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Studying the Process of Formation of Precambrian Period Limestone Dolomite Fossils of Stromatolites in Hot Mineral Water Interacting with $CaCO_3$

Ignat Ignatov[1*] Oleg Mosin[2]
1. DSc, Professor, Scientific Research Center of Medical Biophysics (SRCMB), N. Kopernik Street, 32, Sofia 1111, Bulgaria
2. PhD (Chemistry), Biotechnology Department, Moscow State University of Applied Biotechnology, Talalikhina Street, 33, Moscow 109316, Russian Federation

Abstract

We studied the conditions of formations of stromatolites in hot mineral water. Studying these formations are very important and interesting as stromatolites store information about nascent life on earth and organic part of the first living organisms - numerous colonies of cyanobacteria, blue-green algae and oil assimilating bacteria, encountered in strata of limestone and dolomite in the crater of extinct volcanoes and thermal sources. For this aim were performed experiments with hot mineral and seawater from Bulgaria using IR-, and DNES-spectroscopy. There were discussed the reactions of condensation and dehydration occurring in alkaline aqueous solutions at 65–95 ^{0}C and pH = 9–10, resulting in synthesis from separate molecules larger organic molecules as polymers and short polipeptides. It was shown that hot alkaline mineral water with temperature from +65 ^{0}C to +95 ^{0}C and pH value from 9 to 11 is more suitable for the origination of life and living matter than other analyzed water samples. The pH value of seawater on contrary is limited to the range of 7.5 to 8.4 units. In hot mineral waters the local maximums in IR-spectra are more manifested compared to the local maximums obtained in IR-spectra of the same water at a lower temperature. The difference in the local maximums from +20 ^{0}C to +95 ^{0}C at each 5 ^{0}C according to Student *t*-criterion – $p < 0.05$. These data indicate that the origination of first organic forms of life depends on the structure and physical chemical properties of water, as well as its temperature and pH value. **A**lso it was demonstrated the role of limestone and silica in the formation of the first organic life forms.

Keywords: stromatolites, hot mineral water, origin of life, IR, DNES.

1. Introduction

Previous biological experiments with D_2O and structural-conformational studies with deuterium substituted molecules, performed by us, enable to modeling conditions under which the first living forms of life might be evolved (Ignatov & Mosin, 2013a; Ignatov & Mosin, 2013b; Ignatov & Mosin, 2013c). The content of deuterium in hot mineral water may be increased due to the physical chemical processes of the deuterium accumulation. It can be presumed that primary water might contain more deuterium at early stages of evolution of first living structures, and deuterium was distributed non-uniformly in the hydrosphere and atmosphere (Ignatov & Mosin, 2012). The primary reductive atmosphere of the Earth consisted basically of gas mixture CO, H_2, N_2, NH_3, CH_4, lacked O_2–O_3 layer protecting the Earth surface from rigid short-wave solar radiation carrying huge energy capable to cause radiolysis and photolysis of water. The processes accompanying accumulation of deuterium in the hydrosphere are solar radiation, volcanic geothermal processes and electric discharges in the atmosphere. These natural processes could lead to the enrichment of the hydrosphere by deuterium in the form of HDO which evaporates more slowly than H_2O, and condenses faster. If this is true, this is a significant fact regarding thermal stability of deuterated macromolecules in the preservation of life under thermal conditions, because chemical bonds with participation of deuterium are somewhat stronger than those ones formed of hydrogen (Mosin & Ignatov, 2012a; Mosin & Ignatov, 2012b).

Natural prevalence of deuterium makes up approximately 0.015–0.020 at.%, and depends strongly on the uniformity of substance and the total amount of matter formed in the course of early Galaxy evolution (Linsky, 2007). The constant sources of deuterium are explosions of nova stars and thermonuclear processes frequently occurring inside the stars. Perhaps this explains the fact that the total amount of deuterium is slightly increased in the period of global warming.

The gravitational field of the Earth is insufficiently strong for the retaining of lighter hydrogen, and our planet is gradually losing hydrogen as a result of its dissociation into interplanetary space. Hydrogen evaporates faster than heavy deuterium, which can be collected by the hydrosphere. Therefore, as a result of this natural process of fractionation of H/D isotopes throughout the process of Earth evolution there should be an accumulation of deuterium in the hydrosphere and surface waters, while in the atmosphere and in water vapour deuterium content tends to be low. Thus, on the planet there occurs a natural process of separation of H and D isotopes, playing an essential role in the maintenance of life on the planet.

The second point regards the influence of temperature on the life processes. Recent studies performed by us showed that the most favorable for the origin of life and living matter seem to be hot alkaline mineral waters interacting with $CaCO_3$ (Ignatov, 2010; Ignatov & Mosin, 2013d). According to the law for conservation of energy the process of self-organization of primary organic forms in water solutions may be supported by thermal energy of magma, volcanic activity and solar radiation. According to J. Szostak, the accumulation of organic compounds in open lakes is more possible compared to the ocean (Szostak, 2011). Life began near a hydrothermal vent: an underwater spout of hot water. Geothermal activity gives more opportunities for the origination of life. In 2009 A. Mulkidjanian and M. Galperin demonstrated that the cell cytoplasm contains potassium, zinc, manganese and phosphate ions, which are not particularly widespread in the sea aquatorium (Mulkidjanian & Galperin, 2009). J. Trevors and G. Pollack proposed in 2005 that the first cells on the Earth assembled in a hydrogel environment (Trevors & Pollack , 2005). Gel environments are capable of retaining water, oily hydrocarbons, solutes, and gas bubbles, and are capable of carrying out many functions, even in the absence of a membrane. Hydrocarbons are an organic compounds consisting entirely of hydrogen and carbon. The previous data showed that the origination of living matter most probably occurred in hot alkaline mineral water containing $CaCO_3$ at $t = 75\text{-}95\ ^0C$ and pH = 9-11 (Ignatov, 2012). This might occur in ponds and hydrothermal vents in seawater or hot mineral water. An indisputable proof of this is the presence of stromatolites fossils. They lived in warm and hot water in zones of volcanic activity, which could be heated by magma and seem to be more stable than other first marine organisms.

The purpose of the research was studying the process of formation of stromatolites in hot mineral water for possible processes for origin of life and living matter in hot mineral water. Within the frames of the research the various samples of water from Bulgaria were studied.

2. Material and Methods

2.1. Objects of Studying

2.1.1. Biological Objects

As model systems were used cactus juice of *Echinopsis pachanoi* and Mediterranean jellyfish *Cotylorhiza tuberculata* (Chalkida (Greece), Aegean Sea).

2.1.2. Water Samples

The research by the IR-spectrometry (DNES-method) was carried out with samples of water taken from various water springs of Bulgaria:

1 – Mineral water (Rupite, Bulgaria);
2 – Seawater (Varna resort, Bulgaria);
3 – Mountain water (Teteven, Bulgaria);
5 – Deionized water (the control).

2.1.3. IR-Spectroscopy

IR-spectra of water samples were registered on Brucker Vertex (“Brucker”, Germany) Fourier-IR spectrometer (spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; permission – 0.5 cm^{-1}; accuracy of wave number – 0.1 cm^{-1} on 2000 cm^{-1}) and on Thermo Nicolet Avatar 360 Fourier-transform IR (M. Chakarova)

2.1.4. DNES-Spectroscopy

The research was made with the method of differential non-equilibrium spectrum (DNES). The device measures the angle of evaporation of water drops from 72 ^{0} to 0 ^{0}. As the main estimation criterion was used the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in water’s samples. The spectra of water were measured in the range of energy of hydrogen bonds 0,08–0,1387 eV with using a specially designed computer program.

