The Formation of Thermal Proteinoids in Hot Water

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Abstract
It was demonstrated the formation of thermal proteinogenic microspheres in hot water at increasing the pH value. Proteinogenic microspheres doubled in hot water with increasing the pH up to 6.0. The hot alkaline mineral water with temperature from +65 ^{0}C to +95 ^{0}C and the pH value from 9 to 11 is more suitable for the origination of life and living matter than other analyzed water samples. 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 the Student *t*-criterion makes up $p < 0,05$.

Keywords: deuterium, hydrosphere, evolution, origin of life, condensation-dehydratation, IR-spectroscopy.

1. Introduction

Previous biological experiments with D_2O and structural-conformational studies with deuterated 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 stronger than those ones formed of hydrogen.

The 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***)**. Constant sources of deuterium are explosions of nova stars and thermonuclear processes frequently occurring inside the stars. Probably, it could explain a known fact**,** why the amount of deuterium is slightly increased during the global changes of climate in warming conditions. 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 biochemical processes in living matter. Recent studies have shown 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 demonstrate 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 data presented in this paper show that the origination of living matter most probably occurred in hot mineral water. This occurred 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 sea organisms (Ignatov, 2012).

Therefore, the purpose of the research was studying the conditions of primary hydrosphere (temperature, pH, isotopic composition) for possible processes for origin of life and living matter in hot mineral water. Various samples of water from Bulgaria were studied within the frames of the research.

2. Material and Methods

2.1. Chemicals

For preparation of water with varying content of deuterium (HDO) was used D_2O (99.9 atom.%) purchased from the Russian Research Centre "Isotope" (St. Petersburg, Russian Federation). D_2O was preliminary distilled over $KMnO_4$ with the subsequent control of isotope enrichment by ^{1}H-NMR-spectroscopy on a Brucker WM-250 device ("Brucker", Germany) (working frequency: 70 MHz, internal standard: Me_4Si).

2.2. Biological Objects

The objects of the study were used the cactus juice of *Echinopsis pachanoi* and the Mediterranean jellyfish *Cotylorhiza tuberculata* (Chalkida, Greece, Aegean Sea).

2.3. Water Samples

The samples of water were 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).
6 – Water with varrying deuterium content (HDO).

2.4. 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).

2.5. 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.

2.6. 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.

3. Results and Discussion

We have 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.
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 with 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. Another important parameter was measured by the DNES method – the average energy ($\Delta E_{H...O}$) of hydrogen H…O-bonds among individual molecules H_2O, which makes up -0.1067±0.0011 eV. When the water temperature is changed, the average energy of hydrogen H...O-bonds alternates. This testified about the restructuring of average energies among individual H_2O molecules with a

statistically reliable increase of local maximums in DNES-spectra.

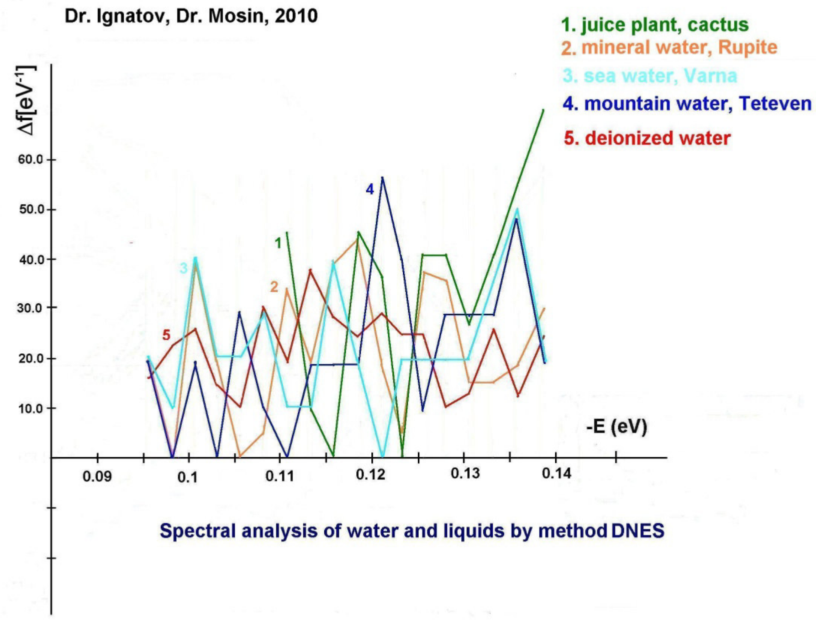

Fig. 1: DNES-spectra of water samples of various origin: 1 – cactus juice; 2 – mineral water from Rupite village (Bulgaria); 3 – seawater (Varna, Bulgaria); 4 – mountain water (Teteven, Bulgaria); 5 – deionized water (the control)

