Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.3, No.14, 2013

Modeling of Possible Processes for Origin of Life and Living Matter in Hot Mineral and Seawater with Deuterium

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Abstract

The isotopic compositions of water its temperature and pH value were analyzed in experiments with modeling of primary hydrosphere. We performed experiments for the research of hot mineral and seawater from Bulgaria with IR-spectroscopy (differential non-equilibrium energy spectrum (DNES), Brucker Vertex Fourier-IR and Thermo Nicolet Avatar 360 Fourier-transform IR). It was shown that hot alkaline mineral water with temperature from +65 ^{0}C to +95 ^{0}C with pH = 9–11 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. In frames of the research the conditions imitating primary hydrosphere enriched with deuterium were also studied. The prokaryotic and eukaryotic cells of various microorganisms realizing methylotrophic, chemoheterotrophic, photoorganotrophic, and photosynthetic pathways of assimilation of carbon substrata were adapted to the maximal concentration of D_2O, all biological material of which contained deuterium instead of hydrogen. Their studying would clear up the mechanism of function of deuterated macromolecules in conditions of hot D_2O-solutions.

Keywords: deuterium, heavy water, hydrosphere, evolution, origin of life and living matter

1. Introduction

Natural prevalence of deuterium (^{2}H or D) makes up approximately 0.015 atom %, 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 well-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 vapor 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 absolute content of deuterium (isotopic shifts, δ, ppm) according to the international standard VSMOW, corresponding to Pacific ocean water which is rather stable on isotopic composition, compiles for D/H = (155.76 ± 0.05) · 10^{-6} (155.76 ppm) (Lis et al., 2008). For the international standard SLAP of natural water of the Antarctic Region containing less deuterium, the absolute content of deuterium compiles D/H = 89–· 10^{-6} (89 ppm). The average ratio of H/D in nature makes up approximately 1: 5700. In mixtures of D_2O with H_2O the isotopic exchange occurs with high speed with the formation of semi-heavy water (HDO): $D_2O + H_2O = HDO$. For this reason deuterium presents in smaller content in aqueous solutions in form of HDO, while in the higher content – in form of D_2O.

In natural waters deuterium distributes non-uniformly: from 0.015 atom % D for water from the Antarctic ice – the most deuterium depleted natural water with deuterium content being 1.5 times smaller, than in sea water, up to 0.02–0.03 at.% D for river and sea water. Melted snow and glacial waters in mountains and some other regions of the Earth usually contain 3–5 % less deuterium than drinking water. On the average, 1 ton of river water contains approximately 150–300 grams of deuterium. Other natural waters contain varying levels of deuterium from δ = +5.0 D,%, SMOW (Mediterranean Sea) up to δ = -105.0 D,%, SMOW (Volga River).

The structure of the D_2O molecule is the same as that of H_2O, with very small distinction in values of lengths of covalent bonds. D_2O boils at 101.44 ^{0}C, freezes at 3.82 ^{0}C, has density at 20 ^{0}C 1.105 g/cm^3, and the maximum

of density is not on 4 ^{0}C, as for H_2O, but on 11.2 ^{0}C (1.106 g/cm^3). These effects are reflected in energy of a chemical bond, kinetics and chemical reactions rates in D_2O–H_2O mixtures. Enzymatic reactions in D_2O are considerably slowed down. However, there are reactions, catalyzing by D_3O^+ or H_3O^+ ions or OD^- and OH^- ions, which rates in D_2O are higher, than in H_2O. According to the theory of chemical bond, breaking up of H–O bonds can occur faster, than D–O bonds, mobility of D_3O^+ ion is lower on 28..5 % than H_3O^+ ion, and OD^- ion is lower on 39.8 % than OH^- ion, the constant of ionization of D_2O is less than that of H_2O (Lobishev and Kalinichenko, 1978; Bild et al, 2004). The maximum kinetic isotopic effect at ordinary temperatures in a chemical reaction leading to rupture bonds involving H and D was calculated, and the maximum ratio k_h/k_d in macromolecules is in the range of 6 to 8 for C– H versus C–D, N–H versus N–D, and O–H versus O–D bonds (Vertes, 2004).

