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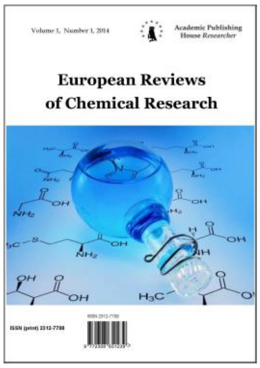


Articles and Statements

Origin of Life in Hot Mineral Water from Hydrothermal Springs and Ponds. Effects of Hydrogen and Nascent Hydrogen. Analyses with Spectral Methods, pH and ORP

Ignat Ignatov [a,*]

[a] Scientific Research Center of Medical Biophysics (SRCMB), Bulgaria

Abstract

The studies were performed of the composition of water, its temperature, pH and oxidation reduction potential (ORP) value in experiments with modelling of primary hydrosphere and possible conditions for origin of first organic forms of life in hot mineral water in hydrothermal springs and open ponds. Experiments with hot mineral and seawater from Bulgaria by IR-spectroscopy with DNES-method and Thermo Nicolet Avatar 360 Fourier-transform IR were conducted. Cactus juice of *Echinopsis pachanoi* and Jellyfish *Aurelia aurita* from Black Sea were used as model systems. The reactions of condensation and dehydration in alkaline aqueous solutions with pH = 9–11, resulting in synthesis of larger organic molecules as polymers and short polipeptides from separate molecules, were considered and scrutinized. It was shown that hot alkaline mineral water with temperature from +65 °C to +95 °C and pH value from 8.5 to 10 and ORP with negative value is more suitable for the origination of life and living matter than other analyzed water samples. The pH value of seawater on the contrary is limited to the range of 7,5 to 8,4 units. The research was connected with estimation of the common local extremums in hot mineral and sea water, cactus juice and jellyfish.

Keywords: origin of life, hot mineral water, hydrothermal conditions, IR-spectroscopy.

1. Introduction

Previous biological experiments with D_2O and structural-conformational studies with deuterated molecules, enable to modeling conditions under which the first living forms of life might be evolved (Ignatov, Mosin, 2013). 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.

* Corresponding author
E-mail addresses: mbioph@dir.bg (I. Ignatov)

The point regards the influence of temperature on the 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, 2013). 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). Probably the life has begun 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 (K), zinc (Zn), manganese (Mn) and phosphate ions (P), which are not particularly widespread in the sea aquatorium (Mulkidjanian, Galperin, 2009). Colín-García and co-authors also summarize a set of experiments proposed to test the role of hydrothermal vents in prebiotic synthesis (Colín-García et al., 2016). B. Damer and D. Deamer have come to the conclusion that cell membranes cannot be formed in salty seawater. Before the continents formed, the only dry land on Earth would be volcanic islands, where rainwater would form ponds where lipids could form the first stages towards cell membranes. Only when true cells had evolved they gradually would adapt to saltier environments and enter the ocean (Damer, Deamer, 2015). J. Trevors and G. Pollack were proposed 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 (H) and carbon (C). The analyses show the possible scenario of the syntheses of periodically molecules of life (Colón Santos, 2019). The data presented in this paper show that the origination of living matter most probably occurred in hot mineral water. This occurred in hydrothermal springs and ponds with hot mineral water. There had been possible also in hydrothermal vents in seawater with 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).

The purpose of the research was studying the conditions of primary hydrosphere (temperature, pH, ORP, isotopic composition) for possible processes for origin of life and living matter in hot mineral water. There was studied primary atmosphere and interaction with hydrosphere and with effects of gas discharge. Various samples of water from Bulgaria were studied within the frames of the research.

2. Material and methods

2.1. Objects of Studying

The research by the IR-spectrometry with DNES-method (Antonov, 1990; Antonov, Ignatov, 1998) was carried out with samples of water taken from various water sources:

1 – mineral water (Rupite, Bulgaria);

2 – seawater (Varna, Bulgaria);

Sediments from hot mineral spring and pond in Rupite, Bulgaria and sea salt from Black Sea were studied using the Thermo Nicolet Avatar 360 Fourier-transform IR;

Cactus juice of *Echinopsis pachanoi* and Jellyfish *Aurelia aurita* (Varna, Bulgaria, Black Sea) were used as two model systems which were both investigated by the IR-spectrometry with DNES method.

