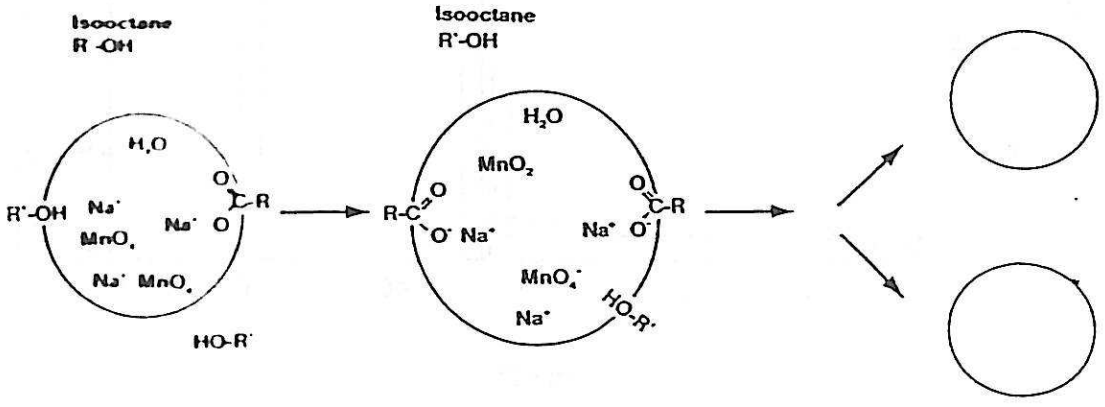

Fig. 3: Schematic representation of self-replicating reverse micelles based on octanol oxidation. 3 CH_3-$(CH_2)_7$-OH + 4 $NaMnO_4$ → 3 CH_3-$(CH_2)_6$-COO^-Na^+ + 4 MnO_2 + NaOH + 4 H_2O

Another simple system which we have studied is based on the hydrolysis of ester molecules (Bachmann et al., 1990, 1991a, 1991b). Again, reverse micelles of

sodium octanoate and octanol as surfactant and cosurfactant are used in a solvent mixture of isooctane and octanol (9:1, v/v). If now octanoic acid octyl ester molecules are dissolved in the bulk solvent, they can be hydrolyzed to octanoate and octanol either by solubilizing in the interior aqueous pool of the reverse micelles hydroxide ions (LiOH) or - if cosurfactant/cosolvent is octylamine (isooctane:octylamine, 85:15, v/v) - an appropriate enzyme catalyst (a lipase). Since the reaction products are components of the reverse micelles, the number of micelles will increase as time progresses. Starting, for example, with initially 50 mM sodium octanoate, 25 mM octanoic acid octyl ester and 23 mM LiOH (w_o=9.2), the number of reverse micelles increases from initially 1.91 mM to 3.03 mM after 300 hours (Bachmann et al., 1990).

In another approach the self-replicating micelles are initially not present in the system, but first created by a simple chemical reaction (Bachmann et al., 1992). For this an aqueous phase containing 3 N NaOH and a supernatant phase of octanoic acid ethyl ester are heated to almost 100 °C for several days. As time progresses the ester molecules are hydrolyzed to octanoate and ethanol. Initially, the rate of hydrolysis is rather slow until the first micelles are formed in the aqueous phase. These micelles are getting self-replicating by taking up ester molecules which are then hydrolysed at the micellar boundary, Fig. 4.