Deuterated cells of various microorganisms adapted to the maximal concentration of D_2O in growth media (99.9 at.%) are convenient objects for evolutional and adaptation studies as well as structural-functional studies. During the cellular growth on D_2O media in cells are synthesized macromolecules in which hydrogen atoms in carbon backbones are replaced on deuterium (Kushner et al, 1999). Such deuterated macromolecules undergo the structural-adaptive changes necessary for normal functioning of cells in the presence of D_2O.
Practical interest in applying deuterated cells of various microorganisms as biomodels in the research of cellular adaptation mechanisms to D_2O and molecular evolution has predetermined a direction of our studies. The purpose of the present research was studying the isotopic effects of deuterium and the conditions of primary hydrosphere (temperature, pH, isotopic composition) for possible processes for origin of life and living matter. Various samples of water from Bulgaria were studied within the frames of the research.

2. Experimental part

Materials and methods

The research was carried out with using prokaryotic and eukaryotic microorganisms, realizing methylotrophic (obligate and facultative methylotrophic bacteria *Brevibacterium methylicum and Methylobacillus flagellatum*), chemoheterotrophic (*Bacillus subtilis*), photoorganoheterotrophic (halobacterium *Halobacterium halobium*) and photosynthetic (blue-green algae *Chlorella vulgaris*) ways of assimilation of substrata.
Samples of water for the research by the IR-spectroscopy method were taken from various sources of Bulgaria:

1 – mineral water (Rupite, Bulgaria);

2 – seawater (Varna resort, Bulgaria);

As two model systems, the cactus juice of *Echinopsis pachanoi* and the jellyfish *Cotylorhiza tuberculata* were investigated by the IR-spectroscopy method.

For preparation of growth media was used D_2O (99.9 atom %) and DCl (95.5 atom %) received from the "Isotope" Russian Research Centre (St. Petersburg, Russian Federation). Inorganic salts and glucose were preliminary crystallized in D_2O and dried in vacuum before using. D_2O 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).
For cell cultivation and adaptation studies were used growth media with an increasing gradient of D_2O concentration from 0; 24.5; 49.0; 73.5 up to 98 % (v/v) D_2O. Cultivation of methylotrophic bacteria was carried out on minimal salt medium M9 (g/l): KH_2PO_4 – 3; Na_2HPO_4 – 6; NaCl – 0.5; NH_4Cl – 1. Cultivation of chemoheterotrophic bacteria was carried out on HW medium (w/w.%): glucose – 12; yeast extract – 2.5; NH_4NO_3 – 3; $MgSO_4 \cdot 7H_2O$ – 2; chalk – 2. Cultivation of photoorganoheterotrophic bacteria was carried out on TS medium (g/l): yeast extract – 2.5; NaCl – 250; $MgSO_4 \cdot 7H_2O$ – 20; KCl – 2; NH_4Cl – 0.5; KNO_3 – 0.1; KH_2PO_4 – 0.05; K_2HPO_4 – 0.05; Na^+-citrate – 0.5; $MnSO_4 \cdot 2H_2O$ – $3 \cdot 10^{-4}$; $CaCl_2 \cdot 6H_2O$ – 0.065; $ZnSO_4 \cdot 7H_2O$ – $4 \cdot 10^{-5}$; $FeSO_4 \cdot 7H_2O$ – $5 \cdot 10^{-4}$; $CuSO_4 \cdot 5H_2O$ – $5 \cdot 10^{-5}$; glycerin – 1.0. Blue-green algae grew on Tamia growth medium (g/l): KNO_3 – 5.0; $MgSO_4 \cdot 7H_2O$ – 2.5; KH_2PO_4 – 1.25; $FeSO_4$ – 0.003; $MnSO_4 \cdot 2H_2O$ – $3 \cdot 10^{-4}$; $CaCl_2$ $6H_2O$ – 0.065; $ZnSO_4$ $7H_2O$ – $4 \cdot 10^{-5}$; $CuSO_4 \cdot 5H_2O$ – $5 \cdot 10^{-5}$, $CoCl_2 \cdot 6H_2O$ – $5 \cdot 10^{-6}$.
For adaptation were used solid 2 % (w/v) agarose media with gradually increasing concentration of D_2O, combined with the subsequent selection of clones resistant to deuterium. The cells were grown in 250 ml Erlenmeyer flasks containing 20 ml of the medium at 32–34 ^{0}C and vigorously aerated on an orbital shaker Biorad ("Biorad Labs", Poland). Photoorganoheterotrophic bacteria and blue-green algae were grown at 38 ^{0}C at illumination by fluorescent lamps. Bacterial growth was defined on ability to formation of separate colonies on a surface of 2 % (w/v) agarose media, and on absorbance of cell suspension measured on Beckman DU-6 (Beckman Coulter, USA) spectrophotometer at λ = 620 nm.
The analysis of amino acids from protein hydrolyzates was carried out on a Biotronic LC-5001 (230 × 3.2) column ("Eppendorf–Nethleler–Hinz", Germany) with a UR-30 ("Beckman–Spinco", USA) sulfonated styrene (7.25 % cross linked) resin as a stationary phase; the granule diameter was 25 μm; 0.2 N sodium–citrate buffer