2.1.5. High-Frequency Coronal Electric Discharge Experiments

A device for high-frequency coronal electric discharge was used in this study, constructed by I. Ignatov and Ch. Stoyanov (Ignatov & Mosin, 2013e). The frequency of the applied saw-tooth electric voltage was 15 kHz, and the electric voltage – 15 кV. The electric discharge was obtained using a transparent firm polymer electrode on which a liquid sample of water (2–3 mm) was placed. The spectral range of the photons released upon electric discharge was from $\lambda = 400$ to $\lambda = 490$ nm and from $\lambda = 560$ to $\lambda = 700$ nm.

2.1.6. Scanning Electron Microscopy (SEM)

SEM was carried out on JSM 35 CF (JEOL Ltd., Korea) device, equiped with SE detector, thermomolecular pump, and tungsten electron gun (Harpin type W filament, DC heating); working pressure – 10^{-4} Pa (10^{-6} Torr); magnification – ×150.000, resolution – 3.0 nm, accelerating voltage – 1–30 kV; sample size – 60–130 mm.

3. Results and Discussion

3.1. The Research of Various Water Samples on the Feasibility for Origin of Life

We carried out the research of various samples of mineral water obtained from mineral springs and seawater from Bulgaria (Fig. 1, curves *1–5*). For this aim we employed the IR-spectrometry and DNES method relative to the control – deionized water.

For calculation of the function *f(E)* represented the energy spectrum of water, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = \frac{14{,}33 f(\theta)}{[1-(1+bE)^2]^2}, \qquad (1)$$

where $b = 14{,}33$ eV^{-1}

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H_2O molecules is calculated by the formula:

$$\theta = arcos\ (-1 - 14{,}33E) \qquad (2)$$

The cactus juice was also investigated by the DNES method (Fig. 1, *curve 1*). The cactus was selected as a model system because this plant contains approximately 90 % of water. The closest to the spectrum of cactus juice was the spectrum of mineral water contacting Ca^{2+} and HCO_3^- ions (Fig. 1, *curve 2*). DNES-spectra of cactus juice and mineral water have magnitudes of local maximums at –0.1112; -0.1187; -0.1262; -0.1287 and –0.1387 eV. Similar local maximums in the DNES-spectrum between cactus juice and seawater were detected at -0,1362 eV. The spectrum of the control sample of deionized water (Fig. 1, *curve 5*) was substantially different from the spectra of seawater and mineral water.

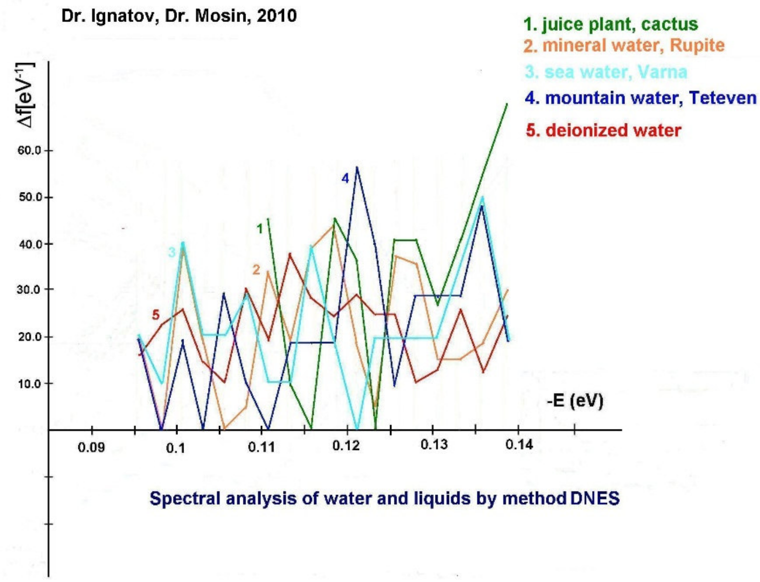


Figure 1: DNES-spectra of water samples of various origin: 1 – cactus juice; 2 – mineral water from Rupite village (Bulgaria); 3 – seawater (Varna, Bulgaria); 4 – mountain water (Teteven, Bulgaria); 5 – deionized water (the control)

As shown from these data, the closest to the IR-spectrum of cactus juice was mineral water from Rupite Village (Bulgaria), which DNES and IR spectrum is shown in Fig. 1 and Fig. 2 (Thermo Nicolet Avatar 360 Fourier-transform IR). IR-spectra of cactus juice and mineral water with HCO_3^- (1320–1488 mg/l), Ca^{2+} (29–36 mg/l), pH (6.85–7.19), have local maximums at 8.95; 9.67; 9.81; 10.47 and 11.12 μm (Fourier-IR spectrometer Brucker Vertex). Common local maximums in the IR-spectrum between cactus juice and seawater are detected at 9.10 μm. The local maximums obtained with IR method at 9.81 μm (1019 cm^{-1}) and 8.95 μm (1117 cm^{-1}) (Thermo Nicolet Avatar 360 Fourier-transform IR) are located on the spectral curve of the local maximum at 9.7 μm (1031 cm^{-1}) (Fig. 2). With the DNES method were obtained the following results – 8.95; 9.10; 9.64; 9.83; 10.45 and 11.15 μm, or 897; 957; 1017; 1037; 1099 and 1117 wave numbers (Table 1).

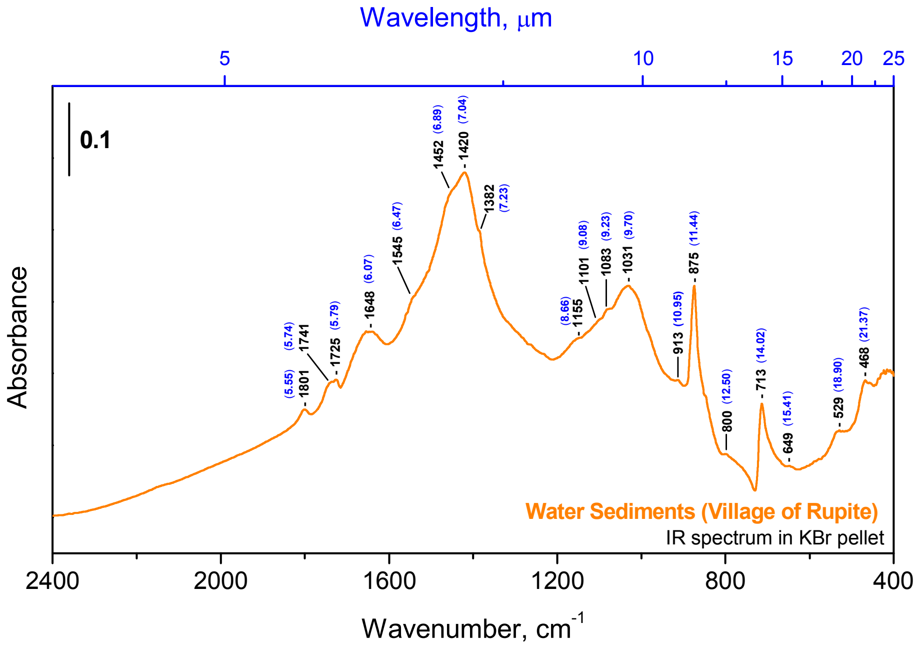


Figure 2: IR-spectrum of water obtained from Rupite Village (Bulgaria)

Table 1: Characteristics of spectra of water of various origin obtained by DNES-method*

-*E*, eV			λ, μm	k, cm^{-1}
Cactus juice	Mineral water from Rupite Village (Bulgaria)	Seawater		
0.1112	0.1112	–	11.15	897
0.1187	0.1187	–	10.45	957
0.1262	0.1262	–	9.83	1017
0.1287	0.1287	–	9.64	1037
0.1362	–	0,1362	9.10	1099
0.1387	0.1387	–	8.95	1117

The note:

*The function of the distribution of energies Δf among individual H_2O molecules was measured in reciprocal electron volts (eV^{-1}). It is shown at which values of the spectrum -*E* (eV) are observed the biggest local maximums of this function; λ – wave length; κ – wave number.