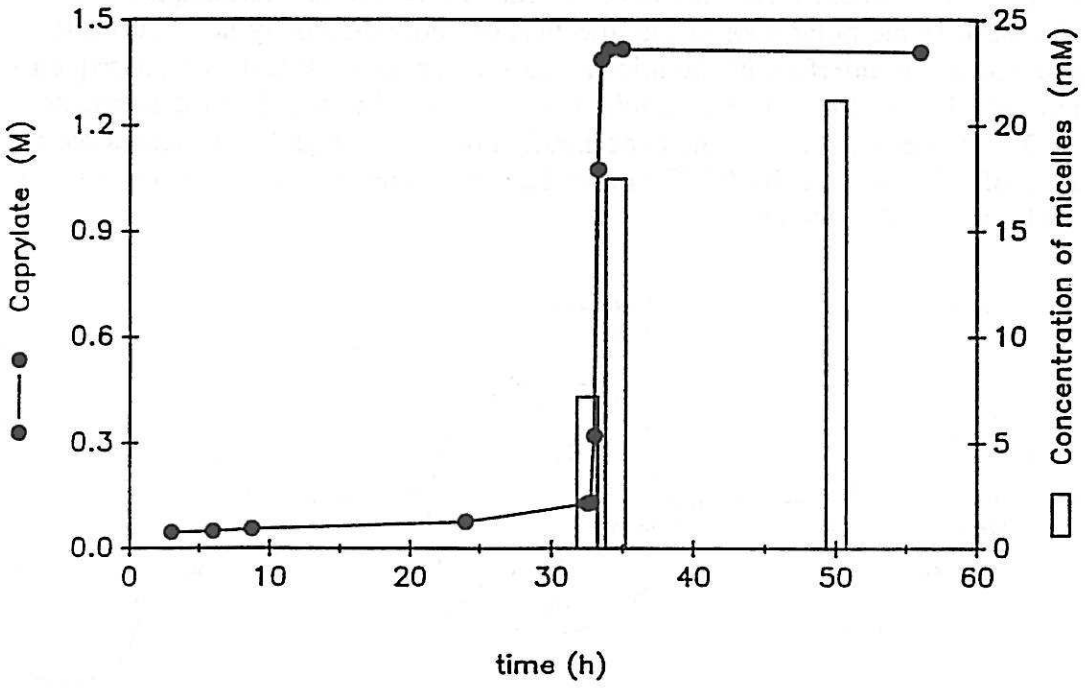

Fig. 4: Time progress of octanoate concentration and micelle concentration following the hydrolysis of octanoic acid ethyl ester by NaOH in a biphasic system at 100 °C ca. Starting conditions: 20 ml 3 N NaOH (stirred at 150 rpm) and 6 ml octanoic acid ethyl ester in a 100 ml round bottom flask (Bachmann et al., 1992).

Self-replicating vesicles

A rather more complex approach is that of self-replicating vesicles, and, so far, we have investigated two conceptually different type of systems.

(i): In the first one, compounds which constitute the bilayer of the vesicles are synthesized enzymatically. This has been studied in a first approach by reconstituting into phosphatidyl choline vesicles the whole enzymatic 'machinery' (four enzymes) involved in the biosynthesis of phosphatidylcholine; and the synthesis is basically starting with acyl coenzyme A and sn-glycerol-3-phosphate as substrates (salvage pathway). Preliminary measurements have shown that this reconstituted chain of enzyme reactions catalyzes the synthesis of new phosphatidylcholine molecules which are spontaneously incorporated into the host vesicles, leading to a change in the equilibrium size - and under certain conditions to an increase in the number of vesicles (Schmidli et al., 1991).

In a second enzymatic approach, the hydrolysis of ethyl oleate, incorporated into oleic acid/oleate vesicles was catalyzed by the help of *chromobacterium viscosum* lipase at pH 9. During the reaction at room temperature, all ethyl oleate is converted into oleic acid/oleate and ethanol and under a set of conditions, the size and the number of the vesicles change with time. Starting with 100 nm unilamellar vesicles, most of the vesicles get smaller (95% ca. of the population with a diameter of 70 nm) which leads to an increase in the number of vesicles (Vonmont-Bachmann et al., submitted).

(ii). In a first non-enzymatic approach towards self-reproduction of vesicles, the principles of the two-phase system of Fig. 4 have been extended. At this aim, we have modified the two-phase reaction system to the hydrolysis of octanoic anhydride (Fig. 5). As time progresses, anhydride molecules are hydrolyzed,

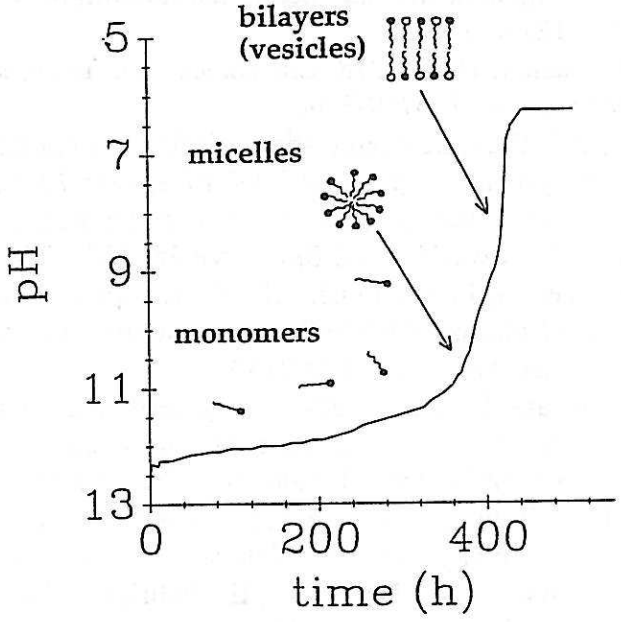

Fig. 5: Hydrolysis of octanoic anhydride at 40 °C in a two-phase system composed of 10 ml 265 mM NaOH, 200 mM NaCl (lower aqueous phase) and 2.5 mmoles octanoic anhydride (upper phase).

leading to a decrease in the pH of the aqueous phase and to the formation of octanoate molecules which spontaneously assemble into micelles as soon as the critical concentration of micelle formation is reached. The hydrolysis reaction is then accelerated by the presence of micelles, and as a consequence of the continuous drop in pH, the octanoate micelles are transformed into bilayer structures (polydisperse vesicles), as soon as the pH of the aqueous phase is approaching the pK value of octanoic acid in the bilayer (pH~6.8, Hargreaves and Deamer, 1978). The vesicles themselves catalyze the hydrolysis reaction, which leads to an increase in the concentration of vesicles, until all anhydride molecules initially present in the system are consumed (Walde et al., submitted).

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