(pH = 2.5) was used as an eluent; the working pressure – 50–60 atm; the eluent input rate – 18.5 ml/h; the ninhydrin input rate – 9.25 ml/h; detection at λ = 570 and 440 nm (for proline).

Carbohydrates were analyzed on a Knauer Smartline chromatograph ("Knauer", Germany) equipped with a Gilson pump ("Gilson Inc.", Germany) and a Waters K-401 refractometer ("Water Associates", Germany) using Ultrasorb CN as a stationary phase: the column size – 250 × 10 mm; the granule diameter – 10 μm; the mobile phase – acetonitrile–water (75 : 25, % (w/w); the input rate – 0.6 ml/min.

Fatty acids were analyzed on a Beckman Gold System (USA) chromatograph, equipped with Model 126 Detector (USA). Stationary phase: Ultrasphere ODS, particel size 5 μm, 4.6 × 250 mm; mobile phase: linear gradient of 5 mM KH_2PO_4-acetonitrile (shown in phantom), elution rate 0.5 ml/min, detection at λ = 210 nm.

The levels of deuterium enrichment were measured on pulse mass spectrometer VG-70 SEQ ("Fisons VG Analytical", the USA), supplied with caesium source Cs^+ on a glyceric matrix with accelerating pressure 5 кВ and an ionic current 8 мА and ^{1}H-NMR-spectroscopy on Brucker WM-250 device ("Brucker", Germany) (working frequency 70 MHz, internal standard Me_4Si).

IR-spectra of water samples were registered on Fourier-IR spectrometer Brucker Vertex ("Brucker", Germany) (a spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; the permission – 0.5 cm^{-1}; accuracy of wave number – 0.1 cm^{-1} on 2000 cm^{-1}); Thermo Nicolet Avatar 360 Fourier-transform IR (Chakarova); Differential Non-equilibrium Spectrum (DNES).

A device for high-frequency coronal electric discharge was used, constructed by I. Ignatov and Ch. Stoyanov. The frequency of the applied saw-tooth electric voltage was 15 kHz, and the electric voltage - 15 кV. The method of the color coronal spectral analysis was applied (Ignatov, 2007). The electric discharge was obtained using a transparent polymer electrode on which a sample liquid was placed. The spectral range of the photons released upon electric discharge was from 400 to 490 and from 560 to 700 nm.

3. Results and discussion

3.1. Adaptation to D_2O

We have investigated isotopic effects of deuterium in prokaryotic and eukaryotic cells of various taxonomic groups of microorganisms realizing methylotrophic, hemoheterotrophic, photoorganotrophic and photosynthetic ways of assimilation of carbon substrates (methylotrophic bacteria, halobacteria, blue-green algae) using ^{1}H-NNR-, IR-, and mass-spectrometry technique. The strategy of adaptation to D_2O is shown in Table 1 on an example of facultative methylotrophic bacterium *B. methylicum*, adapted to D_2O via multistage process on growth media with 2 % (v/v) deutero-methanol and increasing gradient of D_2O concentration (0; 24.5; 49.0; 73.5 and 98.0 % (v/v) D_2O). The step-by-step adaptation method consisted in planting initial cells on solid 2 % (w/v) agarose growth media with increasing gradient of D_2O concentration (from 0; 24.5; 49.0; 73.5 to 98.0 % (v/v) D_2O) and the subsequent selection of clones resistant to deuterium. Cells grown on media with a low gradient of D_2O concentration were transferred on media with higher gradient, up to 98 % (v/v) D_2O. The degree of cell survival on maximum deuterated media was about 40 %. Then the colonies were transferred onto the liquid growth medium of the same D_2O-content, prepared on the basis of 98% (v/v) D_2O and grown for 5 days at 34 ^{0}C. The survival rate in the maximal deuterated growth medium was not more than 40–50 %. The progress of adaptation was observed by the changes of lag-time period, time of cell generation and output of the microbial biomass, as well as by the ability of cells to form single colonies on the surface of solid agarose media with ^2H_2O and cell counting.