2.2. IR-Spectroscopy

IR-spectra of water samples were registered on Thermo Nicolet Avatar 360 Fourier-transform IR (K. Chakarova) and Differential Non-equilibrium Spectrum (DNES).

2.3. pH indicator and oxidation reduction potential (ORP)

The research of pH and ORP with Hanna instruments was performed.

3. Results and discussion

Research of various samples of mineral water from mineral springs and seawater from Bulgaria was carried out. The hot mineral spring and ponds of Rupite are located in eastern foot of the extinct volcano Kozhuh (Figure 1).

Fig. 1. Hot mineral ponds of Rupite, Bulgaria, foot of the extinct volcano Kozhuh

For this DNES method was employed for research of cactus juice *Echinopsis pachanoi* (Table 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 from Rupite, Bulgaria (Table 1).

DNES-spectra of cactus juice and mineral water from Rupite, Bulgaria with HCO_3^-(1320–1488 mg/L), Ca^{2+} (29–36 mg/L) t = 76 °C (source) and t = 52-54 °C (open ponds depending the season) have magnitudes of local extremums at –0.1112 (11.15); -0.1187 (10.45); -0.1262 (9.83); -0.1287 (9.64) and –0.1387 eV (9.85 μm). Similar local extremum in the DNES-spectrum between cactus juice and seawater were detected at -0,1362 eV (9.10 μm).

Table 1. Results with DNES spectral method and Thermo Nicolet Avatar 360 Fourier-transform IR of cactus juice, jelly fish, sea water and salt, sea water and salt, mineral water and sediments from Rupite, Bulgaria

-E (eV); λ (μm); k (cm⁻¹) DNES-method	-E (eV); λ (μm); k (cm⁻¹) DNES-method	λ (μm); k (cm⁻¹) Thermo Nicolet Avatar 360 Fourier-transform IR	-E (eV); λ (μm); k (cm⁻¹) DNES-method	λ (μm); k (cm⁻¹) Thermo Nicolet Avatar 360 Fourier-transform IR	-E (eV); λ (μm); k (cm⁻¹) DNES-method
Cactus juice	Mineral water Rupite (Bulgaria)	Mineral water Rupite (Bulgaria)	Sea Water Black Sea	Sea salt Black Sea	Jelly fish Black Sea
0.1112 (**11.15**; 897)	0.1112 (**11.15**; 897)	(**11.44**; 875)			
0.1187 (**10.45**; 957)	0.1187 (**10.45**; 957)	(**10.95**; 913)			0.1200; (**10.33**; 968)
0.1262 (**9.82**;1018)	0.1262 (**9.82**; 1018)				
0.1287 (**9.63**; 1038)	0.1287 (**9.63**; 1038)	(**9.78**; 1031)			
0.1362 (9.10;1099)	–	(**9.08**;1101)	0.1362 (**9.10**;1099)		(0.1375; **9.02**; 1109)
0.1387	0.1387 (**8.95**; 1117)	–		(**8.93**;1120)	

Note: *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) the biggest local maximums of this function are observed; λ – wave length; κ – wave number.

Common extremums in the IR-spectrum between cactus juice (DNES method) and minerals from the sediments (Thermo Nicolet Avatar 360 Fourier-transform IR) were detected at 11.44; 10.95; 9.78; 9.08; μm. Similar local extremum between cactus juice and sea salt was detected at 8.93 μm.

Study of the local extremums in DNES spectrum of jellyfish from Black Sea was performed (Table 1). There are two local extremums at 9.02 and 10.33 μm. The local extremum at 9.02 μm corresponds to the local extremums in sea salt (8.93 μm).

The local extremums of sediments from hot mineral spring in Rupite, Bulgaria were studied with Thermo Nicolet Avatar 360 Fourier-transform IR (Figure 2).

