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Research on the Origin of Life: Membrane-Assisted Polycondensations of Amino Acids and Peptides

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Abstract: The question as to whether and to what extent the phospholipid membrane of liposomes can assist in the polymerization of amino acids and peptides has been investigated. It has been found that the membrane can select hydrophobic amino acids and peptides, thus operating as a selection tool in the polymerization reaction and thus permitting the formation of oligopeptides not possible in water – in the absence of liposomes – as a result of the too low solubility.

Keywords: Amino acids · COST · Origin-of-life research · Membrane-assisted polymerization · Peptides

Research on the origin of life is actually based on a simple chemical question: how can molecular complexity be created from very simple reagents (prebiotic molecules) and simple (spontaneous) reactions? How can a succession of spontaneous chemical steps turn later into molecular organization, up to the extraordinary complexity of a living cell?

The first step of this prebiotic molecular evolution is the formation of simple moieties such as amino acids, sugars, and aromatic bases of nucleic acids. Stanley Miller's famous experiment in 1953 – the synthesis of amino acids by the action of electric discharge in a reducing atmosphere of H_2O, CH_4, NH_3, and H_2 [1][2] – signified the beginning of research on prebiotic chemistry, which has proceeded successfully ever since.

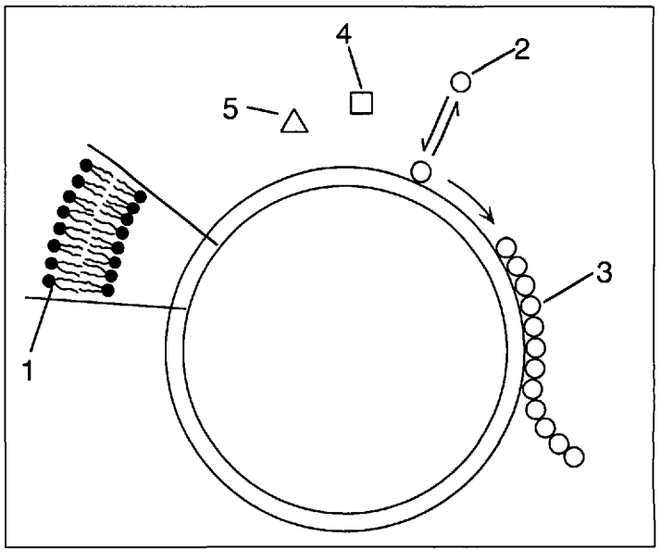
It is clear, however, that the availability of low molecular weight compounds does not solve the problem of how life originated on our planet. For that, the next level of molecular complexity needs to be clarified, namely the formation of macromolecules such as proteins and nucleic acids. In particular there are two problems associated with the origin of biological macromolecules: one is the *polymerization* itself, namely the process by which long chains are formed starting from the random mixture of monomers. This is a process that would afford statistically a random mixture of an astronomical number of macromolecules. For example the theoretical number of polypeptide chains with polymerization degree 60 that can be formed from the 20 different amino acids is 10^{78}, a number which corresponds approximately to the estimated number of atoms in the universe (9×10^{78}) [3]!

The second problem is *selection*, namely the process that would select only a few polypeptides with specific functions from this random mixture. Consider that the estimated number of different proteins existing on our planet is probably in the order of 10^{12}, an infinitesimal fraction with respect to the theoretical possible number pointed out before. (Each organism contains less than ~60000 essential proteins [4], and there may be as many as 100 million – or even more – different species on Earth, although at the moment only ~1.5 million have been identified [5].)

These two questions – polymerization and selection – are still basically unanswered in the current research on the origin of life. Clearly, these are typical questions within the field of macromolecular chemistry.

How can one induce polymerization, say of amino acids, in such a way that there is simultaneously some sort of selection – a process that selects only a small number of possible chains out of the large theoretical number?

In our work, we are using the hydrophobic membrane of lipid vesicles (liposomes) as a matrix for polymerization. This idea is based on the hypothesis shared by several researchers in the field of the origin of life, that lipidic aggregates were very important in prebiotic times, *e.g.* [6][7]. Liposomes are spherical bilayer aggregates formed by lipidic surfactants with typical dimensions between 50 and 500 nm in diameter, containing an aqueous pool as their inner cavity. In this sense, they resemble the lipid matrix of a single cell membrane. The lipidic surfactant can be cationic, anionic, or neutral (including zwitterionic). The most well-known amphiphiles to form bilayers are the lipidic surfactants based on glycerol, such as POPC (1-palmitoyl-2-oleoly-*sn*-



Schematic representation of a cross-section through an unilamellar lipid vesicle composed of phospholipid molecules (**1**). Dipeptides (**2**) for which the vesicle membrane shows affinity are adsorbed onto the vesicle surface and polymerized (**3**). Dipeptides **4** and **5** are not bound to the vesicles and therefore not polymerized.