Table 1. The isotopic composition of growth media and growth characteristics of methylotrophic bacterium *B. methylicum* in the process of adaptation to D_2O*

Experiment number	Media components, % (v/v)		Lag-period, (h)	Yield of biomass, gram from 1 liter of liquid culture	Cell generation time (h)
	H_2O	D_2O			
1	98.0	0	20±1.40	200.02±1.40	20±1.40
2	73.5	24.5	34±0.89	171.8±1.81	2.6±0.23
3	49.0	49.0	44±1.38	121.3±1.83	3.2±0.36
4	24.5	73.5	49±0.91	94.4±1.74	3.8±0.25
5	0	98.0	60±2.01	60.2±1.44	4.9±0.72
6	0	98.0	40±0.88	174.0±1.83	2.8±0.30

Notes: * The data in Expts. 1–5 is submitted for *B. methylicum* at growing on growth media, containing 2% (v/v) deutero-methanol and specified amounts (% , v/v) of D_2O.

The data in Expt. 6 is submitted for adapted to D_2O bacterium.

As the control used experiment 1 where used protonated water and methanol.

All microorganisms adapted to D_2O retained the ability to grow on growth media with high content of D_2O. The general feature of bacterial growth in D_2O was the proportional increase in duration of the lag-period and time of cellular generation and simultaneous reduction of outputs of microbial biomass. These parameter values were correlated with the content of D_2O in growth media with the lowest fixing values of these parameters on maximum deuterated media. The added gradually increasing concentrations of D_2O into growth media caused the proportional increasing lag-period and yields of microbial biomass in all isotopic experiments (Table 1). In contrast to the adapted microorganisms, the growth of non-adapted microorganisms on the maximal deuterated media with D_2O was inhibited. The yields of biomass on deuterated growth media were varied 85-90 % for different taxonomic groups of microorganisms. Adapted microorganisms possessed slightly reduced levels of microbial biomass accumulation and increased cell generation times on maximal deuterated media.

Our experiments demonstrated that the effects observed at the cellular growth on D_2O possess a complex multifactor character connected to changes of physiological parameters – magnitude of the lag-period, time of cellular generation, outputs of biomass, a ratio of amino acids, protein, carbohydrates and fatty acids synthesized in D_2O, and with an evolutionary level of organization of investigated object as well. The cell evidently implements the special adaptive mechanisms promoting functional reorganization of work of the vital systems in the presence of D_2O. Thus, the most sensitive to replacement of H on D are the apparatus of biosynthesis of macromolecules and a respiratory chain, i.e., those cellular systems using high mobility of protons and high speed of breaking up of hydrogen bonds. Last fact allows the consideration of adaptation to D_2O as adaptation to the nonspecific factor affecting simultaneously the functional condition of several numbers of cellular systems: metabolism, ways of assimilation of carbon substrates, biosynthetic processes, and transport function, structure and functions of macromolecules. There is evidence that during adaptation to D_2O the ratio of synthesized metabolites is changing. Furthermore, deuterium induces physiological, morphological and cytological alterations on the cell. This leads to the formation in D_2O large atypical cells (Mosin and Ignatov, 2012). They are usually 2–3 times larger in size and have a thicker cellular wall compared to the control cells grown on H_2O. The structure of DNA in deuterated cells in D_2O may alter; the distribution of DNA in them was non-uniform. Our data generally confirms a stable notion that adaptation to D_2O is a phenotypic phenomenon as the adapted cells return back to the normal growth after some log–period after their replacement back onto H_2O-medium. At the same time the effect of reversibility of growth on H_2O/D_2O does not exclude an opportunity that a certain genotype determines the displaying of the same phenotypic attribute in D_2O-media. Experiments with D_2O have shown, that green-blue algae is capable to grow on 70 % (v/v) D_2O, methylotrophic bacteria – 75 % (v/v) D_2O, chemoheterotrophic bacteria – 82 % (v/v) D_2O, and halobacteria – 95 % (v/v) D_2O.