The results with Mediterranean jellyfish *Cotylorhiza tuberculata* indicated that jellyfish has local maximums in IR-spectra at 8.98 and 10.18 µm (Fig. 3). Before measurements the jellyfish was kept in seawater for several days. On comparison seawater has a local maximum at 8.93 µm in IR-spectra. These results were obtained with Thermo Nicolet Avatar 360 Fourier-transform IR. With DNES method the local maximums in spectra for jellyfish are at 8.95 and 10.21 µm, and for seawater at 9.10 µm. A differential spectrum was recorded between jellyfish and seawater by using the Thermo Nicolet Avatar 360 Fourier-transform IR method. In IR-spectrum of jellyfish are observed more pronouncedly expressed local maximums, detected by Thermo Nicolet Avatar 360 Fourier-transform IR and DNES method. Measurements demonstrate that two common local maximums are observed in IR-spectra of jellyfish and seawater. These maximums are not observed in the IR-spectrum of cactus juice and mineral water from Rupite (Bulgaria). Jellyfish contains approximately 97 (w/w) % of water and is more unstable living organism compared to those ones formed stromatolites. The explanation for this is the smaller concentration of salts and, therefore, the smaller number of local maximums in the IR-spectrum in relation to seawater.

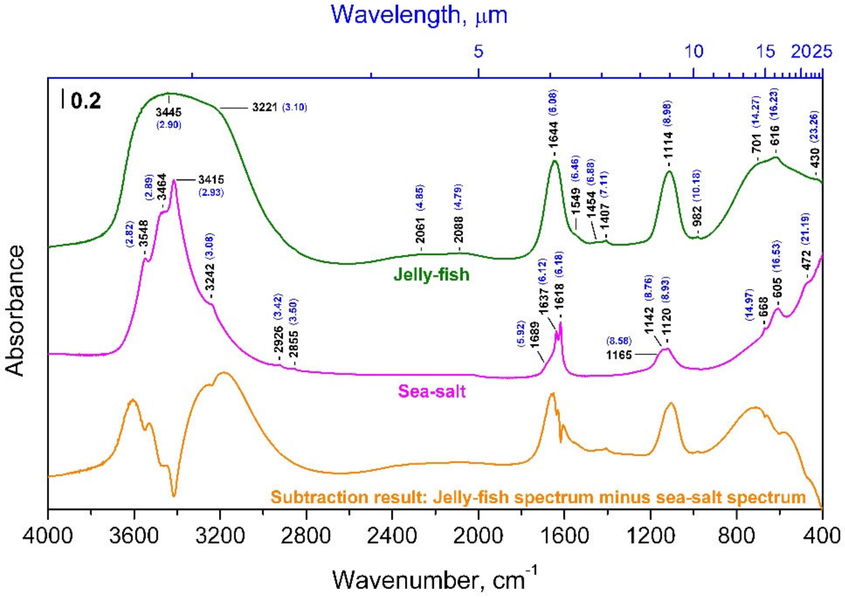


Figure 3: IR-spectrum of seawater obtained from Varna (Bulgaria) and jellyfish *Cotylorhiza tuberculata*, Chalkida (Greece), Aegean Sea

Such a character of IR- and DNES-spectra and distribution of local maximums may prove that hot mineral alkaline water is preferable for origin and maintenance of life compared to other types of water analyzed by these methods. Thus, in hot mineral waters the local maximums in the IR-spectrum are more manifested compared to the local maximums obtained in IR-spectrum of the same water at a lower temperature. The difference in the local maximums from +20 ^{0}C to +95 ^{0}C at each 5 ^{0}C according to the Student t-criterion makes up – $p < 0.05$. These data indicate that the origination of life and living matter depends on the structure and physical chemical properties of water, as well as its temperature and pH value. The most closed to the IR- and DNES-spectrum of water, which contains bicarbonates and calcium ions typical for the formation of stromatolites is the IR-spectrum of cactus juice. For this reason cactus juice was applied as a model system. The most closed to local maximums in IR-spectrum of cactus juice are local maximums in IR-spectra of alkaline mineral water interacting with $CaCO_3$ and then seawater. In connection with these data the following reactions participating with $CaCO_3$ in aqueous solutions are important:

$$CO_2 + 4H_2S + O_2 = CH_2O + 4S + 3H_2O, \quad (3)$$

$$CaCO_3 + H_2O + CO_2 = Ca(HCO_3)_2, \quad (4)$$

$$CO_2 + OH^- = HCO_3^- \quad (5)$$

$$2HCO_3^- + Ca^{2+} = CaCO_3 + CO_2 + H_2O \quad (6)$$

The equation (3) shows how some chemosynthetic bacteria use energy from the oxidation of H_2S and CO_2 to S and formaldehyde (CH_2O). The equation (4) is related to one of the most common processes in nature: in the presence of H_2O and CO_2, $CaCO_3$ transforms into $Ca(HCO_3)_2$. In the presence of hydroxyl OH^- ions, CO_2 transforms into HCO_3^- (equation (5). Equation (6) is valid for the process of formation of the stromatolites – the limestone and dolomite layered acretionary structures (thin-reef columns or mounds of various shapes) formed in shallow seawater or in areas with a periodic change of fresh and salt water by colonies of cyanobacteria. Cyanobacteria release free oxygen into water, while chemically binding hydrogen and carbon. Cyanobacteria are remarkable in that that they are able to use atmospheric nitrogen and convert it into organic forms of nitrogen. During photosynthesis, they may use carbon dioxide as the sole carbon source. In contrast to photosynthetic bacteria, cyanobacteria isolated molecular oxygen in the process of photosynthesis. During the past 3 billion years before the Cambrian they were basic, along with photochemical reactions in the upper atmosphere, free source of oxygen in the Earth's atmosphere.

Stromatolites mostly consist of calcium carbonate because the carbonate type of sedimentation in the sea is most common, but under other hydro-chemical conditions were formed phosphate, silica, glandular stromatolites. The multi-layer coloring of stromatolites can be varried, as the inhabitants of the lower layer can rise in dark time of the top and vice versa. Bacteria glide up and down at a speed of up to 2 cm per hour.

Formation of stromatolites can be represented as follows:

1. Capture of sediment particles;
2. Biomineralization of organic tissue;
3. The precipitation of minerals on the surface and the sludge organisms.

In 2010 D. Ward described fossilized stromatolites in the Glacier National Park (USA) [13]. Stromatolites aged 3,5 billion years had lived in warm and hot water in zones of volcanic activity, which could be heated by magma. This suggests that the first living forms evidently evolved in hot geysers [14].

