Lipid vesicles as possible intermediates in the origin of life

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Lipid vesicles (liposomes) are closed structures in which (at least) one lipid bilayer separates an aqueous inner compartment from the bulk external aqueous medium, as in membranes of contemporary biological cells. Lipid vesicles have therefore been considered as possible cell precursors during the prebiological era on Earth. Recently, it has been shown that lipid vesicles form spontaneously. Furthermore, it has been demonstrated that thermodynamically controlled peptide binding to and controlled polymerization reactions on vesicles are possible, thus leading to an increase in the molecular complexity of lipid vesicles. This may have been relevant during the prebiological evolution.

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Current Opinion in Colloid & Interface Science 1999, **4**:33–39

Electronic identifier: 1359-0294-004-00033

 ISSN 1359-0294

Introduction

Research on the origin of life is based on the elementary idea of molecular evolution, as proposed by Oparin many years ago [1,2]. According to this approach, life originated from inanimate matter via a spontaneous increase of molecular complexity and specificity. A number of books have been written on this subject; for example by Bernal [3], Miller and Orgel [4], Fox and Dose [5], Folsome [6], Dyson [7], and De Duve [8]. We know that cellular life was already present on our planet at least 3,500 million years ago (see the fossil records reported by Schopf [9,10]), and that the Earth is ~4,500 million years old. It is also assumed that the chemical transformations of organic molecules into, for example, amino acids or nucleotides, cannot have started earlier than 3,900 million years ago, when the Earth was sufficiently cold and equilibrated. What happened between this time and the time of the earlier fossils (~400 million years) is not known — and the reconstruction of this pathway leading to the transition to life is the interest of researchers engaged in the study of the origin of life.

A current view of the origin of life, which is popularized in a number of biology and biochemistry textbooks, see for example [11–14], is based on the RNA-world [15–17]. It is proposed that RNA is the key prime molecular entity, since it is endowed with both self-replication and catalytic properties — the so-called ribozymes [18,19]. Once a self-replicating RNA family is made, because of its capability to mutate in the process of self-replication, ribozymes could be formed which might subsequently catalyze the formation of proteic enzymes and DNA. This scenario is very appealing, and in fact has gained a large measure of acceptance within the scientific community. There is a problem, however, in that this scenario is based on the spontaneous, prebiotic formation of an RNA 'family'; namely a highly stereoregular polymer with a specific sequence. Textbooks assume that this specific RNA family can form spontaneously from the prebiotic soup, but this, at the present state of our knowledge, is highly improbable. Calculations of the probability of such an event occurring have been presented in the literature, and, for example, Joyce and Orgel [20] define this view as 'the molecular biologist's dream', while showing how unrealistic this dream is. This point is discussed in other papers as well [21].

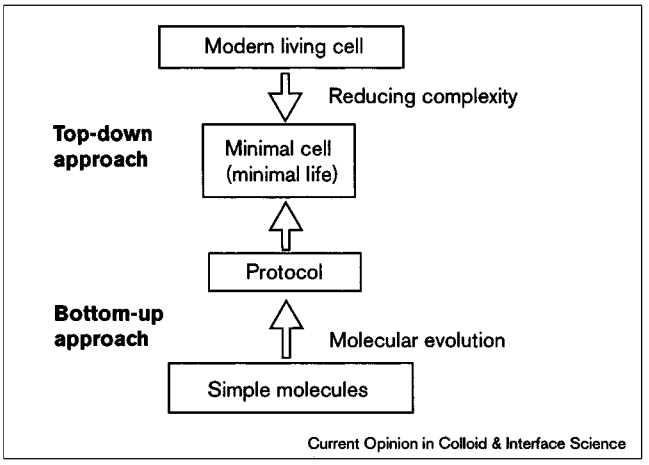
Perhaps there are other, until now, undiscovered patterns by which a stereoregular specific RNA sequence — or some other suitable precursor — may spontaneously come into existence, and it would indeed be very appealing if this was to be the case. However, for the time being, it is right and reasonable to look for alternative scenarios relevant to the origin of life.