In the process of adaptation to D_2O, most important for the intermacromolecular structure are dynamic short-lived hydrogen (deuterium) bonds formed between the electron deficient H(D) atoms and electronegative O, C, N, S- heteroatoms, acting as acceptors of H-bond. The hydrogen bond, based on weak electrostatic forces, donor-acceptor interactions with charge-transfer and intermolecular Van der Waals forces, are of vital importance in the chemistry of intermolecular interactions and maintenance of a spatial structure of macromolecules in aqueous solutions. The substitution of H with D affects the stability and geometry of hydrogen bonds in an apparently rather complex way and may, through the changes in the hydrogen bond zero-point vibration energies, alter the conformational dynamics of hydrogen (deuterium)-bonded structures of DNA and proteins in D_2O. It may cause disturbances in the DNA-synthesis, leading to permanent changes on DNA structure and consequently on cell genotype (Thomson, 1960). Isotopic effects of deuterium, which would occur in macromolecules of even a small difference between hydrogen and deuterium, would certainly have the effect upon the structure. The sensitivity of enzyme function to the structure and the sensitivity of nucleic acid function (genetic and mitotic) would lead to a noticeable effect on the metabolic pathways and reproductive behavior of an organism in the presence of D_2O (Cleland et al, 1976). And next, the changes in dissociation constants of DNA and protein ionizable groups when transferring the macromolecule from H_2O to D_2O may perturb the charge state of the DNA and protein molecules.

Another important property is defined by the three-dimensional structure of the D_2O molecule having the tendency to pull together hydrophobic groups of macromolecules to minimize their disruptive effect on the hydrogen (deuterium)-bonded network in D_2O. This leads to stabilization of the structure of protein and nucleic acid macromolecules in the presence of D_2O (Mosin et. al., 1999). At placing a cell into D_2O-media lacking protons, not only H_2O is removed from a cell due to isotopic (H–D) exchange, but there also occurs a rapid isotopic (H–D) exchange in hydroxyl (-OH), sulfohydryl (-SH) and amino ($-NH_2$) groups in all molecules of organic substances, including proteins, nucleic acids, carbohydrates and lipids. It is known that in these conditions only covalent C–H bond is not exposed to isotopic (H–D) exchange and, thereof only molecules with bonds such as C–D can be synthesized de novo (Mosin et al., 1996).

It should be noted that due to isotopic effects of deuterium in D_2O are synthesized molecules with other structural and functional properties and activity than molecules formed with hydrogen. These differences can

cause variations in nucleic acid synthesis, which can lead to structural changes and functional differences in the cell and its organelles. Thus, the structural and dynamic properties of the cell membrane, which depends on qualitative and quantitative composition of membrane fatty acids, can also be modified in the presence of D_2O. The cellular membrane in the bacteria is one of the most important organelles for metabolic regulation, combining apparatus of biosynthesis of polysaccharides, transformation of energy, supplying cells with nutrients and involvement in the biosynthesis of proteins, nucleic acids and fatty acids. Obviously, the cell membrane plays an important role in the adaptation to D_2O. But it has been not clearly known what occurs with the membranes – how they react to the replacement of H to D and how it concerns the survival of cells in D_2O-media devoid of protons.

Comparative analysis of the fatty acid composition of deuterated cells of chemoheterotrophic bacteria *B. subtilis*, obtained on the maximum deuterated medium with 99.9 at.% D_2O, carried out by HPLC method, revealed significant quantitative differences in the fatty acid composition compared to the control obtained in ordinary water (Fig. 1 *a, b*). Characteristically, in a deuterated sample fatty acids having retention times at 33.38; 33.74; 33.26 and 36.03 min are not detected in HPLC-chromatogram (Fig. 1*b*). This result is apparently due to the fact that the cell membrane is one of the first cell organelles, sensitive to the effects of D_2O, and thus compensates the changes in rheological properties of a membrane (viscosity, fluidity, structuredness) not only by quantitative but also by qualitative composition of membrane fatty acids. A similar situation was observed with the separation of other natural compounds (proteins, amino acids, carbohydrates) extracted from deutero-biomass obtained from maximal deuterated D_2O-medium.