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. 2 and Fig. 3 (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. 3). 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: Characteristics of spectra of water of various origin obtained by the 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

Notes:
*The function of the distribution of energies Δf 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.

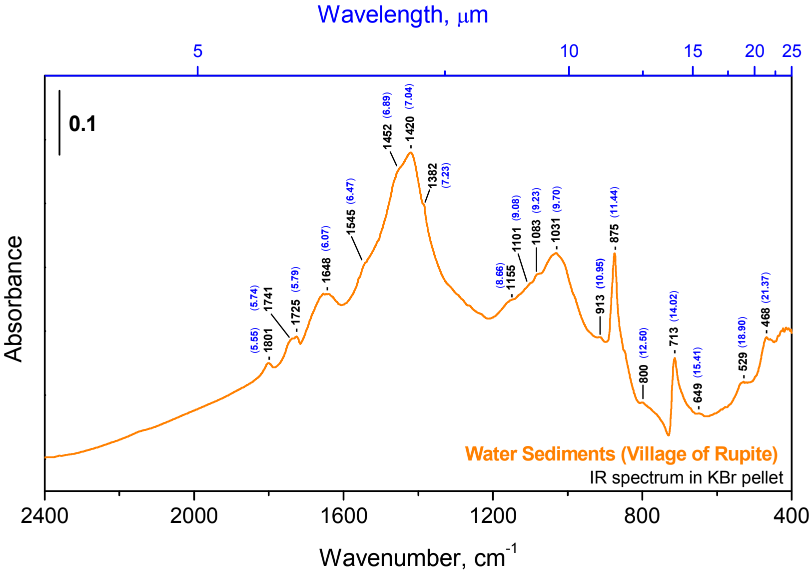

Fig. 2: IR-spectrum of mineral water obtained from Rupite Village (Bulgaria)

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 the measurement 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 the 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.

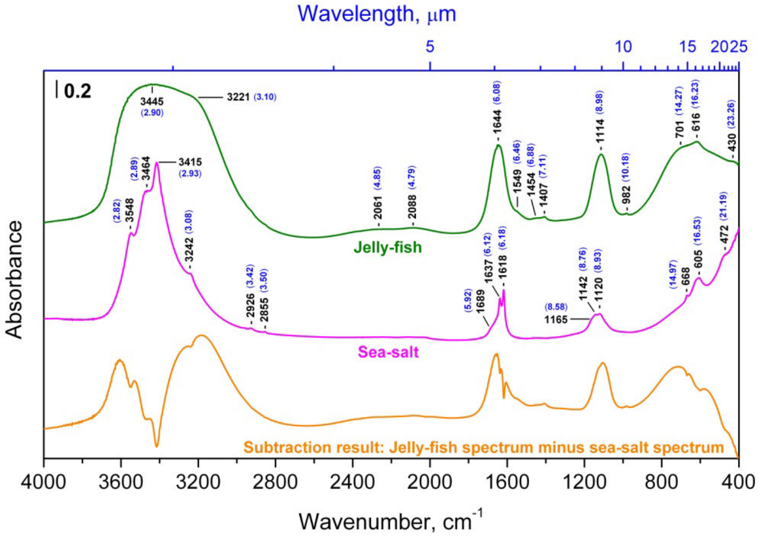

Fig. 3: IR-spectrum of seawater obtained from Varna (Bulgaria) and jellyfish *Cotylorhiza tuberculata*, Chalkida (Greece), the Aegean Sea

Such a character of IR- and DNES-spectra and distribution of local maximums in spectra relative to the spectra of the jellyfish 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 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:

$CO_2 + 4H_2S + O_2 = CH_2O + 4S + 3H_2O,$ (3)
$CaCO_3 + H_2O + CO_2 = Ca(HCO_3)_2,$ (4)
$CO_2 + OH^- = HCO_3^-$ (5)
$2HCO_3^- + Ca^{2+} = CaCO_3 + CO_2 + H_2O$ (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 dolomite layered accretion structures formed in shallow seawater by colonies of cyanobacteria. In 2010 D. Ward described fossilized stromatolites in the Glacier National Park (USA) (Schirber, 2010).