In nature, there are places with a different composition and origin of water being under the same external conditions, but having different temperatures from ^{0}C to 10 ^{0}C and above 12,5 ^{0}C up to 100 ^{0} C in geothermal sources. For example, in the town of Zlatna Panega, Teteven region, Bulgaria in the lake formed by the water spring with the river Vit is only a few kilometers away, the average temperature is 21 ^{0}C. The average river temperature is around 15 ^{0}C. The photos in Fig. 4 and 5 show the obvious difference between the fauna of karst water sources and rivers. This is proof of optimally favorable active living algae places with the same external conditions. The only difference is in the physical composition of water. The mineral water which interacts with calcium carbonate, as well as sea water is alkaline.

Figure 4: Karst and mineral springs, vegetation of Zlatna Panega, Teteven region, Bulgaria. Photo Alexander Ignatov

Figure 5: The river Vit, Teteven region, vegetation, 3 km from the source of Zlatna Panega, Bulgaria. Photo Alexander Ignatov

With calcium-silicate rocks are associated the development of the oldest forms of life on Earth. The earliest evidence for the existence of living organisms with calcareous structures layered on Earth date back 3,5 billion years. These ancient limestone fossils (dolomites) of Precambrian period – stromatolites, which built a skeleton of limestone and silica (SiO_2) (Fig. 6–8).

Figure 6: Colonial (cocci) form of the Late Proterozoic of Australia (850 million years ago)

Figure 7: Filamentous forms of *Palaeo Lyngbya* from the same location

Figure 8: Stromatolites (Australia)

Stromatolites consisting of calcium carbonate and sand-clay material, formed on the bottom of shallow ponds back in Archean in the most ancient geological era of the earth – 2,5–3,5 billion years ago. Studying these formations are very important and interesting as stromatolites store information about nascent life on earth and organic part of the first living organisms – numerous colonies of cyanobacteria, blue-green algae and bacteria, encountered in strata of limestone and dolomite in the crater of extinct volcanoes and thermal sources. As an example, the photograph in Fig. 9 shows the numerous colonies of blue-green algae in the Rupite, Bulgaria. The place is located in the crater of an ancient extinct volcano. At a temperature of +75 ^{0}C plant life is growing in full force.

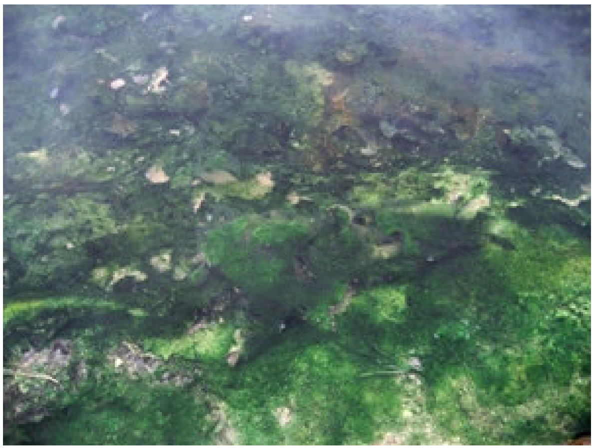


Figure 9: Algae in mineral water, +75 ^{0}C, Rupite, Vanga place, Bulgaria. Photo Alexander Ignatov

At the beginning of the evolution of life there has been an increasing volcanic activity on Earth, even at the bottom of the primary ocean. At that same time on the Earth there had been more silicon and silicon compounds, and they reacted with water. For this reason, they were absorbed in the early living organisms – diatoms and radiolarians. These planktonic forms of microorganisms are found in the upper layers of the sea water along with other organisms with calcareous (foraminifera) and chitin shells. The size of the skeleton of silicon organisms reaches several tens of micrometers (Fig. 10). After the death these organisms sank to the bottom of the sea, and their substance was chemically reacted with seawater. Calcium carbonate and chitin of foraminifera plankton organisms was dissolved in water better than silica of diatoms and radiolarians, forming silica precipitations. The siliceous shales with siliceous deposits of microorganisms were formed in the Phanerozoic era of deep ocean basins at depths of about 2–3 km. The heyday of the microorganisms with silica skeleton could lead to such an accumulation of silicon in the ocean, so that at the sea bottom could be formed the silica gel. Then the silica could crystallize around scattered in the limestone crystallization centers, gradually replacing the molecules of calcium carbonate. Later, the organisms - foraminifera that have calcareous shells began to absorb calcium from the limestone rocks.

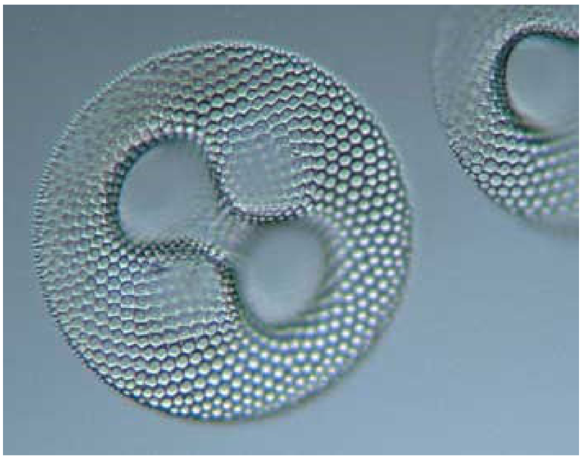


Figure 10: Organism with a silicon skeleton

The most common mineral of the earth's crust quartz SiO_2, apparently played a large role in the origin of life. The quartz crystal has a tetrahedral structure, which is composed of chain silicate structure (Fig. 11–17). The uniqueness of quartz is that its crystals are optically active, e.g. they can affect the polarized light. But quartz crystals not merely affect the light passing through them, they also have optically active properties on the surface of the crystal. Consequently, on the surface of *D*- and *L*- enantiomeric quartz crystals it was theoretically possible the selective absorption of *L*- and *D*-isomers.

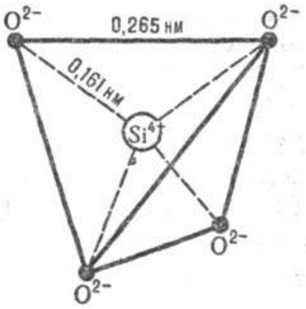


Figure 11: Elementary correct silicon-oxygen tetrahedron SiO_4^{4-}

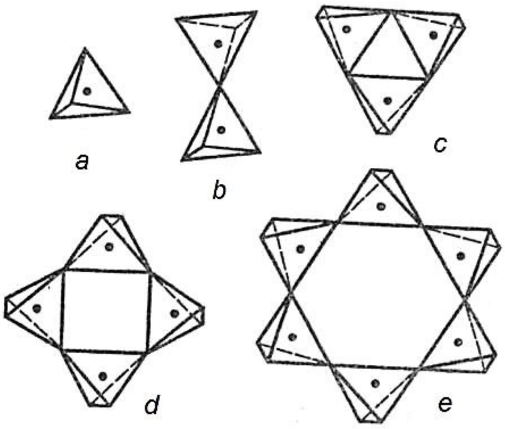


Figure 12: The simplest types of silicon anionic groups: *a* – SiO_4; *b* – Si_2O_7; *c* – Si_3O_9; *d* – Si_4O_{12}; *e* – Si_6O_{18}