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glycero-3-phosphocholine) and similar compounds with different alkyl chains and head groups. The corresponding liposomes consist of a membrane with a hydrophobic core that is able to bind hydrophobic compounds, such as hydrophobic amino acid derivatives and peptides, as well as lipophilic condensing agents (Figure).

We have been able to bind the barely water-soluble condensing agent EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) to POPC liposomes. When these liposomes were exposed to a library of tryptophan-containing dipeptides such as H-Asp-Trp-OH, H-Trp-Trp-OH, H-Glu-Trp-OH, H-Gly-Trp-OH, only H-Trp-Trp-OH was bound to the liposomes and therefore oligomerized with significant yields (*i.e.* out of the theoretically possible 16 tetrapeptides, H-Trp-Trp-Trp-Trp-OH makes about 70% of all tetrapeptides formed) [8]. The other peptides are too hydrophilic and were not selected. With this type of selection, tryptophan oligomers can be formed. The products of such an oligomerization become more hydrophobic with increasing length and remain attached to the liposomal matrix. We have therefore two selection steps, one arising from the choice of the monomer, and one from the possibility of polymerizing water-insoluble compounds on the liposome membrane.

As an alternative to the use of a hydrophobic condensing agent, we have carried out direct polymerization of N-carboxy anhydride (NCA) amino acids, such as NCA-Trp. Also in this case, we could obtain long oligomers (up to a polymerization degree of 29), which cannot be obtained by aqueous polymerization methods in the absence of liposomes [8].

The selectivity and specificity of the liposome membrane can be changed and regulated by the type of lipidic surfactants (*i.e.* addition of ionic co-surfactants). For example, the positively charged DDAB (didodecyldimethylammonium bromide) forms stable mixed liposomes with POPC. This lipidic bilayer can now also attract negatively charged amino acid derivatives – for example NCA-glutamic acid – and induce polymerization.

The possibility of obtaining co-oligopeptides made out of hydrophobic and ionic amino acids in the same molecule is the next challenge. As a matter of fact, we have been able to condense the dipeptide H-His-Trp-OH to the corresponding hexapeptide. Recognizing the catalytic character of the imidazole group, this may open up ways to construct simple, catalytically active polypeptides.

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Enantioselective Proton Transfer Chemistry: Asymmetric Synthesis with Chiral Lithium Amide Bases

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Enantioselective proton transfer chemistry has been relatively underdeveloped, considering the potential power and broad applicability of this approach [1]. Notable early contributions in this area include the enantioselective protonation of enolates by Duhamel and Plaquevent [2], and the asymmetric rearrangement of cyclohexene oxide by reaction with a chiral lithium amide, described by Whitesell and Feldman [3].

We have devoted a substantial part of our research effort to the development of desymmetrisation reactions using chiral lithium amide bases, including **1** and **2**, and have helped to establish this approach as a tool for asymmetric synthesis. Much of this chemistry involves the reaction of a cyclic (or polycyclic) prochiral ketone with a chiral lithium amide base in a process that involves the base selecting between enantiotopic hydrogens. For example, the use of base **1** allows the conversion of ketone **3** into the enol silane **4**, and the azabicyclic ketone **5** into the aldol product **6**, both in high enantiomeric excess (Scheme 1).

This type of transformation has obvious applications in target synthesis. For example we have prepared the unique alkaloid toxin anatoxin-a **7** (the unnatural enantiomer) by this method [4], as well as a protected form of thymine polyoxin C **8** [5]. Current activities in this area involve the development of a strategy for the synthesis of functionalised intermediates possessing the [5-8-5] framework found in natural products such as the ophiobolins and fussicoccins, *e.g.* conversion of ketone **9** into the complex intermediate **10**, as shown in Scheme 2 [6].

In addition to applications in the area of enolate chemistry, chiral lithium amides have also been used to generate reactive anions, in non-racemic form, from a considerable range of non-ketonic substrates. Examples include the enantioselective synthesis of

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