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 (Ponsa et al., 2011). 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.

The above-mentioned data can 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 alkaline conditions, with pH = 9–11. The important factor in reaction of condensation of two amino acid molecules into 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 ribonucleic acids. 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.

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 are 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. 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 hydrolysates. 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.

Under certain conditions (temperature, pH) in hot mixture of proteinoids in water solutions are formed elementary structures like proteinoid microspheres with diameter 5–10 µm (Fig. 4) (Nakashima, 1987). 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. By morphological features the proteinoid microspheres with a diameter ~5–10 µm resemble cell membrane, which in certain conditions (the increased pH value) may be double (Fig. 4-7). The catalyst for their formation could serve sulfur and its derivatives which were found in ancient rocks in the form of grains of sulfides, as well as pyrite sands. Synthesis of protenoid microspheres from a mixture of thermal proteinoids is important because it provides material for the next stage of the evolution of life. This is the stage from disparate organic molecules to organized aggregates having organized structure (coacervates) and separated from the surrounding environment by the primitive membrane.

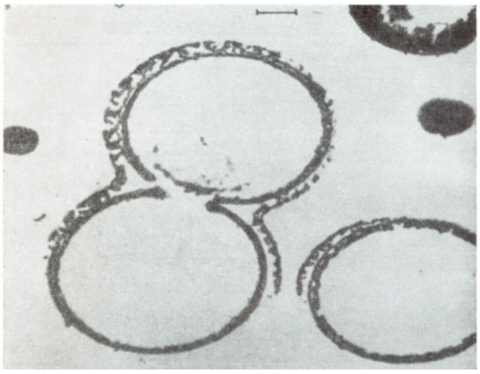

Fig. 4: Electron micrographs of sections of thermal proteinoid microspheres in scanning electron microscope (magnification × ~1000 times) (Nakashima, 1987).

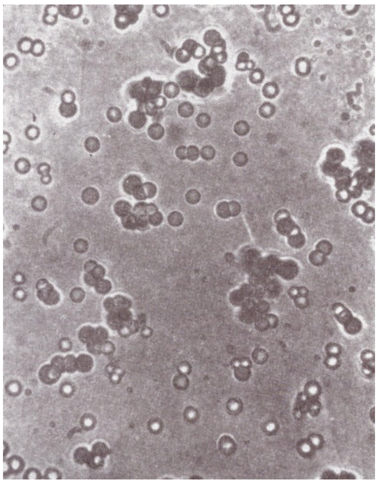

Fig. 5: Proteinoid microspheres prepared by washing of hot proteinoid mixtures (average diameter of 2 µm) (Nakashima, 1987).

Fig. 6: Proteinoid microspheres with low pressure on the preparation being combined in a chains like algae (Nakashima, 1987).

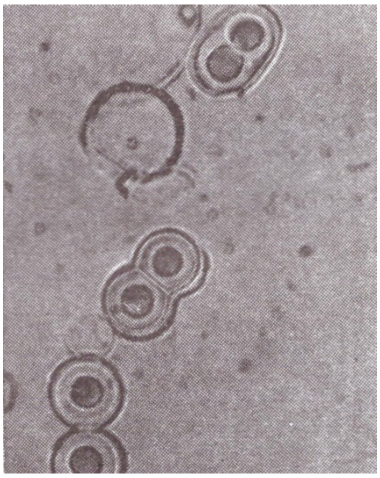

Fig. 7: Proteinogenic microspheres doubled with increasing pH up to 6.0 (Nakashima, 1987).

Coacervates are important in a number of hypotheses about the origin of life on Earth in which they serve as some protoorganisms.

The author of the coacervates theory is the Russian biochemist A.I. Oparin (1924). Later A.I. Oparin and English scientist John Haldane independently came to similar conclusions. A. Oparin believed that the transition from chemical to biological evolution required the emergence of individual phase-separate systems that can interact with the environment. According to the A. Oparin's theory the coacervation played a major role in one of the early stages of the origin of life on Earth.