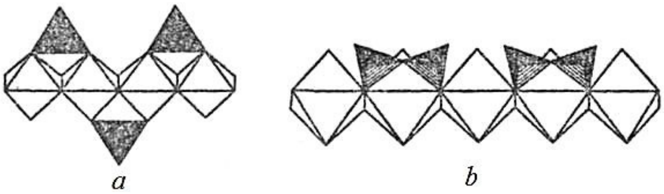


Figure 13: Elementary-silicon-oxygen units of ortho-group SiO_4^{4-} in the structure of Mg-pyroxene enstatite (*a*) and diorto-group $Si_2O_7^{6-}$ in Ca-piroksenoide in wollastonite (*b*)

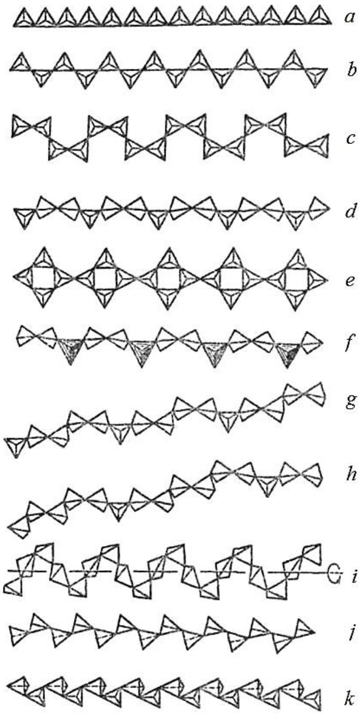


Figure 14: The most important types of silicon-chain anionic groups: *a* – meta-germanate; *b* - pyroxene; *c* – batisite; *d* – wollastonite; *e* - vlasovite; *f* – melilite; *g* – rhodonite; *h* – piroksmangite; *i* – metaphosphate; *j* – fluoro-barite; *k* – barite.

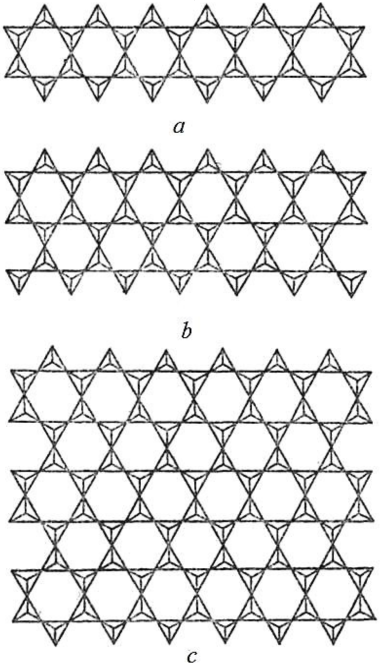


Figure 15: Condensation of pyroxene silicon-oxygen anions in the belt double row amphibole (*a*), three-row amphibole (*b*), layered talc and anions close to them (*c*)

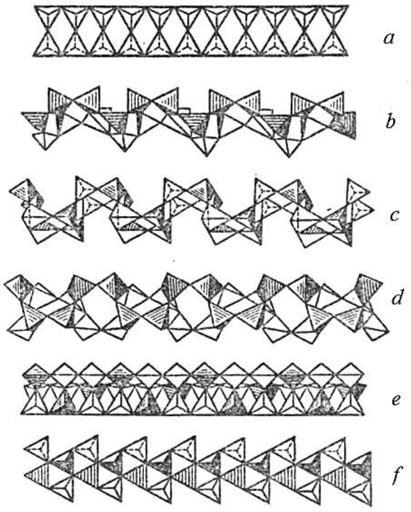


Figure 16: The most important types of silicon band groups: *a* – sillimanite; *b* – epididymitis; *c* – orthoclase; *d* – narsarsukite; *e* – fenakite prismatic; *f* – evklazovaya inlaid.

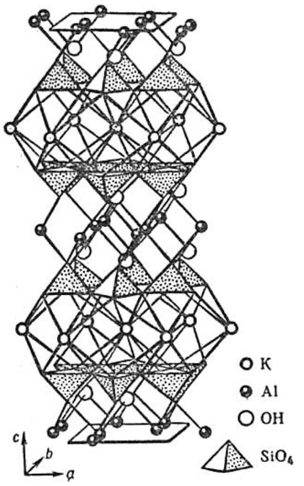


Figure 17: A fragment (elementary packet) of the layered crystal structure of muscovite $KAl_2(AlSi_3O_{10}XOH)_2$, illustrating the alternation of aluminum-silicon-oxygen layers of polyhedral meshes with the large aluminum and potassium cations, reminiscent of the DNA chain

Another interesting property of quartz is that its structure resembles that of water. The discoverers of hydrogen bonds Bernal and Fowler in 1932, compared the structure of iquid water with the crystalline structure of quartz, and the associates of water were considered as tetramers $4H_20$, in which four water molecules were joined in a compact tetrahedron with twelve internal hydrogen bonds. The result of the structure is a four-sided pyramid - the tetrahedron.

According to some researchers's opinions these hydrogen bonds can form tetramers of both right- and left-handed so as quartz crystals have right- and left-rotational crystalline forms (Fig. 18). As such each tetramer has four unused external hydrogen bonds, the tetramers can be connected to these external bonds to polymer chains, such as a DNA molecule. Since there are only four external bonds and internal – in 3 times more, this allows to tetramers in liquid water to bend, rotate, and even to break these loose by thermal vibrations the hydrogen bonds. According to this hypothesis, it causes the water flow.

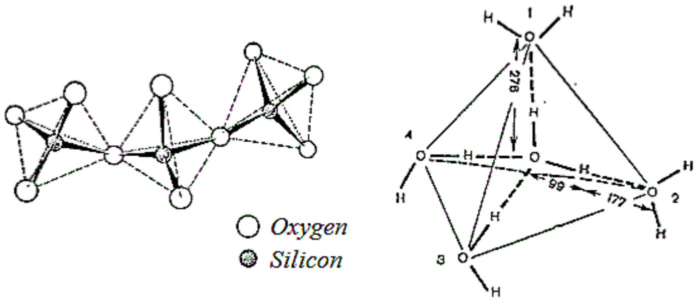


Figure 18: The crystal structure of quartz (left) and the tetrahedral structure of water (right)

The estimated water structure could have been due to its rheological ancient bond with quartz and other silicon-oxygen minerals prevailing in the earth's crust, from the depths of which once there appeared water on the Earth. As a small crystal of salt makes the surrounding solution to crystallize into the crystals like it, so quartz could initiate water molecules to line up into the tetrahedral structure, which is the most energetically favorable.

It is known that water in geysers is rich in carbonates, while the temperature is ranged from +100 ^{0}C to +150 ^{0}C. In 2011 a team of Japanese scientists under the leadership of T. Sugawara showed that life originated in warm or, more likely, hot water (Kurihara et al., 2011). From aqueous solution of organic molecules, DNA and synthetic enzymes were created proto cells. For this the initial solution was heated to a temperature close to water's boiling point +95 ^{0}C. Then its temperature was lowered to +65 ^{0}C with formation of proto cells with primitive membrane. This laboratory experiment is an excellent confirmation of the possibility that life originated in hot water.

