LIPID AGGREGATES INDUCING SYMMETRY BREAKING IN PREBIOTIC POLYMERISATIONS

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Abstract. It is a long-standing and still open problem to determine the origin of biomolecular homochirality, and many scenarios have been suggested. Amphiphilic molecules are renowned for their capability to reorganize themselves in a variety of different morphologies and topologies, and for their capability to partition chemicals in well defined domains. Here a possible role for amphiphilic molecules inducing symmetry breaking is suggested in the framework of the research on origin of life.

Keywords: chiral selection, compartment, cubic phase, origin of life

1. Introduction

The organic molecules associated with living organisms, as well as their metabolic by-products are generally chiral and optically active, having either a dextro-(+) or a levo-(–) optical rotation. The polymeric molecules forming the basic structural units of living matter, such as proteins, polysaccharides, and nucleic acids, are generally homochiral, that is, they are made up of monomer units having uniform chirality, with no traces of the enantiomeric mirror-image monomer units in their chains. There are basically two different scenarios that are usually evoked to rationalize the emergence of chiral specificity. The first postulates that chiral specificity of the bioorganic world is a result of evolution of structures and functions of the biological level of complexity. The second scenario is opposite and postulates that the mirror symmetry of the organic material was broken already at the stage of chemical evolution.

Avetisov and Goldanskii (1996) have stressed the imperative for chiral purity in the context of informational and functional biopolymers. They pointed out that the primary structures of RNA, DNA and protein enzymes are without exception homochiral, and that the polymer chains of RNA and DNA are matrices for assembling complementary replicas. Without a complete homochirality, the coupling between appropriate bases cannot take place (Avetisov and Goldanskii, 1991). They also pointed out that biological information carriers could not evolve without chirally specific macromolecular structures and enantioselective functions assembling these structures.

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The creation and the amplification of enantiomeric excess from a racemic mixture are only the first steps on the long pathway toward living structures.

Another crucial task is the formation of a boundary that separates the aqueous medium containing optically pure material from the external environment.

All known living organisms are cellular and each cell is separated from the essentially aqueous outside environment by a thin bilayer water-insoluble membrane composed of both lipid and protein (Singer and Nicolson, 1972). In the following two sections the aspects of compartmentalization and symmetry breaking will be reviewed, and finally in Section 4 the two aspects will converge into a common perspective.

2. Compartmentalization

The presence of a boundary that separates the inside from the outside appears to be *per se* fundamental to individuate a living organism (Goldrach, 1958). In all known living beings, the boundary that separates a cell from the outside environment is based on thin membranes made of lipid and proteins. Then, from the knowledge of contemporary living organism it seems only reasonable to include the self-assembly of amphiphilic molecules into bilayer membrane as an essential step in the evolution of life on earth (Szostak *et al*., 2001). The self-organization of lipid molecules can lead to a variety of aggregates, such as lamellar, hexagonal or cubic phases depending on the external conditions. At high water content and mild condition of temperature and pressure, lamellar phases can be easily formed both in form or extended lamellae with no curvature, or in form of vesicles, which consist of curved bilayer membrane encapsulating water. Since the membrane is extremely flexible, vesicles can show a huge variety of morphologies and topologies depending on thermal fluctuation and environment condition. Possible routes that bring from simple molecules to the synthesis of amphiphiles able to form vesicles have been hypothesized (Ourisson and Nakatani, 1999) and experimental works that show the evolutionary pathway of this, aggregates in presumable prebiotic environment have been also published (Deamer and Barchfeld, 1982). If one assumes that a vesicle, during its formation, entraps a solution containing an enantiomeric excess of organic material, the first optically pure polymers can be matrices for assembling complementary replicas.

3. Breaking of Symmetry in Lipid Systems

In literature many examples are known of systems in which the mirror symmetry is broken as well as systems in which compartmentalization provides the environment where Darwinian selection can take place (Pickett *et al*., 2000; Weissbuch *et al*., 2002; Zepik *et al*., 2000). One extremely simple example of symmetry breaking

was provided by Okuyama (Pickett *et al*., 2000), that showed how hard-sphere exclusion and cylindrical confinement are sufficient conditions for the emergence of spontaneous twisting conformations.