The general principles of the application of surfactant aggregates in investigating the origin of life

One main alternative avenue is to look for 'spontaneous' processes, such as self-assembly and hydrophobic interactions, and to consider the level of molecular complexity and specificity which can be reached on this basis. The general belief underlying this research is that in prebiotic times only very simple reactions would have been operative.

On this basis, it is appropriate to look at self-assembly as an initial step for the construction of an organized system which demonstrates molecular complexity and which will have a useful function. Lipid vesicles — and by lipids we mean in this context simple as well as complex amphiphiles, for example carboxylic acid soaps and phospholipids — have been considered as precursors of the living cell [22–25]. Connected with this is the idea that compartmentalization is an important principle for the development of life on Earth since it leads to the first working cellular unit [25]. Pioneering work in this field has been carried out by Deamer et al. [26,27] who also showed that boundary structures are formed

Figure 1



'Bottom-up' and 'top-down' approaches in research on the origin.

from organic compounds extracted from the Murchison meteorite [28,29].

Starting from small molecules, the procedure by which one advances up the ladder of molecular complexity to (eventually) a minimal living cell, can be defined as the 'bottom-up' approach; see Figure 1. In this experimental set up, one cannot use enzymes or nucleic acids, as these macromolecules, by definition, did not exist at the start of evolution.

Still using lipid vesicles as the central theme, we can also define a 'top-down' approach. The rationale is the following: even the simplest modern living cell contains several hundred proteins and several hundred families of nucleic acids. Presumably, this high complexity is the result of cellular evolution; when cellular life started, the cell did not require such a large number of enzymes or nucleic acids to function. This brings us to the question 'what is the minimum number of enzymes and nucleic acids which a cell requires to still perform the most basic functions of life (basically self-maintenance and reproduction)?' This question can in principle be investigated by transforming a simple liposome into a functional biological cell, by sequential addition of enzymes and nucleic acids. We will return to this approach later on in this article.

The 'bottom-up' approach

Early work on self-replication of surfactant assemblies was carried out with micelles and reverse micelles [30–33], but in later years research has shifted towards lipid vesicles (liposomes) [34,35] because of their closer analogy to biological cells, both in terms of a bilayer membrane and a close to micron-sized aqueous interior core.

Vesicles provide a simple example of self-assembly and self-organization; an ordered compartment is formed either spontaneously, in the case of surfactants and soaps, or by the use of energy (sonication) [36–43•,44•,45,46]. The observation that spontaneous formation of order and compartmentalization, which are so central to living structures, can be formed by simple surfactant molecules should put these aggregates at a focal point for research into the origin of life. The next question we can ask is 'can lipid vesicles display some additional property which can make them more interesting from the point of view of molecular evolution?'

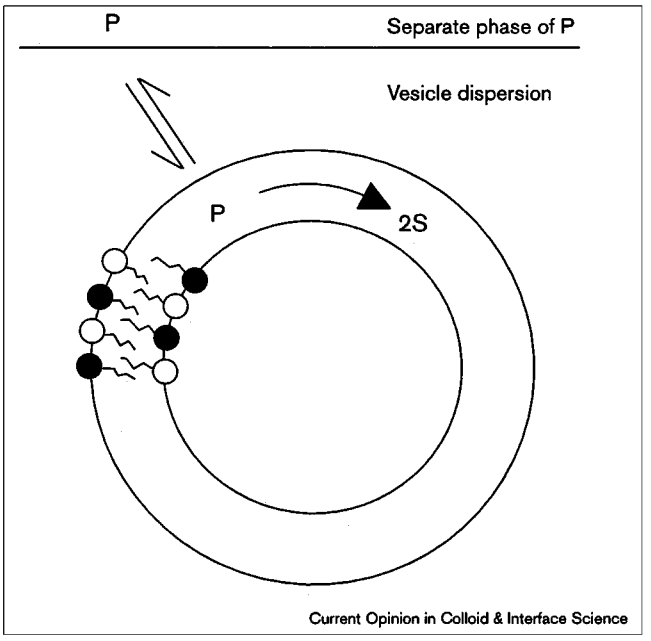
The answer to this question represents the main thrust of our research at the ETH in Zürich, and we have tackled the question by looking at two distinct aspects: the self-reproduction of vesicles, and their capacity to bind hydrophobic, biologically-active compounds. These two topics are related to each other, as self-reproduction will relate to the capability of vesicles to bind hydrophobic, water-insoluble compounds — even molecules of their own kind. Most of our work on self-reproduction has been carried out with vesicles formed by fatty acids, most notably octanoic acid (caprylic acid), oleic acid (cis-9-octadecenoic acid), and 2-methyldodecanoic acid, which are much simpler molecules (from a chemical viewpoint) than typical phospholipids. It has been known for several years that medium and long chain fatty acids form vesicles, depending on the degree of deprotonation, when dispersed in aqueous solution [47–50]. Research on these systems continues to attract the interest of researchers [51,52].