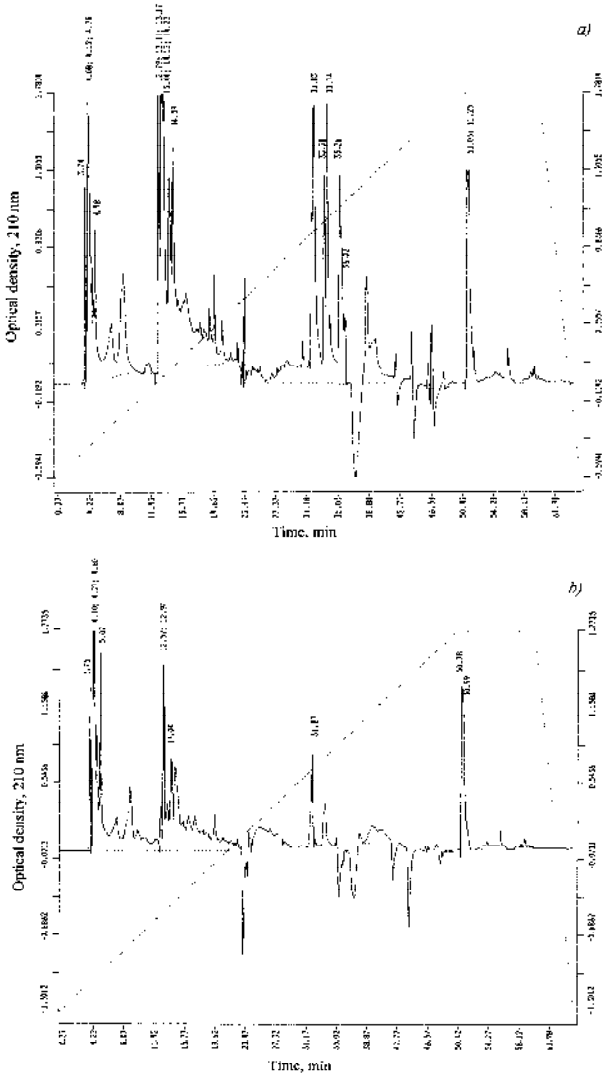


Fig. 1. HPLC-chromatograms of fatty acids obtained from protonated (*a*) and deuterated (*b*) cells *B. subtilis*, on the maximum deuterated D_2O-medium (HW-medium); a chromatograph Beckman Gold System (USA) Model 126 Detector (USA) stationary phase: Ultrasphere ODS, 5 μm; 4.6 × 250 mm; mobile phase: linear gradient 5

mM KH_2PO_4-acetonitrile (shown in phantom), elution rate: 0.5 ml/min, detection at λ = 210 nm. The peaks on the chromatogram with retention time 3.75 min (instead of 3.74 minutes in the control), 4.10; 4.27; 4.60 (instead of 4.08; 4.12; 4.28 in the control), 5.07 (instead of 4.98 in control) 12.57; 12.97 (instead of 12.79; 13.11; 13.17 in control) 14,00 (instead of 14.59 in the control), 31.87 (instead of 31.83 in the control); 33.38; 33.74; 33.26; 36.03; 50.78; 50.99 (instead of 51.03; 51.25 for control) correspond to individual intracellular fatty acids.

Amino acid analysis of protein hydrolyzates and intracellular carbohydrates isolated from deuterated cells of *B. subtilis*, also revealed the differences in quantitative composition of amino acids synthesized in D_2O-medium. Protein hydrolyzates contains fifteen identified amino acids (except proline, which was detected at λ = 440 nm) (Table 2). An indicator that determines a high efficiency of deuterium inclusion into amino acid molecules of protein hydrolyzates are high levels of deuterium enrichment of amino acid molecules, which are varied from 50 atom % for leucine/isoleucine to 97.5 atom % for alanine

Table 2. Amino acid composition of the protein hydrolyzates of *B. subtilis*, obtained on the maximum deuterated medium and levels of deuterium enrichment of molecules*.