3.2. The Reactions of Condensation-Dehydration in Hot Water

The prognosis was made to predict a possible transition from synthesis of small organic molecules under high temperatures to more complex organic molecules as proteins. There are reactions of condensation-dehydration of amino acids into separate blocks of peptides that occur under t = +75-95 ^{0}C and alkaline conditions, with pH = 9–11. The important factor in reaction of condensation of two amino acid molecules into the dipeptide is allocation of H_2O molecule when a peptide chain is formed, as the reaction of polycondensation of amino acids is accompanied by dehydration, the H_2O removal from reaction mixture speeds up the reaction rates. This testifies that formation of early organic forms may have occured nearby active volcanoes, because at early periods of geological history volcanic activity occurred more actively than during subsequent geological times. However, dehydratation accompanies not only amino acid polymerization, but also association of other small blocks into larger organic molecules, and also polymerization of nucleotides into nucleic acids. Such association is connected with the reaction of condensation, at which from one block a proton is removed, and from another – a hydroxyl group with the formation of H_2O molecule.

In 1969 the possibility of existence of condensation-dehydration reactions under conditions of primary hydrosphere was proven by M. Calvin (Calvin, 1969). From most chemical substances hydrocyanic acid (HCN) and its derivatives – cyanoamid (CH_2N_2) and dicyanoamid ($HN(CN)_2$) possess dehydration ability and the ability to catalyze the process of linkage of H_2O from primary hydrosphere (Mathews & Moser, 1968). The presence of HCN in primary hydrosphere was proven by S. Miller's early experiments (Miller, 1953). Chemical reactions with HCN and its derivatives are complex with a chemical point of view; in the presence of HCN, CH_2N_2 and $HN(CN)_2$ the condensation of separate blocks of amino acids accompanied by dehydration, can proceed at normal temperatures in strongly diluted H_2O-solutions. These reactions show the results of synthesis from separate smaller molecules to larger organic molecules of polymers, e.g. proteins, polycarboxydrates, lipids, and nucleic asids (Fig. 19). Furthermore, polycondensation reactions catalyzed by HCN and its derivatives depend on acidity of water solutions in which they proceed (Abelson, 1966). In acid aqueous solutions with pH = 4–6 these reactions do not occur, whereas alkaline conditions with pH = 9–10 promote their course. There has not been unequivocal opinion, whether primary water was alkaline, but it is probable that such pH value possessed mineral waters adjoining with basalts, i.e. these reactions could occur at the contact of water with basalt rocks, that testifies our hypothesis.

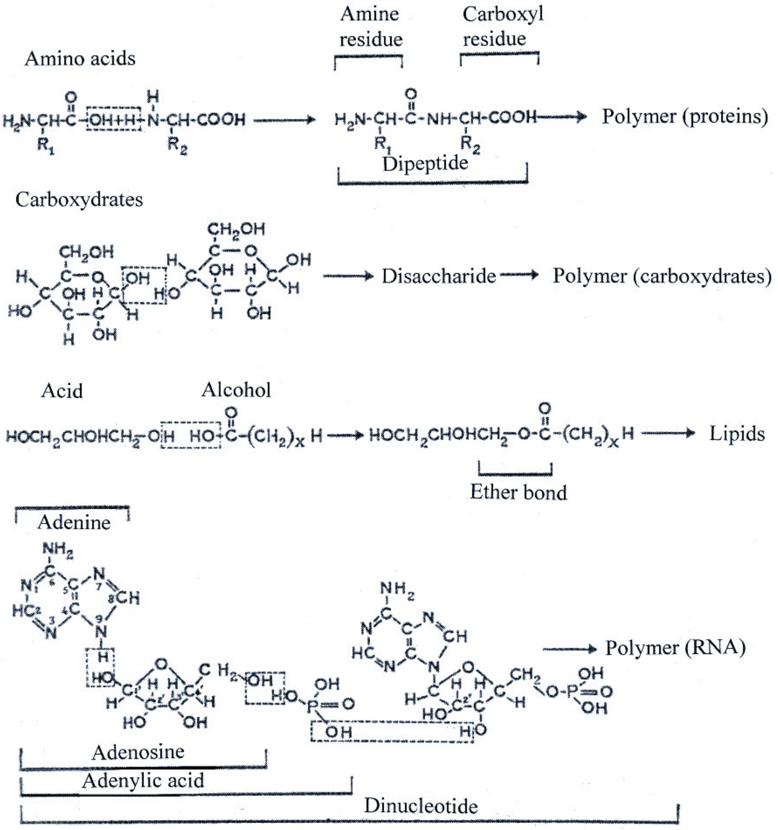
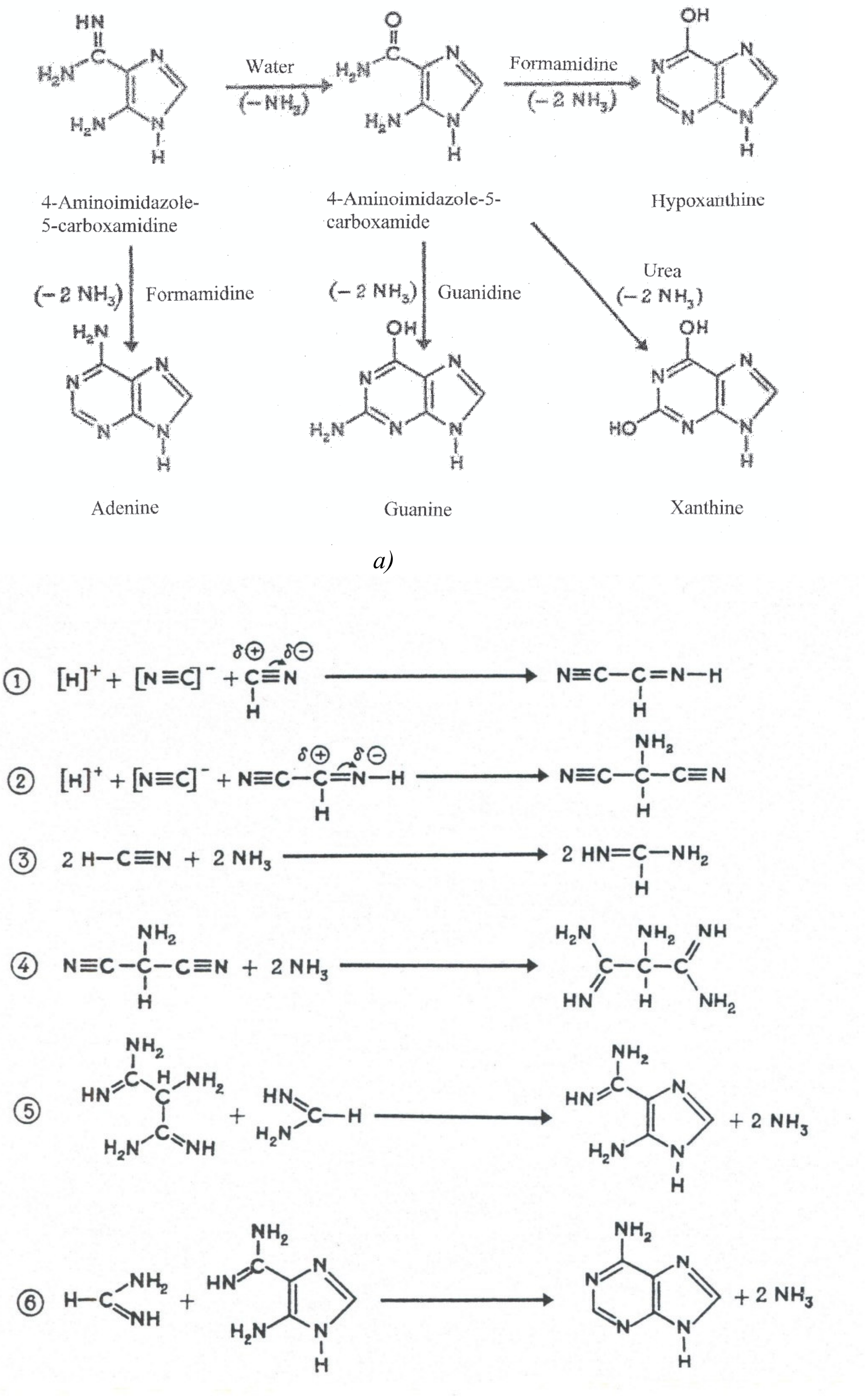


