

## **Catalysis by metalloenzymes and the origin of life**

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A living cell is a highly complex chemical system characterized by a spatio-temporal order. It can be seen as a chemical reactor in which a large number of reactions that follow extremely precise temporal sequences take place. Time control in the cell is achieved by catalysts called enzymes. A reaction will take place when the necessary enzyme is present while in its absence the rate of the reaction will be so low it can in general be neglected. The presence of a specific enzyme depends on the expression of a gene which itself is controlled by other molecules called effectors. In this short paper, we will focus on the origin of these biological catalysts whose roles are so fundamental that a living system without enzymes is inconceivable. We would however like to insist that this does not mean that we consider that the “metabolism first” model for the origin of life is better than the “genes first” model.<sup>1</sup> From our point of view, the search for “the” origin of life is vain as the transition from “non living” to “living” was necessarily a continuous process and it would therefore be impossible to find the discontinuity that one could eventually consider as “the origin” of life. As a result, when confronted with the transition from “non living” to “living” systems, Aristotelian logic cannot be used and we think should be replaced by fuzzy logic or multi-valued logic.<sup>2</sup> In this context, the choice between the “metabolism first” scenario and the “genes first” scenario is irrelevant. What is however clear, is that catalysis was certainly one of the most important aspects of the chemical evolution which led to the first living systems.

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<sup>1</sup> A. Pross (2004). Causation and the origin of life. *Metabolism or replication first?* *Orig. Life Evol. Biosph.* **34**, 307-321

<sup>2</sup> G. Bruylants, K. Bartik and J. Reisse. Is it useful to have a clear-cut definition of life? On the use of fuzzy logic in prebiotic chemistry. *Orig. Life Evol. Biosph.* (accepted for publication)

Biological catalysts are essentially proteins but a number of catalytical polynucleotides have been discovered in the past two decades. The term “ribozymes” has been coined for these catalysts as they are small RNA molecules. The discovery of ribozymes was very important because it offered a solution to a problem which could appear to be a catch-22 situation. Until the discovery of ribozymes, scientists were convinced that the synthesis of proteinic enzymes depended on other proteinic enzymes. When the mechanism of protein synthesis inside the ribosome was finally fully elucidated, it was discovered that a ribozyme was a key catalytic element of the very complex ribosome structure.<sup>3,4</sup> On the basis of this crucial observation, but also of other observations, it is now generally accepted that a “RNA world” predated a “RNA-DNA-protein world”.<sup>5,6</sup> Of course, this does not resolve all the difficulties encountered when explaining the chemical evolution leading to life because polyribonucleotides are very complex molecules and their abiogenic synthesis seems highly improbable.

One could of course ask what was present before the “RNA world”. This question, as many others related to the origin of life, remains unsolved. For some authors, small peptides with catalytic properties could have played a role in the synthesis of the first ribozymes but, until now, no experimental arguments have been found to support this scenario. Nonetheless, even if we accept the hypothetical scenario of a “polypeptide world” before the “RNA world” (which itself came before the present “DNA-RNA-Protein world”) a major problem remains. Indeed, peptides, ribozymes and proteins are organic polymers which formally result from the condensation of monomers through the elimination of water molecules. Obviously, this type of condensation reaction cannot take place spontaneously in liquid water as hydrolysis would be the dominant process. Today, in the living world, the condensation of monomers proceeds easily because, after billions of years of biological evolution, strategies have been developed to circumvent this difficulty. Activation of the monomers and isolation of the reaction site within internal hydrophobic cavities of enzymes or supramolecular enzymatic complexes are examples of these strategies. For people interested in the transition from “non-living” to “living” systems, a further question arises immediately: which synthetic pathways lead to the formation of peptides or small RNA when proteinic enzymes or ribozymes were not yet present?

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<sup>3</sup> N.Ban, P.Nissen, P.B. Moore and T.A. Steitz (2000). The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289**, 905-920

<sup>4</sup> P. Nissen, J. Hansen, N. Ban, P.B. Moore and T.A. Steitz (2000). The structural basis of ribosome activity in Peptide Bond Synthesis. *Science* **289**, 920-930

<sup>5</sup> W. Gilbert (1986). The RNA world. *Nature* **319**, 618

<sup>6</sup> G.F. Joyce (1989). RNA Evolution and the Origin of Life. *Nature* **333**, 217-224