Self-reproduction of vesicles and the notion of vesicular autocatalysis

A basic mechanism for vesicle self-reproduction is represented in Figure 2. A water-insoluble surfactant precursor, for example the anhydride of a fatty acid, is added in excess to a dispersion already containing preformed fatty acid vesicles. In the absence of vesicles, the hydrolysis of the water insoluble precursor, the fatty anhydride, occurs extremely slowly. In the presence of pre-added vesicles, the rate of hydrolysis is increased, and each water-insoluble precursor molecule, P (the fatty anhydride) is hydrolyzed to two molecules, 2S, of fatty acid, which are bilayer-forming compounds. The accumulation of surfactant within the bilayer of the original vesicle gives rise to growth, and eventually to new vesicles. The mechanisms involved are unclear at present, but 'budding off' of vesicles is presently an active area of investigation from both theoreticians and experimentalists [53–57].

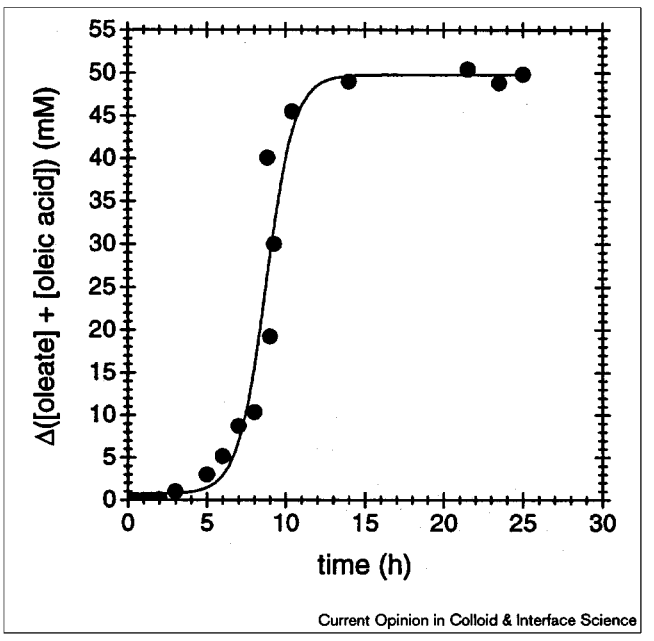
As more vesicles are produced, more water-insoluble precursor is hydrolyzed, producing more vesicles, and so

Figure 2



Schematic representation of the vesicle self-reproduction. P: precursor (fatty anhydride); S: surfactant (fatty acid and soap with filled and empty head groups, respectively).

Figure 3



Hydrolysis of oleic anhydride at pH 8.5 and 40°C. Initial conditions: 10 ml 0.2 M *N,N*-bis(2-hydroxyethyl) glycine buffer, pH 8.5, 250 μmol oleic anhydride and 25 μmol oleic acid.

forth in a typical autocatalytic fashion. This process may be referred to as vesicular autocatalysis.