Coacervates are tiny colloidal particles - drops possessing osmotic properties. Coacervates are naturally formed in concentrated solutions of proteins and nucleic acids, and capable of adsorbing various substances. Due to the interaction of opposite electrical charges there occur aggregation of molecules in them. Small spherical particles are produced because water molecules create around the formed unit an interface unit.

Numerous studies have shown that coacervates possess a fairly complex organization, and have a number of properties that bring them closer to the simplest living systems and proto-cells. For example, they are able to absorb from the surrounding environment different substances that interact with the compounds of the drop itself, and the increase in size. These processes are in some way reminiscent of a primary form of assimilation. However, in the coacervates may occur processes of decay and isolation of degradation products. The relationship between these processes in different coacervates is different. In coacervtes separate dynamically more stable structures with a predominance of synthetic activity are allocated . But all this still does not give grounds for attributing coacervates to living systems because they lack the ability to self-replication and self-regulation of synthesis of organic substances. But they kept the preconditions for the emergence of living matter in them.

Coacervates may explain how biological membranes appeared. The formation of the membrane structure is considered the most "difficult" stage of chemical evolution of life. True living being (in the form of a cell, even the most primitive) could not take shape before the appearance of the membrane structure and enzymes. Biologic membranes are aggregates of protein and lipid capable to distinguish the substance from the environment and to give strength to the packing of molecules.

The increased concentration of organic substances in coacervates increased the possibility of interaction between the molecules and the complexity of organic compounds. Coacervates formed in water at contact of two weakly interacting polymers.

Besides coacervates in the "primordial soup" accumulated polynucleotides, polypeptides, and various catalysts, which are indispensable for the formation of the capacity for self-reproduction and metabolism. As catalysts may be served inorganic substances. Thus G. Bernal hypothesized that at that time the most successful conditions for the emergence of life evolved in small calm warm lagoons with a lot of silt and clay turbidity. In this environment very quickly occurred the amino acid polymerization, because in that case the polymerization process herein requires no heating since the sludge particles act as a catalyst.

Coacervate drops also occur by simply mixing of a variety of protein and lipid. In this case the polymer molecules "gather" in multimolecular phase-separate formations.

Recently, the problem of formation of coacervates was shed new light with a slightly unexpected side. Oceanographers found that particles suspended in the form of organic matter in seawater occurs much more frequently than it was previously thought. For the first time such a coacervate-like aggregates were found in Long Island Sound. Now it is shown that they are present not only in coastal waters, but also in the high seas. Some researchers have reported that these units have the form of thin platelets from 5 μm up to several millimeters in diameter. Empirically it was found that such particles can be obtained by passing through seawater air bubbles. Therefore foaming is apparently plays a fundamental role in the formation of these aggregates. Air bubbles occur frequently in all natural water reservoirs under the influence of wind and turbulent flows (Fig. 11).

It is believed that at the formation of coacervates for this type may take polar molecules having at the ends of the polar and nonpolar groups, for example, soaps, steroids and others. Such molecules exhibit surface-active properties due to the surface tension forces so that they may tend to create films on the water surface water with a thickness in one molecule. As a result, on the interface of air-water appear the monomolecular films which are destroyed under the influence of waves. The molecules in the droplets, taken out of the film tend to be reconstructed in a spherical body, placing it in the radially polar end outward (Fig. 8-10). Once being in the water, these droplets may penetrate the surface film and be immersed in it forming small coacervate droplets.

Another apparently sufficiently close in time evolution step was the formation of membranes. Due to membranes living matter may differ in composition and energy level from the environment. The osmotic pressure of fluids of many invertebrates is most close to the osmotic pressure of sea water, irrespective whether the animal lives - in the sea, on land or in fresh water. Activity, inherent to membranes appeared at the early stages of metabolism. Because metabolism always change the concentration of one or more substances, that is why there is a difference between the composition of the external and internal environment.

This difference is maintained through membranes important property - they are arranged so that they pass one molecule and detain others. This property of the membrane is determined primarily by the size, or electric charge of molecules that make up the membrane. Such membranes are called semipermeable, as a result of their operation there occurs a difference in osmotic pressures on both sides of the membrane.