To solve this question, a suggestion was formulated many years ago, and reformulated under various forms since then. Bernal, one of the pioneers of prebiotic chemistry, suggested that lamellar materials like clays could have played a role in the first steps of prebiotic chemistry.<sup>7</sup> Clays have catalytic properties and their lamellar structure protects the adsorbed molecules from water. They are generally hydrated silica and contain water molecules but adsorbed organic molecules would not be confronted to as large an excess of water molecules as when they are dissolved in liquid water. In 1982, Cairns-Smith<sup>8</sup> was the first to discuss in detail a scenario in which minerals like clays were not only involved in prebiotic stages as catalysts or adsorbents but he also suggested that the first “living organisms” were based on inorganic solid state chemistry. The title of his book “Genetic takeover and the mineral origins of life” shows that Cairns-Smith was interested by the origin of the genetic code. In this book he tries to convince his readers that crystals with defects can play the role of information support and that this “crystallographic information” can even be copied and transferred. The work of Cairns-Smith, even if frequently cited, has not had a very deep impact, probably because his main point was the origin of the genetic code but also because the hypothetical transition from a mineral living world towards an organic living world remains puzzling. Cairns-Smith tried to address this fundamental question in chapter 8 of his book, entitled “The entry of carbon”, but his comments on this topic are very general and devoted essentially to the hypothetical mechanisms by which a genetic code based on clays could lead to a hypothetical code based on polynucleotides.

We wish to examine here how catalysis by biopolymers (peptides, small RNA) could have emerged from “non-living” systems in which this task was achieved by inorganic molecules. Catalysis by acids, bases and transition metals (at various oxidation levels) is well known and studied for more than a century, not only from the fundamental point of view but also because of the numerous industrial applications<sup>9</sup>. Indeed, a majority of chemical reactions used in the organic and polymer industries are catalysed by transition metals, acids or bases. We will focus on catalysis by transition metals and more precisely on homogenous catalysis involving transition metal cations. Homogeneous catalysts are generally organic complexes of transition metal cations.<sup>10</sup> It is interesting to observe that the catalytic activity of a large number of

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<sup>7</sup> J.D. Bernal (1960). The problem of stages in biopoesis. p 30-45 in Aspects of the origin of Life. Editor M. Florkin, Pergamon

<sup>8</sup> A.G. Cairns-Smith (1982). Genetic Takeover and the Mineral Origin of Life. Cambridge University Press

<sup>9</sup> B.J. Söderberg (2004). Transition metals in organic synthesis: highlights for the year 2002. Coord. Chem. Rev. **248**, 1085-1158

<sup>10</sup> G.P. Parshall and S.D. Ittel (1992). Homogeneous Catalysis. The Applications and Chemistry of Catalysis by Soluble Transition Metal Complexes, J. Wiley (2d edition)

present-day ribozymes is strongly dependent on the presence of cations,<sup>11,12,13</sup> sometimes  $Mg^{++}$  but more frequently transition metal cations (a search in Pub.Med based on the key words “ribozyme” and “transition metal” gives 482 entries). For ribozymes, which are polyanions, the cations certainly play a structural role but they also play a direct catalytical role. The hypothetical but plausible “RNA world” would have been a world where metallic cations played a very important role. We would like to suggest that metallic cations would probably also have played an important role in the hypothetical peptidic “pre-RNA world”. Our main point is the following: in the long and progressive evolution from a hypothetical “peptide world” towards the plausible “RNA world”, and then towards our “DNA-RNA-Protein world”, metallic cations were always key factors, as structural entities but much more importantly, as catalysts or as components of catalytical systems.

A peptide is nothing more than a small protein and we therefore wish to focus on the very important class of bio-catalysts called proteinic metalloenzymes. In these systems, the metal ions are part of a non-proteinic organic moiety, called co-factor, which is inserted inside the protein and which interacts with it through non-covalent bonds. The most ancient, ubiquitous and diverse class of co-factors is, according to Bandyopadhyay et al., iron sulphur clusters which are found in more than 200 types of enzymes.<sup>14</sup> This observation must be correlated to the scenario suggested by Gunther Wächterhauser for the origin of life<sup>15,16</sup> for whom, an “iron-sulfur world” was an important stage in prebiotic chemistry. In his numerous papers, he suggests reactions which could be favoured by the  $H_2S-FeS$  couple.  $FeS$  and  $H_2S$  react to give  $FeS_2$  and  $H_2$  and this reaction is highly exergonic (it liberates free energy) and according to Wächterhauser, this could be the driving force for a rich organic chemistry based on the reduction of  $CO_2$  under mild conditions. Many other authors have pointed out the possible role of iron and the iron-sulfur system in the first stages of the origin of life,<sup>17,18</sup> by

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<sup>11</sup> E.L. Christian, N.M. Kaye and M.E. Harris (2000). Helix P4 is a divalent ion binding site in the conserved core of the ribonuclease P ribozyme. *RNA* **6**, 511-519

<sup>12</sup> M. Roychowdhury-Saha and D.H. Burke (2006). Extraordinary rates of transition metal ion-mediated ribozyme catalysis. *RNA* **12**, 1846-1852