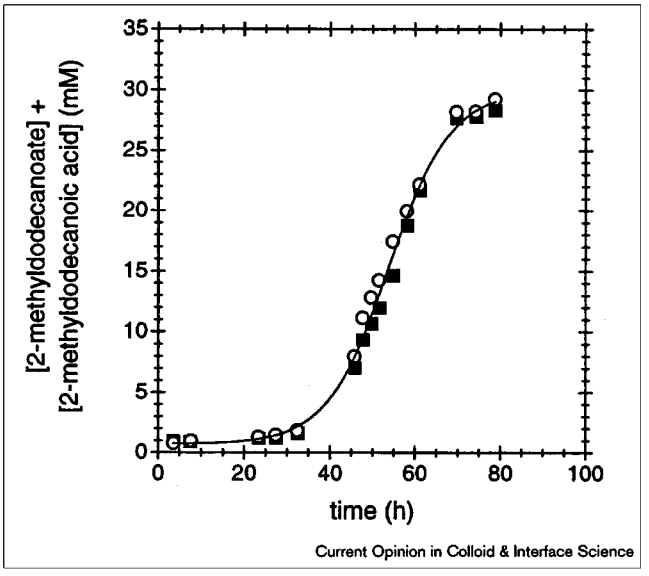
Let us consider data for a typical vesicle catalysis experiment for oleic acid vesicle self-reproduction, as shown in Figure 3 [34]. If the hydrolysis of oleic anhydride at pH 8.5 is followed, the reaction is initially rather slow until after approximately 4–5 h (at 40°C), the rate of hydrolysis then increases autocatalytically, as vesicles are formed. The formation of vesicles during the reaction is confirmed by light and electron microscopy [34]. If fatty acid vesicles are already present at the start of the experiment, the rate of hydrolysis is considerably faster, and no slow initial phase is observed. This does not mean that the aggregates act as a conventional catalyst which simply decrease the activation energy of a particular chemical step in the reaction. Rather, the aggregates influence the reaction rate because of their solubilization power, so the driving force is probably hydrophobicity and the favorable partitioning of the anhydride into the bilayer. Thus, vesicular catalysis (like micellar catalysis [58–62•]) is dominated by concentration and surface effects [63,64].

Other work has been concerned with vesicles from chiral fatty acids, in particular with 2-methyldodecanoic acid [35], in order to investigate the possibility of a connection between self-reproduction and chirality. In particular, autocatalytical processes leading to self-reproduction could lead to an increase in population of one enantiomer over the other. Using homochiral or racemic 2-methyldodecanoic anhydride as precursor molecules, the product-time profile was again sigmoidal, as shown in Figure 4, just as in the case of oleic anhydride, measured under similar conditions (compare with Figure 3). The rate of hydrolysis was, again, initially slow until vesicles were formed. The reaction rates for the hydrolysis of (*R,R*)-2-methyldodecanoic anhydride and (*S,S*)-2-methyldodecanoic anhydride, catalyzed by (*R*)-2-methyldodecanoic acid vesicles or (*S*)-2-methyldodecanoic acid vesicles were the same within experimental errors; the initial presence of chiral vesicles could not induce a significant enantioselectivity under the conditions used.

Enhancement of catalysis of peptides by binding to liposomes

Hydrophobic binding is indeed a fundamental property of vesicles [65] and can be seen as a driving force to increase the molecular complexity of the liposome. Hydrophobic amino acids and simple dipeptides come to mind, as they are conceivably prebiotic compounds. The idea that the interaction between peptides and membranes might be important for the origin of life has been considered [66], but few focused experiments on oligomerization/polymerization have been described so far [67–70]. It has recently been shown, however, that controlled polymerization is possible in vesicle assem-

Figure 4



Hydrolysis of (*S,S*)-2-methyldodecanoic amhydride (empty circles) and of (rac)-2-methyldodecanoic anhydride (filled squares) at pH 8.2 and 25 C. Initial conditions: 10 ml 0.05 M *N,Ny*-bis(2-hydroxyethyl) glycine buffer, pH 8.2 and 150 μmol of the corresponding 2-methyldodecanoic anhydride. Adapted.

blies [71•]. There are a large number of studies carried out on binding of peptides to membranes [72–76] but the implications for the origin of life have not been considered.