It is believed that the formation of the primitive membranes was similar in principle with that of coacervate droplets. It could have started with alignment of polar molecules at the interface - the water or lipid-water (Fig. 13). Modern cell membranes are composed of a double monomolecular lipid layers. Lipids play a major role in the membranes, although there are known non-lipid membrane.

In 2011, Japanese researchers have reproduced in the laboratory of the occurrence of the "primordial soup" protocells with a cationic shell and DNA elements inside (natural segments of DNA were part of the initial components, capable of division as a result of the polymerase chain reaction, DNA replication.

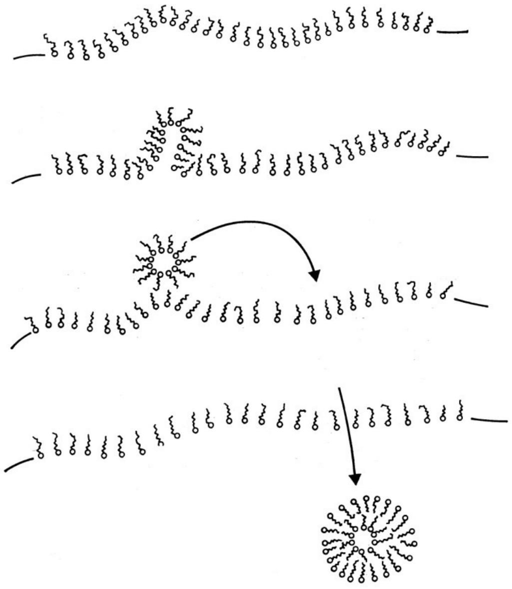

Fig. 8: Formation of coacervate drops from mono-layer polar lipid molecules on the surface of the water-air interface under the influence of excitement of water. Circle - polar end of the molecule, a zig-zag tail - a non-polar hydrocarbon radical

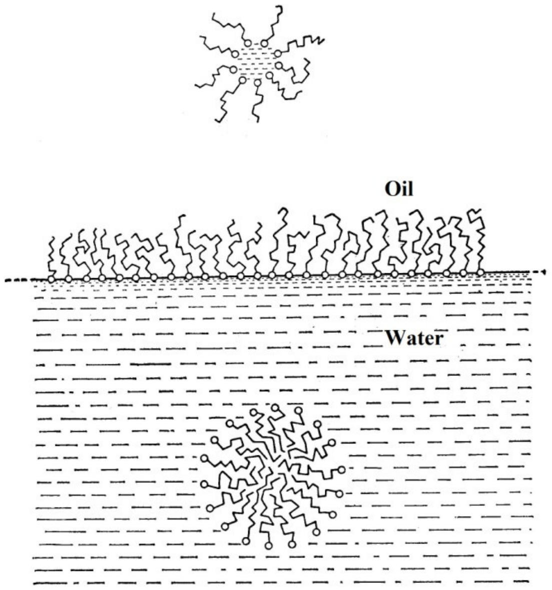

Fig. 9: Location of polar molecules in coacervate droplets in water and oil, and the location of molecules of water in a monomolecular layer at the interface water – oil

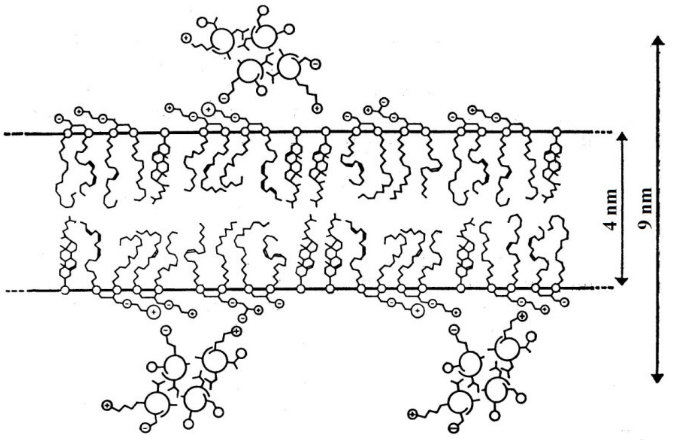

Fig. 10: Schematic representation of two monomolecular layers of polar molecules that play a role of membrane, with adsorbed on them globular protein molecules. Monomolecular layers forming the membrane are composed of identical building blocks. Each unit is composed of two lipid molecules and one molecule of cholesterol. Such a structure is developed in the process of organic evolution: it is not absolutely essential for the functioning of the membrane. Any film of polar organic molecules may serve as a membrane if it does not prevent the diffusion of certain molecules