Another possible way to obtain oligopeptides of homochiral sequence from racemic mixtures is the self-assembly of the precursor molecules into ordered architectures followed by lattice-controlled reactions. Lahav and coworkers recently proposed two-dimensional self-assemblies of enantiomeric crystalline self-assemblies on the water surface of amphiphilic activated analogs of lysine and glutamic acid (Weissbuch *et al*., 2002).

Amphiphilic molecules are renowned for their ability to partition chemically immiscible components into nano scale domains (Seddon, 2001). Often these domains exhibit intriguing complex periodic geometries with long-range order. Surprisingly, the diverse systems that self assemble in this manner, surfactants, lipids, soaps, and block copolymers, exhibit topologically identical geometries, suggesting to researchers that a common set of principles governs amphiphilic phase selection (Hamley, 2000; Imai *et al*., 2000; Luzzati *et al*., 1992; Seddon, 2001; Seddon *et al*., 1996; Tschierske, 2002).

Since the early work of Luzzati (Luzzati *et al*., 1968) on lipid systems, there have been many observations of self-organized mesostructures of amphiphiles such as cubic phases. In particular conditions of solvent concentration and temperature, lipid cubic phases are formed spontaneously from lamellar or hexagonal phases. In terms of topology cubic phases are the most complex of the mesomorphic structures occurring in lipid-water systems known so far. Concerning the numbers of layers, however, they are the simplest structures, because there is only one bicontinuous layer that extends throughout the volume (Fontell, 1990; Luzzati *et al*., 1968).

One of the most intriguing aspects of amphiphilic polymorphism is the existence of bicontinuous phases that can be traversed in any direction in both the hydrophilic (water-like) and the hydrophobic (oil-like) regions. This bicontinuity was demonstrated by measuring diffusion properties with nuclear magnetic resonance (Strey *et al*., 1992). In contrast to sponge and micro emulsion phases, which are also bicontinuous, bicontinuous cubic phases show a long-range order that can be demonstrated by the appearance of Bragg-peaks in diffraction patterns. The amphiphilic interfaces of bicontinuous cubic phases form triply periodic surfaces (TPS). A TPS divides space into two unconnected but intertwined labyrinths. Both labyrinths percolate space and provide the pathways that can be used to traverse the structure both in the hydrophilic and the hydrophobic regions. In most relevant cases, the two labyrinths are congruent to each other; i.e., the structure is balanced. A balanced structure is characterized by two (rather than by one) space groups: the space group H of a single labyrinth and the space group G of both labyrinths together. H can also be considered to be the space group of the oriented TPS as opposed to G, the space group of the unoriented TPS. H is a subgroup of index 2 of G since it comprises all symmetry operations of G with the exception of the

operations α that interchange the two labyrinths (i.e., that map one side of the TPS onto the other side). G is a super group of H, since it comprises all the symmetry operations of H plus α. Although bicontinuous ordered phases may have many of the 230 three-dimensional space groups, experiments show that most of them belong to a subset of the 36 cubic space groups. Lipid cubic phases exhibit a long-range order and mainly they belong to the space groups $Pn\bar{3}m$, $Im\bar{3}m$, and $Ia\bar{3}d$ (Hyde *et al*., 1997) as expressed in the Hermann-Mauguin notation (Hahn, 1989).

4. Enantioselection via Lipid Self Organization

In spite of all the works and the ideas that have been proposed, a unifying and generally accepted model able to cover the two aspects related with the emergence of life in prebiotic conditions, namely the creation of an enantiomeric excess and the amplification of this excess, has not been proposed yet.