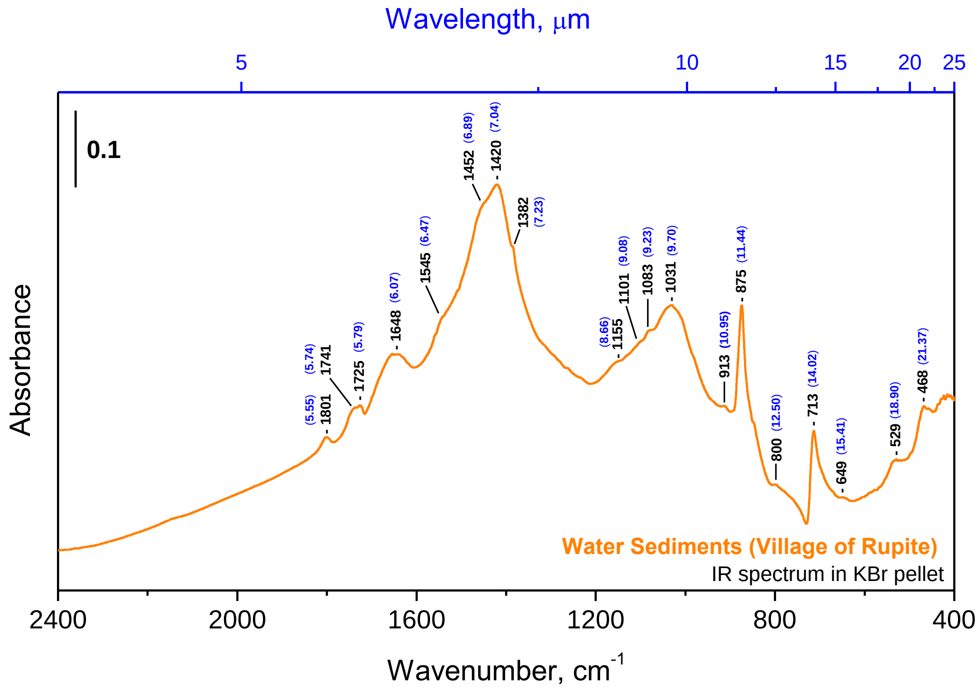


Fig. 2. IR-spectrum of water obtained from Rupite Village (Bulgaria)

The results with DNES method indicated that jellyfish *Aurelia aurita* had local exremums at 9.02 and 10.33 μm in IR-spectra (Table 1). Before measurements the jellyfish was kept in seawater for several days. For comparison seawater has a local extremum at 9.10 μm with DNES method and Sea salt 8.93 μm with Nicolet Avatar 360 Fourier-transform IR (Figure 3). Jellyfish contains approximately 97 (w/w) % of water and is the most unstable living organism compared to those ones that form stromatolites. The explanation for this is the smaller concentration of salts and, therefore, the smaller number of local extremums in the IR-spectrum in relation to seawater.

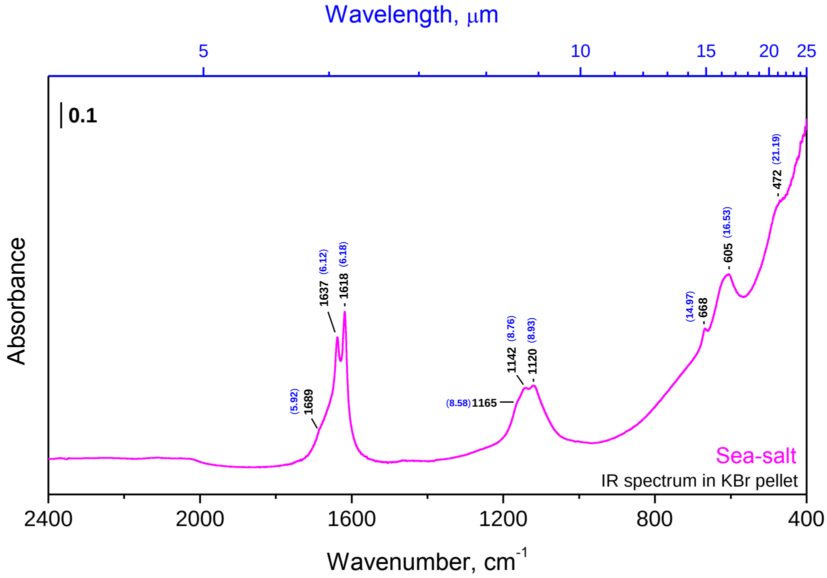


Fig. 3. IR-spectrum of seasalt obtained from Varna (Bulgaria)

Such a character of IR-spectrums and distribution of local extremums may prove that hot mineral alkaline water is preferable for origin and maintenance of life compared to other types of water analyzed by DNES and Nicolet Avatar 360 Fourier-transform IR. Thus, in hot mineral waters the local extremums in the IR-spectrums are more manifested compared to the local extremums obtained in IR-spectrum of the same water at a lower temperature (Ignatov, 2013). The difference in the local extremums in the interval from +20 °C to +95 °C at each 5 °C step is significant at $p < 0.05$ according to Student's t-criterion. 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. The IR-spectrum of cactus juice is the closest to the IR- and DNES-spectrum of water, which contains bicarbonates and calcium ions typical for the formation of stromatolites. For this reason cactus juice was applied as a model system. The local extremums in IR-spectra of alkaline mineral water interacting with $CaCO_3$ and then seawater are the. The most closed closest to the local extremums in IR-spectrum of cactus juice.