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 first experiments on the modeling of non-equilibrium conditions with gas electric discharge simulating primary atmosphere and electrosynthesis of organic substances from anorganic ones under the energy of the electric field in a primary oxygen-free atmosphere were held in 1953 by S. Miller (USA) (Miller, 1953). For this aim the mixture of water and gases consisted of hydrogen (H_2), methane (CH_4), ammonia (NH_3) and carbon monoxide (CO) was placed into a closed glass container being exposed by pulsating electrical spark discharges at the temperature of boiling water; oxygen was not allowed into the unit. After processing the reaction mixure by the electric discharge it was found that from the initial inorganic substance was synthesized organic compounds – aldehydes and amino acids. Experiments found that approximately ~10–15% of carbon was transferred into an organic form. However, about ~2% of carbon was detected in the amino acids, the most common of which was glycine. Initial analysis showed the presence in the reaction mixture obtained after the processing by spark electric discharge 5 amino acids. A more complete analysis carried out in 2008, showed the formation by electrosynthesis in the reaction mixture 22 amino acids having from 5 to 20 carbon atoms in the molecule (Fig. 11). Interestingly is that along with the amino acids in the reaction mixture after the treatment with electric spark discharges were detected trace amounts of nucleic acid precursors – nucleosides.

It should be noted that in the implementation of the gas discharge effect as well as in experiments of S. Miller are modeled extreme non-equilibrium conditions with gas electric discharge, resulting that in a thin layer of air gap with the thickness ~100 μm are formed reactive radicals reacting with each other to form new compounds (electrosynthesis). Such extreme conditions are thought to have occurred in the primary oxygen-free atmosphere of the Earth, which supposedly consisted of a mixture of water and gases – H_2, CH_4, NH_3 and CO, subjected to spark electrical discharges (lightning) under the conditions of high solar (UV) and geothermal activity.

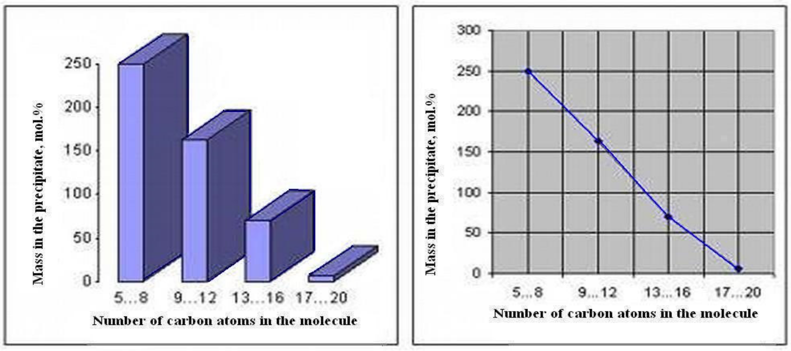

Fig. 11: Distribution of carbon compounds obtained in the experiments of S. Miller, mass and number of carbon atoms in the molecule (diagrams constructed by the authors according to the S. Miller experiments)

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). 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 coronal electric discharge on water drops placed on the electrode of the device for gas coronal electric discharge formation. Water drops were heated to the boiling point in an electric field of high frequency and voltage and an electric discharge was applied, analogous as in the primordial atmosphere. As a result, an organized structure with a size of ~1.2–1.3 mm was formed in inter-electrode space (Fig. 12). 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 space where the basic electric voltage was applied.

Fig. 12: 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 original size for some time in the absence of the electric discharge.

This experiment shows that the organization of structures in water under certain external conditions as the temperature my 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 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 membrane like structures.

Our data confirm 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 – temperature, 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 content of deuterium in hot mineral water may be increased due to the physical chemical processes of the deuterium accumulation as the 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 the primary hydrosphere really contained HDO, that this may explain the thermal stability of the first organic life forms in the hot mineral water, as the thermal stability of deuterated macromolecules like DNA and proteins in D_2O solutions is somewhat higher than their protonated forms due to the isotopic effects of deuterium.

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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 modelling 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. (2013b) 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. (2013c) *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.

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

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).

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.