In the present work the polymorphism of lipid system is utilized to deal with both the aspects of compartmentalization and symmetry breaking. The requirements of a creation of a population of optically pure vesicular system can be achieved in three steps. The first is the formation of an extended 3 dimensional chiral aggregate. The second is a polymerization in a chiral and confined environment, and finally the third one is the transformation of this aggregate into a collection of vesicles entrapping an optically active mixture of biopolymers.

Whereas the polymerization and the formation of vesicles from a cubic phases are better understood processes, the first step, that is the creation of a three dimensional chiral aggregate, is still missing a reasonable model.

The cubic phases are by definition isotropic but the case of $Ia\bar{3}d$ is peculiar. This space group has been observed in fatty acid systems (Luzzati, 1997), where the surfactants can spontaneously organize in form of curved bilayers, with negative Gaussian curvature everywhere (see Figure 1), or they can organize in form of periodical continuous monolayers (see Figure 2).

In the case of continuous monolayers the space group $I4_132$ gives the symmetry of the surface. The space group $I4_132$ is a subgroup of index 2 of $Ia\bar{3}d$ since it comprises all symmetry operations of $Ia\bar{3}d$ with the exception of the operations that interchange the two labyrinths.

The amphiphilic monomers partition the space in two balanced sub volumes shown as dark grey and light grey space in Figure 2. The surface shown in the picture represents the surface where the hydrophilic heads lie. As consequences the light grey sub volume is filled with the lipid tails and the hydrophilic heads, the water and eventually the counter ions occupy the dark grey sub volume.

Topologically, the two sub volumes are two interwoven and not intersecting chiral labyrinths. The chirality of the dark grey labyrinth is shown in Figure 3. Water-soluble molecules can be found only in the ‘water’ channel (dark grey), whereas organic molecules can be found only in the ‘oil’ channel (light grey). The

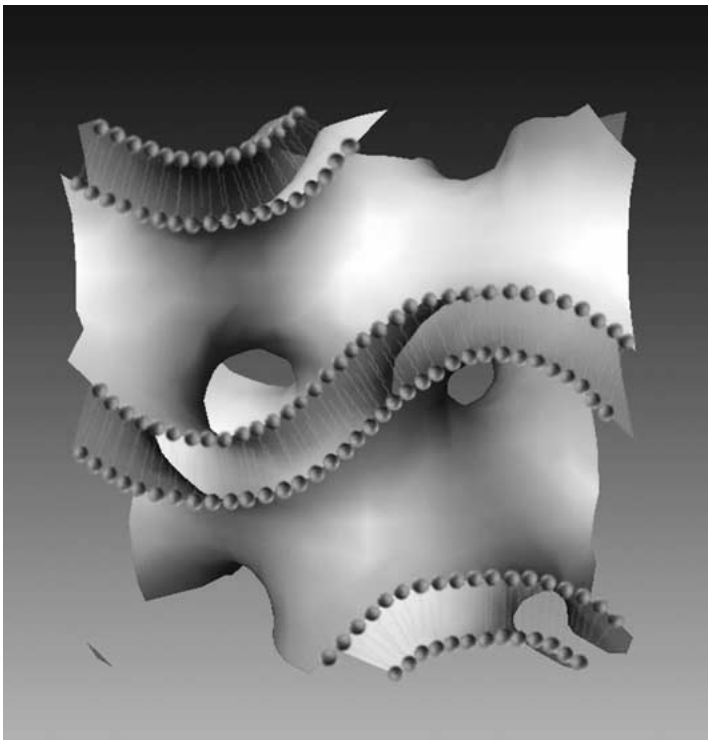

Figure 1. Two parallel surfaces enveloping a gyroid type surface. The spheres represent the amphiphilic heads.

labyrinths are balanced and molecules present inside the labyrinth can experiment enantiomorph environments. Inside each labyrinth with space group $I4_132$ two kinds of chiral channels can be found.

The case of lipid cubic phases is extremely interesting for the extension of chiral organization (Luzzati *et al*., 1993) and several billions of surfactant molecules can be arranged in a single phase.