In connection with these data the following reactions participating with $CaCO_3$ in aqueous solutions are important:

$CO_2 + 4H_2S + O_2 = CH_2O + 4S + 3H_2O$, (1)
$CaCO_3 + H_2O + CO_2 = Ca(HCO_3)_2$, (2)
$CO_2 + OH^- = HCO_3^-$ (3)
$2HCO_3^- + Ca^{2+} = CaCO_3 + CO_2 + H_2O$ (4)

The equation (1) shows how some chemosynthetic bacteria use energy from the oxidation of H_2S and CO_2 to S and formaldehyde (CH_2O). The equation (2) 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 (3)). Equation (4) is valid for the process of formation of the stromatolites – the dolomite layered acretionary 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 (Pons et al., 2011). It is known that water in geysers is rich in carbonates, while the temperature is ranged from +60 to +100 °C and more than +100 °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). DNA and synthetic enzymes created proto cells from aqueous solution of organic molecules. For this the initial solution was heated to a temperature close to water's boiling point +95 °C. Then its temperature was lowered to +65 °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.

A research is conducted of Oxidation Reduction Potential (ORP) of hot mineral water from Rupite, Bulgaria. With temperature increase ORP gets reduced with (-70 mV) from 50 to 25° C (Table 2). The measured pH is 7.70. The change of ORP shows that in hot mineral water are released electrons in alkaline medium.

When reviewing the processes of life origin it is necessary to consider the composition of primary atmosphere 3.5 billion years ago. It contains H_2, N_2, CO_2, CO. With the temperature rise of the water and boiling in the modern atmosphere, the bubbles contain oxygen and it gets acidified. The author suggests that the bubbles in the water in contact with the ancient atmosphere contain hydrogen, and the water gets more alkaline. Also ORP decreases and may result in negative values. An experiment is conducted with saturation of water from Rupite with hydrogen. The achieved average result is (-215 mV) with temperature 50°C.

Table 2. Results of temperature (°C) and Oxidation Reduction Potential (ORP) of mineral water from Rupite, Bulgaria

Temperature (° C)	Oxidation Reduction Potential (ORP) (mV)
25	57
30	45
35	37
40	30
45	18
50	-13

The difference is (-215 mV)-(-13 mV) = (-202 mV). This indicates that in the ancient atmosphere were gained more electrons in the water. In such a way are achieved more hydroxyl groups (OH^-) and bicarbonate ions (HCO_3^-).

The interaction with calcium ions (Ca^{2+}) during exchange of electric charges as per formulae makes the processes for structuring of stromatolites by formulae (1) and (2) more active.

$CO_2 + OH^- = HCO_3^-$ (3)

$2\ HCO_3^- + Ca^{2+} = CaCO_3 + CO_2 + H_2O$ (4)

The following reaction (5) is valid in electrolysis. In the ancient atmosphere and hydrosphere there was increased gas discharge.

$2H_2O + 2\ e^- = H_2 + 2OH^-$ (5)

The same reaction contributed for the formation of stromatolites. Nowadays it is observed in elecrolyzer devices for waters catholyte and anolyte. In the ancient hydrosphere and land the charge had been negative and in the atmosphere positive. The conditions had been optimal for "nature" electrolysis. In these direction had been the experiments of Miller (Miller, 1953).

The reaction (4) is possible to structure nascent hydrogen H^* (Mehandjiev et al., 2019).

$H_2O + e^- \rightarrow H^* + OH^-$, (6)

The reaction (5) released in the gaseous form after recombination is formed:

$H^* + H^* \rightarrow H_2$ (7)

The nascent hydrogen is very active for chemical reactions in primary hydrosphere for origin of life and additional formation of H_2 makes alkalizing effect.

The allocation of H_2O molecule when a peptide chain is formed is important factor in reaction of condensation of two amino acid molecules into dipeptide. As reaction of polycondensation of amino acids is accompanied by dehydration, the H_2O removal from reaction mixture speeds up the reaction rate. 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 (Ignatov, Mosin, 2012).