Figure 19: Reactions of condensation and dehydration in alkaline conditions with pH = 9–10 catalyzed by HCN and its derivatives, resulting in synthesis from separate molecules larger organic molecules of polymers. The top three equations: condensation and the subsequent polymerization of amino acids in proteins; carbohydrates – in polycarboxydrates and acids and ethers – into lipids. The bottom equation – condensation of adenine with ribose and H_3PO_4, leading to formation of dinucleotide

It should be noted, that geothermal sources might be used for synthesis of various organic molecules. Thus, amino acids were detected in solutions of formaldehyde CH_2O with hydroxylamine NH_2OH, formaldehyde with hydrazine (N_2H_4) in water solutions with HCN, after heating of a reactionary mixture to +95 ^{0}C (Harada & Fox, 1964). In model experiments reaction products were polymerized into peptide chains, that is the important stage towards inorganic synthesis of protein. In a reactionary mixture with a HCN–NH_3 solution in water were formed purines and pyrimidines (Fig. 20). In other experiments amino acid mixtures were subjected to influence of temperatures from +60 ^{0}C up to +170 ^{0}C with formation of short protein-like molecules resembling early evolutionary forms of proteins subsequently designated as thermal proteinoids. They consisted of 18 amino acids usually occurring in protein hydrolyzates. The synthesized proteinoids are similar to natural proteins on a number of other important properties, e. g. on linkage by nucleobases and ability to cause the reactions similar to those catalyzed by enzymes in living organisms as decarboxylation, amination, deamination, and oxidoreduction. Proteinoids are capable to catalytically decompose glucose (Fox & Krampitz, 1964) and to have an effect similar to the action of α-melanocyte-stimulating hormone (Fox & Wang, 1968). The best results on polycondensation were achieved with the mixes of amino acids containing aspartic and glutamic acids, which are essential amino acids occurring in all modern living organisms.



a)

b)

Figure 20: Prospective mechanisms of thermal (+95 ^{0}C) synthesis of purines in aqueous solutions: *a)* – synthesis of hypoxanthine, adenine, guanine and xanthine from 4-aminoimidazole-5-carboxamidine, 4-aminoimidazole-5-carboxamide, water, NH$_3$, formamidine and urea; *b)* – synthesis of adenine from NH$_3$ and HCN (total reaction: 5HCN = adenine)

Under certain conditions in hot mixture of proteinoids in water solutions are formed elementary structures like proteinoid microspheres with diameter 5–10 μm (Nakashima, 1987). Gas electric discharge with color coronal spectral analyses was applied in this type of experiment analogous to S. Miller's experiments (Ignatov & Tsvetkova, 2011). In S. Miller's experiments one of the basic conditions is electric gas discharge. The analogous experiment was conducted by the authors under laboratory conditions. The first living structures were most probably formed in warm and hot mineral water with more bicarbonate and metal ions (Na, Ca, Mg, Zn, K, etc.). According to our previous experiments, the first living structures may have evolved in warm and hot mineral water with a high content of bicarbonate (HCO_3^-) anions, cations of alkali metals (Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}).

There occurred gas electric discharge (lightning) in the primordial atmosphere close to the water surface. In the course of experiment was used the similar gas electric discharge on water drops placed on the electrode of the device for gas electric discharge formation. The similar composition and water temperature were modeled on the electrode of the gas discharge device made of hostafan, with electric voltage – 15 kV, electric impulse duration – 10 μs; electric current frequency – 15 kHz, wherein the air gap layer on the boundary with water sample was formed the electrical discharge, similar to plasma phenomena (lightning) and the electrostatic discharge on the surface of organic and inorganic samples of various kinds (Ignatov & Tsvetkova, 2011). Water drops were heated up to the boiling point in an electric field of high frequency and the electric discharge was applied, analogous to that in the primordial atmosphere. As a result, an organized structure with a size of ~1.2–1.3 mm was formed in interelectrode space (Fig. 21). It was formed as a result of the accretion of elementary structures sized of ~5–10 μm in the biggest structure with size 1.2–1.4 mm and concentrated in a large structure where the basic electric voltage is applied.

Figure 21: The organized structure in water sample subjected to the temperature +100 ^{0}C in the electric field of high voltage and frequency. The material of the electrode – hostafan; electric voltage – 15 kV, electric impulse duration – 10 μs; electric current frequency – 15 kHz.

It should be noted that no structure was organized in a control sample of water placed on the electrode. Before its placement on the electrode, the water was heated to the boiling point and then cooled. The structure organization increased with the increase of the duration of the gas electric discharge. Moreover, in experiments was observed formation of small structures and their further “adjoining” to the larger structure. The large structure was preserved with the original size for some time in the absence of electric discharge.

This experiment shows that the organization of structures in water under certain external conditions may take place. In natural conditions water is heated up to +100 ^{0}C by the magma. The structure formed from heated water was evidently a result of self-organization. Living organisms are complex self-organizing systems. Thermodynamically they belong to the open systems because they constantly exchange substances and energy with the environment. The changes in the open systems are relatively stable in time. The stable correlation between components in an open system is called a dissipative structure. According to I. Prigozhin, the formation of dissipative structures and the elaboration to living cells is related to changes in entropy (Nikolis & Prigozhin, 1979).

Taking into account these views it may be concluded that the initial stage of evolution, apparently, was connected with formation at high temperature of the mixtures of amino acids and nitrogenous substances – analogues of nucleic acids. Such synthesis is possible in aqueous solutions under thermal conditions in the presence of H_3PO_4. The next stage is the polycondensation of amino acids into thermal proteinoids at temperatures 65–95 ^{0}C. After that stage in a mix of thermal proteinoids in hot water solutions were formed the membrane like structures.

Our data are confirmed by experiments of T. Sugawara (Japan), who in 2011 created the membrane like proto cells from aqueous solution of organic molecules, DNA and synthetic enzymes under temperature close to water’s boiling point +95 ^{0}C (Sugawara, 2011). This data confirm the possibility that first organic forms of life originated in hot water.