<sup>13</sup> P.M. Gordon and J.A. Pierilli (2001). Metal ion coordination, by the AGC triad in domain 5 contributes to group II intron catalysis. *Nat. Struct. Biol.* **8**, 893-898

<sup>14</sup> S. Bandyopadhyay, K. Chandramouli and M.K. Johnson (2008). Iron-sulfur cluster biosynthesis. *Biochem. Soc. Trans.* **36**, 1112-1119

<sup>15</sup> G. Wächterhauser (1988). Before enzymes and templates: theory of surface metabolism. *Microbiol. Rev.* **52**, 452-484

<sup>16</sup> G. Wächterhauser (1992). Grounworks for an evolutionary biochemistry: the iron-sulfur world. *Prog. Biophys. Mol. Biol.* **58**, 85-201

<sup>17</sup> R.J.P. Williams (1990). Iron and the origin of life. *Nature* **243**, 213-214

<sup>18</sup> M.R. Edwards (1998). From a soup or a seed ? Pyrite metabolic complexes in the origin of life. *Trends Ecol. Evol.* **13**, 178-181

considering not only chemical reactions taking place at the surface of iron-sulfur crystals but also on iron-sulfur bubbles.<sup>19</sup> The interest for the “iron-sulfur world” is directly related to the discovery of the rich chemistry taking place near submarine hot springs where iron sulfides and H<sub>2</sub>S are present. Already in 1988, Cairn-Smith and his co-workers had established a possible link between this chemistry under extreme conditions and the origin of life.<sup>20</sup> It is indeed tempting to substantiate this iron-sulfur scenario by the observation that iron sulphur clusters play an important role in the present day living world, but we must remember that this kind of extrapolation into the past can, at best, give information about the metabolisms taking place in the last universal common ancestors (LUCA), which were already cells with a DNA code and proteinic enzymes. Delaye and Lazcano have also pointed out the difficulty in extrapolating present day metabolic data towards a very remote past.<sup>21</sup> It is furthermore important to point out that homogeneous catalysts used in synthetic organic chemistry are generally much less efficient than metalloenzymes. The protein polymeric structure of metalloenzymes is, without doubt, a structural element which is important in terms of catalytic efficiency.

The spontaneous formation, under prebiotic conditions, of medium-sized molecules (able to act as co-factors) containing one or a few cations seems an acceptable hypothesis. Indeed, some of the organic molecules found, for example, in the Murchison meteorite,<sup>22</sup> such as nitrogen containing aromatic compounds, are known to be efficient ligands of cations. The exceptional catalytic efficiency of proteinic metalloenzymes could be the consequence of a fortuitous association of a peptide and a organometallic complex, followed by a natural selection process of the Darwinian type leading to a longer peptide with better catalytic properties and finally, to a metalloprotein. To be submitted to Darwinian evolution, the system in which the initial association between the potential co-factor and a peptide occurred must have been sufficiently complex so as to be able to reproduce itself by autocatalysis. Of course, the scenario suggested here is not supported by any observations or experimental simulations: this hypothetical stage of evolution can only be suggested on the basis of the final result i.e. the observation that today, metalloenzymes are extremely efficient biocatalysts.

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<sup>19</sup> M.J. Russell, R.M. Daniel, A.J. Hall and J.D. Sherringham (1994). A hydrothermally precipitated catalytic iron sulphide membrane as a first step towards life. *J. Mol. Evol.* **39**, 231-243

<sup>20</sup> M.J. Russell, A.G. Cairn-Smith and P.S. Braterman (1988). Submarine hot springs and the origin of life. *Nature* **336**, 117

<sup>21</sup> L. Delaye and A. Lazcano (2005). Prebiological evolution and the physics of the origin of life. *Phys. Life Rev.* **2**, 47-64

<sup>22</sup> J. Reisse and F. Mullie (1987). Organic matter in carbonaceous chondrites. *Top. Curr. Chem.* **139**, 83-117

At the end of this note and as a short summary, we would like to suggest that a “pre-RNA world” was a “metallo-peptidic world” involving polypeptides interacting through non-covalent interactions with organometallic complexes (co-factors). Catalysis by transition metal cations is so important in the living world today that it is difficult to imagine that these cations (which were necessarily present since the Earth’s accretion) were not involved from the very beginning in the chemical evolution which led to the first “living” systems. If we apply fuzzy logic to the origin of life problem and if we decide to give a “living index” equal to 0 (non-living system) to the system represented by small sample of the Hadean hydrosphere (including all the dissolved molecules and ions) and a value of 1 (for a living system) to the very first unicellular prokaryotic cell(s), metallic cations together with water, were most probably the only chemical constituents which played a role during the entire process leading from a living index of 0 to a living index of 1.