The results with ORP show that free electrons in water support for amino acid polymerization and small blocks into larger organic molecules (Ignatov, 2019).

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 (Figure 4).

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 = 9–11 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 this hypothesis (Ignatov, Mosin, 2012).

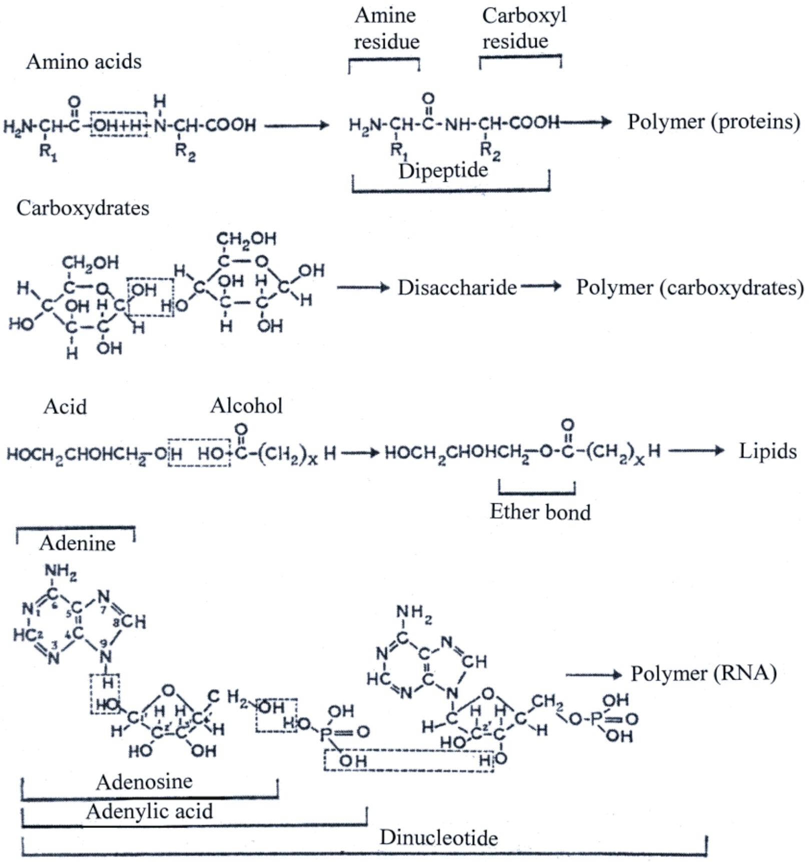
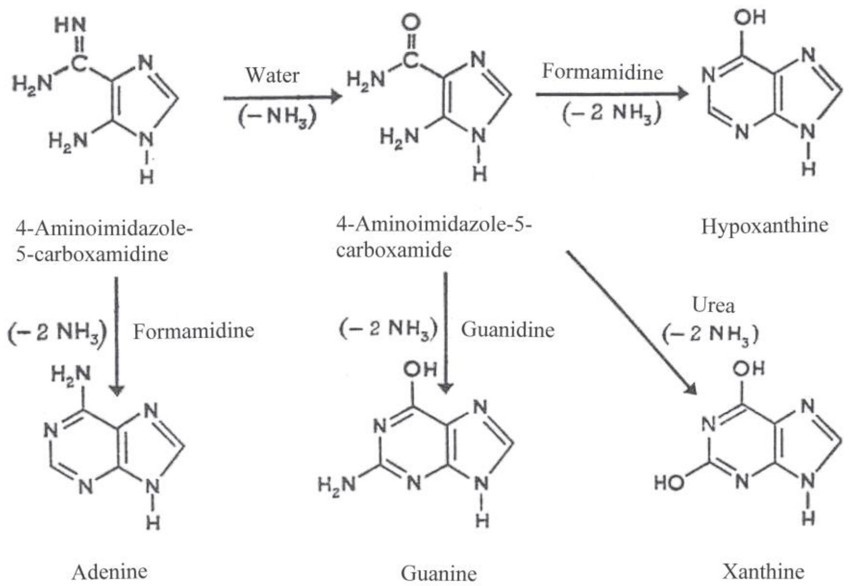
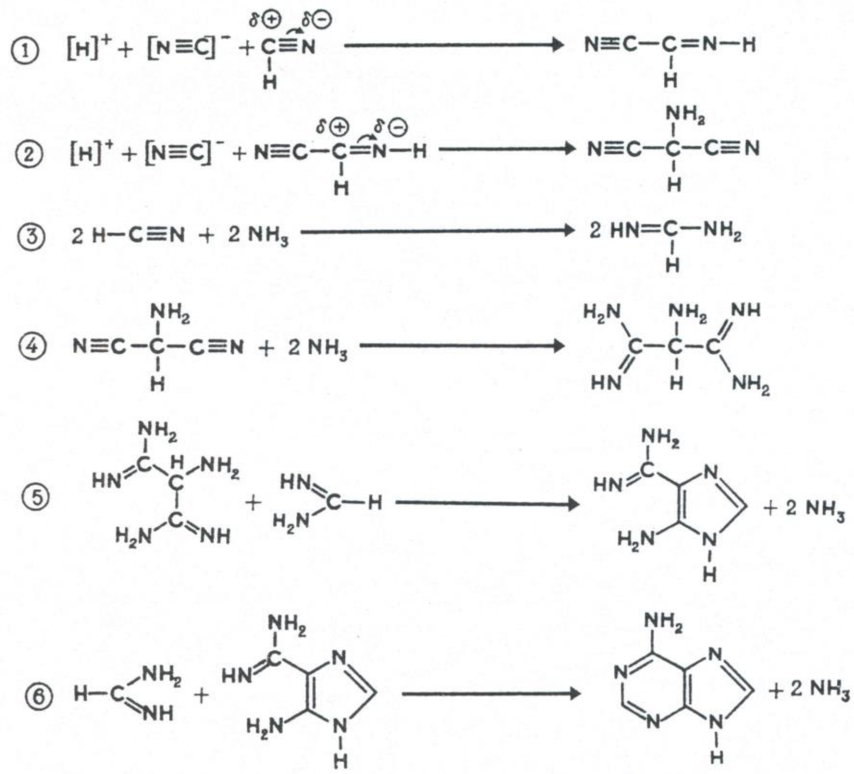