In conclusion, concerning the origin of molecular complexity and the origin of life, it is important to point out that the processes described here in this bottom-up approach are based on spontaneous processes — self-assembly, the binding of hydrophobic substrates and peptides — as well as the hydrolysis of the acid anhydride, which is a precursor for the structural unit of the vesicle. The binding of hydrophobic peptides and/or other substrates can provide a primitive, yet effective form of chemical selection of the molecules, within the bilayer of the vesicle, depending on the partition coefficient.

The 'top-down' approach

In the 'top-down' approach, enzymes and nucleic acids can be used, as the main goal is to establish the conditions for a minimal working cell. The first exercise in this approach is to assess what extent vesicles can be used to host molecular biology reactions. We have considered a series of reactions representative of the biochemistry of enzymes and RNA/DNA, for example the Qβ replicase reaction. This enzyme is able to replicate short ribonucleic acid chains.

The first goal is to implement a system which carries out two processes at the same time: the replication of the nucleic acids previously entrapped in these vesicles along with the simultaneous reproduction of the shell. The reproduction of the vesicles can be performed using an oleic acid/oleate vesicle suspension overlaid with oleic anhydride, as previously discussed. Simultaneously, the whole reaction mixture required for RNA synthesis by Qβ replicase was entrapped in the oleic acid/oleate vesicles and, as evidenced by gel electrophoresis, new radioactively-labeled RNAs were synthesized during the process of vesicle transformation. These experiments can be seen as a first approach towards a minimal cell, in other words a system in which the 'shell' and the 'core' material increase in number simultaneously [77].

A further step in the implementation of protocell models can be considered by carrying out a relatively complex biological reaction in liposomes, such as the polymerase chain reaction leading to DNA synthesis in liposomes [78]. In this experiment it was shown that complex cyclic reactions, involving heating and cooling, can occur in liposomes. It was found that liposomes formed from phospholipids maintain their structural integrity even when heated to 60–95°C for 2–4 h. It has been suggested that life may have originated in an environment comparable to hydrothermal vents and these results showed that vesicles could play an important role as models for protocells even under such extreme conditions.

One property of phospholipid vesicles is that they are almost completely impermeable to hydrophilic compounds, such as nucleotides [79–81] and, therefore, reactions could only occur as long as substrate molecules were present inside the microreactor. For a liposome-derived form of living precursor it would be important to use amphiphilic molecules which allow a 'feeding' from the outside while the enzymes remain entrapped in the microreactor.

When referring to the use of liposomes as compartments for biochemical reactions, one should also mention work with giant vesicles [82–86•]. These are vesicles ranging typically between 10–100 mm in diameter and, therefore, they can be visualized directly by light microscopy. Furthermore, biochemicals can be injected into the cell using special micromanipulation techniques [87–89]. This permits the construction of biochemical reactors in a calibrated, step-by-step procedure, which in turn permits a gradual increase in the extent of complexity. The giant vesicle approach is one which should assist in the development of the 'top-down' approach.

Concluding remarks and outlook

The work described here is based on three main features of vesicles: the spontaneous formation of vesicles,

the hydrophobicity of the bilayer membrane, and the micro-compartimentalization of substrates in the water core, or the bilayer. Vesicles can be perceived as a separate 'microphase' in water, facilitating molecular interactions and processes which are not possible in a homogenous aqueous medium.

The bottom-up approach however, appears, very difficult to implement, and even from a conceptual point of view there are a number of difficulties to understand how specific and functional macromolecules can be obtained by spontaneous chemical processes. The lure of the RNA world probably lies in the fact that, once RNA and ribozymes are present, the construction of other specific macromolecules appears, at least on paper, conceivable. However, as already mentioned, the spontaneous formation of a self-replicating RNA family is certainly not a trivial process.

All this may be relevant for the origin of life. The key question is how much can the simple binding processes, described in this review, contribute to the increase of molecular complexity, and to the origin of macromolecular specificity, which is the basis for the implementation of catalytic and information-rich processes thus leading to minimal life.

Acknowledgements

We like to thank Professor Brian H Robinson for helpful discussions

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