It is worth to notice that a variety of lipid molecules are able to organize spontaneously into cubic phases, regardless the symmetry of the molecules (Seddon, 2001).

The transition from lipid phases with space group $I4_132$ into a lamellar phase, the so-called L_α-phase, and therefore the kind of lipid organization of vesicles was observed to be spontaneous under appropriate conditions (Imai *et al*., 2000; Luzzati *et al*., 1993; Seddon, 2002). A model of this transition based on X-ray analysis (Piotto, 2000) is shown in Figure 4. During this transition the symmetry of the channels geometry is broken because the section of one type of channels reduces, and finally completely vanishes. Molecules present in one labyrinth are then exposed to a chiral environment.

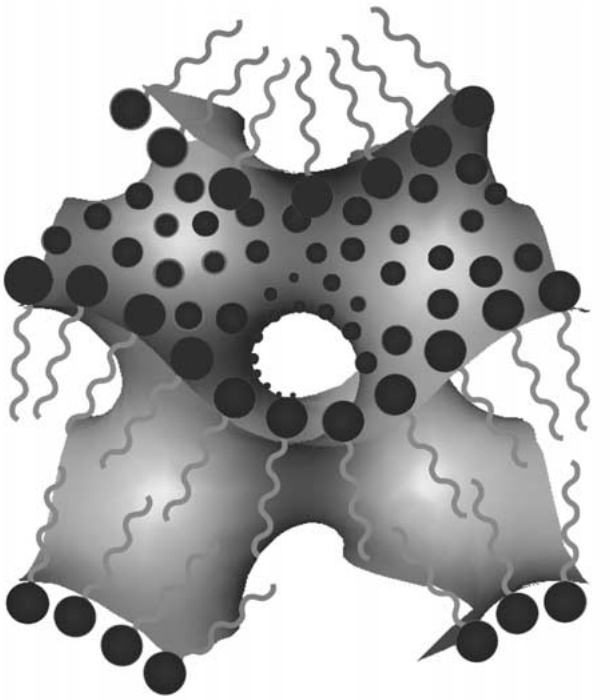

Figure 2. Amphiphiles organization in continuous monolayers based on the $I4_132$ spacegroup.

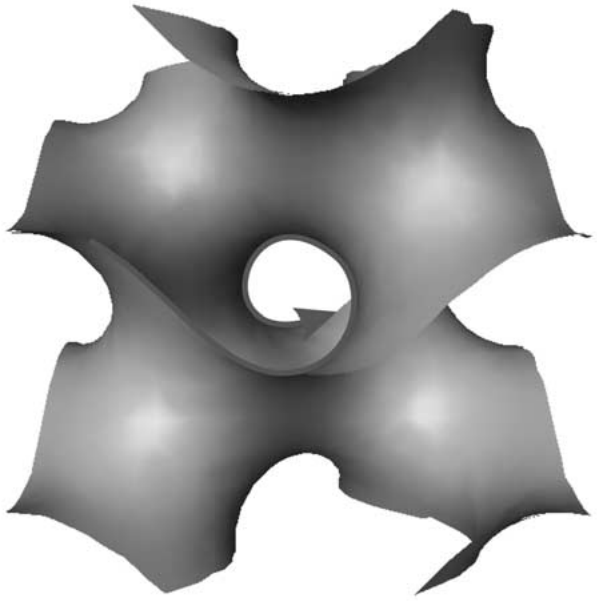

Figure 3. Chirality of the monolayer surface facing the water channel.