Fig. 4. Reactions of condensation and dehydration in alkaline conditions with pH = 9–11 catalyzed by HCN and its derivatives, resulting in synthesis from separate molecules larger organic molecules of polymers. The top three equations: condensation and the subsequent polymerization of amino acids in 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 °C (Harada, Fox, 1964). In model experiments reaction products were polymerized into peptide chains that are the important stage towards inorganic synthesis of protein. Purines and pyrimidines were formed in a reactionary mixture of water solution with a HCN–NH_3 (Figure 5). In other experiments amino acid mixtures were subjected to influence of temperatures from +60 °C up to +170 °C with formation of short protein-like molecules resembling early evolutionary forms of proteins subsequently designated as thermal proteinoids (Ignatov, Mosin, 2012). 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)

Fig. 5. Prospective mechanisms of thermal (+95 °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)

In natural conditions water was heated by the magma. The structure formed from heated water was evidently a result of self-organization. Living organisms are complex self-organizing systems. They are open because they constantly exchange substances and energy with the environment and change the entropy (Ignatov, 2011). 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).

The initial stage of evolution, apparently, was connected with formation of the mixtures of amino acids and nitrogenous substances – analogues of nucleic acids at high temperature. 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 ^oC. After that membrane like structures were formed in a mix of proteinoids in hot water solutions. In 2011 T. Sugawara (Japan) created membrane like proto cells from aqueous solution of organic molecules, DNA and synthetic enzymes under temperature close to water's boiling point +95 ^oC (Sugawara, 2011).

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, ORP, electric discharges and isotopic composition. Hot mineral alkaline water interacting with $CaCO_3$ is closest to these conditions. Next in line with regard to 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 and negative ORP. In warm and hot mineral waters the local extremums in IR-spectrums from 8 to 14 μm were more expressed in comparison with the local extremums measured in the same water samples with lower temperature. The new achievement is connected with chemical composition of ancient atmosphere and alkalization of the water from the hydrogen.

The research for origin of life and living matter in hot mineral water in mineral springs and open ponds (Ignatov, 2010; Szostak, 2011; Damer, Deamer, 2015) and in hydrothermal vents in the ocean (Mulkidjanian, Galperin, 2009) give the possibilities for life at the surface of the moons of the planets in Solar System. 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 (Ignatov, 2010). The candidates are – Europa (moon of Jupiter), Titan and Enceladus (moons of Saturn).

5. Acknowledgements

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