4. Conclusion

The data obtained testify that origination of life and living matter depends on physical-chemical properties of water and external factors – temperatures, pH, electric discharges and isotopic composition. Hot mineral alkaline water interacting with $CaCO_3$ is most closed to these conditions. Next in line with regard to its quality is seawater. For chemical reaction of dehydration-condensation to occur in hot mineral water, water is required to be alkaline with pH range 9–11. In warm and hot mineral waters the local maximums in IR-spectra from 8 to 14 μm were more expressed in comparison with the local maximums measured in the same water samples with lower temperature. The most common minerals of the earth's crust quartz SiO_2 and limestone, apparently played a large role in the origin of life. At the beginning of the evolution of life there has been an increasing volcanic activity on Earth, even at the bottom of the primary ocean. At that same time on the Earth there had been more silicon and silicon compounds, and they reacted with water. For this reason, they were absorbed in the early living organisms - diatoms and radiolarians. After the death these organisms sank to the bottom of the sea, and their substance was chemically reacted with seawater. Calcium carbonate and chitin of foraminifera plankton organisms was dissolved in water better than silica of diatoms and radiolarians, forming silica precipitations. The siliceous shales with siliceous deposits of microorganisms were formed in the Phanerozoic era of deep ocean basins at depths of about 2-3 km. The heyday of the microorganisms with silica skeleton could lead to such an accumulation of silicon in the ocean, so that at the sea bottom could be formed the silica gel. Then the silica could crystallize around scattered in the limestone crystallization centers, gradually replacing the molecules of calcium carbonate. Later, the organisms - foraminifera that have calcareous shells began to absorb calcium from the limestone rocks.

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References:

Abelson, P. (1966) Chemical events on the "primitive" earth. *Proc. Natl. Acad. Sci. U.S.*, 55: 1365–1372.

Calvin, M. (1969) *Chemical evolution*. Oxford: Clarendon, 278 p.

Fox, S.W. & Krampitz, G. (1964) Catalytic decomposition of glucose in aqueous solution by thermal proteinoids. *Nature*, 203: 1362–1364.

Fox, S.W. & Wang, C.T. (1968) Melanocytestimulating hormone: Activity in thermal polymers of alpha-ammo acids. *Science*, 160: 547–548.

Harada, I. & Fox, S.W. (1964) Thermal synthesis of natural ammo-acids from a postulated primitive terrestrial atmosphere. *Nature*, 201: 335–336.

Ignatov, I. (2010) Which water is optimal for the origin (generation) of life? *Euromedica*, Hanover. pp. 34–37.

Ignatov, I. (2012) *Origin of life and living matter in hot mineral water*. Conference on the Physics, Chemistry and Biology of Water, Vermont Photonics, USA, 2012. p. 67.

Ignatov, I. & Tsvetkova, V. (2011) Water for the origin of life and informationability of water. Kirlian (electric images) of different types of water. *Euromedica*, Hanover, pp. 32–35.

Ignatov, I. & Mosin, O.V. (2013a) Color coronal (Kirlian) spectral analysis in modeling of nonequilibrioum conditions with the gas electric discharges simulating primary atmosphere. S. Miller's experiments. *Naukovedenie*, 3(16): 1–15 [in Russian] [Online] Available: URL: http://naukovedenie.ru/PDF/05tvn313.pdf (May 10, 2013).

Ignatov I. & Mosin, O.V. (2013*a*) Possible processes for origin of life and living matter with modeling of physiological processes of bacterium *Bacillus subtilis* in heavy water as model system. *Journal of Natural Sciences Research*, 3(9): 65-76.

Ignatov, I. & Mosin, O.V. (2013*b*) Modeling of possible processes for origin of life and living matter in hot mineral and seawater with deuterium. *Journal of Environment and Earth Science*, 3(14): 103-118.

Ignatov, I. & Mosin, O.V. (2013c) Isotopic composition of water and its temperature in the evolutionary origin of life and living matter. *Naukovedenie*, 1(14): 1–16 [in Russian] [Online] Available: URL: http://naukovedenie.ru/PDF/42tvn113.pdf (February 13, 2013).

Ignatov, I. & Mosin, O.V. (2013d) Possible processes for origin of life and living matter with modeling of physiological processes of bacterium *Basillus subtilis* as model system in heavy water. *Journal of Natural Sciences Research*, 3(9): 65–76.

Ignatov, I. & Mosin, O.V. (2012) Isotopic composition of water and its temperature in modeling of primordial hydrosphere experiments. *Euro-Eco*, Hanover, p. 62.

Ignatov, I. & Mosin, O.V. (2013d) Modeling of possible processes for origin of life and living matter in hot mineral and seawater with deuterium. *Journal of Environment and Earth Science*, 3(14): 103–118.

Ignatov, I. & Mosin, O.V. (2013e) Method for colour coronal (Kirlian) spectral analysis. *Biomedical Radio Electronics*, 1: 38–47 [in Russian].

Kurihara, K., Tamura, M. & Shohda, K. (2011) Self-Reproduction of supramolecular giant vesicles combined with the amplification of encapsulated DNA. *Nature Chemistry*, 4(10): 775–781.

Linsky, J.L. (2007) D/H and nearby interstellar cloud structures / Ed. J.I. Linsky. *Space Science Reviews*, NY:

Springer Science, Business Media. Vol. 130. 367 p.

Mathews, C.N. & Moser, R. (1968) Peptide synthesis from hydrogen-cyanide and water. *Nature*, 215: 1230–1234.

Max, J.J. & Chapados, C. (2009) Isotope effects in liquid water by infrared spectroscopy. III. H_2O and D_2O spectra from 6000 to 0 cm^{-1}. *J. Chem. Phys*., 131: 184505.

Miller, S.L. (1953) A production of amino acids under possible primitive Earth conditions. *Science*, 117(3046): 528–529.

Mosin, O.V. & Ignatov, I. (2012a) Isotope effects of deuterium in bacterial and microalgae cells at growth on heavy water (D_2O). *Voda: Himia i Ecologija*, 3, 83–94 [in Russian].

Mosin, O.V. & Ignatov, I. (2012b) Studying of isotopic effects of heavy water in biological systems on example of prokaryotic and eukaryotic cells. *Biomedicine, Moscow*, 1(1–3): 31–50 [in Russian].

Mulkidjanian, A.Y. & Galperin, M.Y. (2009) On the origin of life in the Zinc world. Validation of the hypothesis on the photosynthesizing zinc sulfide edifices as cradles of life on Earth. *Biology Direct*, 4: 26–28.

Nakashima, T. (1987) Metabolism of proteinoid microspheres / Ed. T. Nakashima. In: *Origins of life and evolution of biospheres*, 20(3–4), pp. 269–277.

Nikolis, P. & Prigozhin, I. (1979) *Self-organization in non-equilibrium systems*. Moscow: Mir, pp. 1–512 [in Russian].

Ponsa, M.L., Quitte G.., Fujii, T. et al. (2011) Early archean serpentine mud volcanoes at Isua, Greenland, as a niche for early life. *Proc. Natl. Acad. Sci. U.S*., 108: 17639–17643.

Schirber, M. (2010) First fossil-makers in hot water. *Astrobiology magazine* [Online] Available: URL: http://www.astrobio.net/exclusive/3418/first-fossil-makers-in-hot-water (January 3, 2010).

Somlyai, G., Jancso, G., Jakli, G *et al.* (2012) Deuterium depletion from tissue culture to human clinical studies. In: *2nd International Congress on Deuterium Depletion*. Budapest, Hungary, 17–18 May, 2012.

Sugawara, T. (2011) Self-reproduction of supramolecular giant vesicles combined with the amplification of encapsulated DNA. *Nature Chemistry*, 1127: 775–780.

Szostak, J.W. (2011) An optimal degree of physical and chemical heterogeneity for the origin of life? *Philos. Trans. Royal Soc. Lond. Biol. Sci*., 366(1580): 2894–901.

Trevors, J.I. & Pollack, G.H. (2005) Hypothesis: origin of live in hydrogel environment. *Progress in biophysics and molecular biology*, 89(1): 1–8.