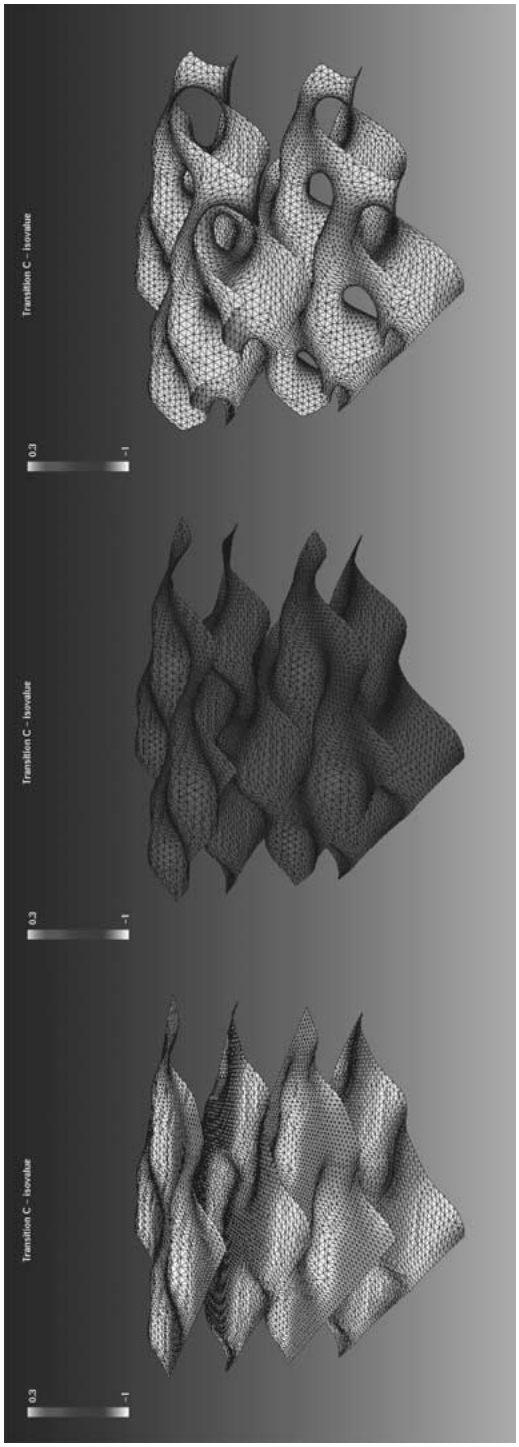

Figure 4. Possible transformation from cubic phase based on the spacegroup $I4_132$ into lamellar phase.

It is worth to recall that excluded volume itself is responsible for a remarkable range of collective structures and pattern formation, like solvation shells in simple fluids, or solvent swelling of polymers. These all come by requiring that no two physical bodies overlap. The excluded volume is merely a constraint, which may be supplemented by further constraints like the homochirality of the channels.

In principle, every polymerisation, taking place in confined systems like vesicles, should take place in the water filled channel. A polymerisation of water-soluble molecules, taking place in only one chiral channel, might experiment a chiral environment and therefore be enantioselective.

The transition from gyroid based lipid phase and lamellar phase is spontaneous in the appropriate conditions of temperature, water concentration and composition.

Vesicles can develop exclusively within the fluid lamellar state; under the conditions that the membranes are soft enough to bend into closed shells and can decrease the overall energy of the system by avoiding unfavorable hydrophobic contributions. The formation and the stability of the respective structures are the result of the interactions between the lipids and water, but also of the bending energy of the lipid arrangement. Under moderate stirring or hand shaking, vesicles form very easily from lamellar phase and the resulting vesicles will entrap the material inside the channel. At the end of the process, an entire population of vesicles, containing enantiomeric enriched material, can be available.

5. Conclusions

Until now breaking of symmetry and compartmentalization have been usually seen as two distinct aspects with a specific role in the frame of the emergence of life. This paper suggests a key role of lipid molecules in both these aspects. In fact the breaking of symmetry can be achieved via polymerization in confining chiral environment such as cubic lipid phases, and such chiral environments can be formed although starting from intrinsically non-chiral monomers.

The formation of an optically active mixture of biopolymers inside the chiral channels of a surfactants macroaggregate can generate a population of optically pure vesicular systems via the well-known transition from a cubic into a lamellar phase.

From these considerations results that amphiphilic molecules can play important roles in the development of compartmentalization as well as in the induction of symmetry breaking in polymerization under prebiotic conditions.

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