Amino acid	Yield, % (w/w) dry weight per 1 gram of biomass		Number of deuterium atoms incorporated into the carbon backbone of a molecule**	Level of deuterium enrichment of molecules, % of the total number of hydrogen atoms***
	Protonated sample (control)	The sample obtained in 99.9 % D_2O		
Glycine	8.03	9.69	2	90.0
Alanine	12.95	13.98	4	97.5
Valine	3.54	3.74	4	50.0
Leucine	8.62	7.33	5	50.0
Isoleucine	4.14	3.64	5	50.0
Phenylalanine	3.88	3.94	8	95.0
Tyrosine	1.56	1.83	7	92.8
Serine	4.18	4.90	3	86.6
Threonine	4.81	5.51	–	–
Methionine	4.94	2.25	–	–
Asparagine	7.88	9.59	2	66.6
Glutamic acid	11.68	10.38	4	70.0
Lysine	4.34	3.98	5	58.9
Arginine	4.63	5.28	–	–
Histidine	3.43	3.73	–	–

Notes:
* The data obtained by mass spectrometry for the methyl esters of N-(dimethylamino) naphthalene-1-sulfonyl chloride (dansyl) amino acid derivatives.
** While calculating the level of deuterium enrichment protons (deuterons) at the carboxyl (COOH-) and NH_2-groups of amino acid molecules are not taken into account because of their easy dissociation in H_2O/D_2O
*** A dash means absence of data.

Qualitative and quantitative composition of the intracellular carbohydrates of *B. subtilis* obtained on maximally deuterated D_2O-medium shown in Table. 3 (the numbering is given to the sequence of their elution from the column) contained monosaccharides (glucose, fructose, rhamnose, arabinose), disaccharides (maltose, sucrose), and four other unidentified carbohydrates with retention time 3.08 min (15.63 %); 4.26 min (7.46 %); 7.23 min (11.72 %) and 9.14 min (7.95 %) (not shown). Yield of glucose in deuterated sample makes up 21.4 % by dry weight, i.e. higher than for fructose (6.82 %), rhamnose (3.47 %), arabinose (3.69 %), and maltose (11.62 %). Their outputs are not significantly different from control in H_2O except for sucrose in deuterated sample that was not detected (Table 3). The deuterium enrichment levels of carbohydrates were varied from 90.7 atom % for arabinose to 80.6 atom % for glucose.

Table 3. Qualitative and quantitative composition of intracellular carbohydrates of *B. subtilis* obtained on the maximum deuterated medium and levels of deuterium enrichment of molecules*.

Carbohydrate	Content in the biomass, % of the dry weight of 1 g biomass		Level of deuterium enrichment, % of the total number of hydrogen atoms***
	Protonated sample (control)	The sample obtained in 99.9 % D_2O**	
Glucose	20.01	21.40	80.6
Fructose	6.12	6.82	85.5
Rhamnose	2.91	3.47	90,3
Arabinose	3.26	3.69	90.7
Maltose	15.30	11.62	–
Sucrose	8.62	ND	–

Notes:
* The data were obtained by IR-spectroscopy.
** ND – not detected
** A dash means the absence of data.

3.2. Hot mineral water for origin of life and living matter

Biological experiments with D_2O and structural-conformational studies with deuterated molecules enable to modeling conditions under which life might be evolved. 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 (Mosin and Ignatov, 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 then H_2O, and condenses faster. According to our opinion this seems to be 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 little stronger than those formed of hydrogen.

The second 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 and Mosin, 2012). 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 and Galperin, 2009). J. Trevors and G. Pollack proposed in 2005 that the first cells on the Earth assembled in a hydrogel environment (Trevors and 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 organic compounds consisting entirely of hydrogen and carbon.
To test our hypothesis for origin of life and living matter in hot mineral water we have carried out the research of various samples of mineral water from mineral springs and seawater from Bulgaria (Fig. 2, curves *1–5*). For this aim we employed the IR-spectroscopy and differential non-equilibrium energy spectrum (DNES) method relative to the control – deionized water. The cactus juice was also investigated by the DNES method (Fig. 2, *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. 2, *curve 2*). DNES-spectra of plant juice and mineral water and water have magnitudes of local maximums at -0.1112; -0.1187; -0.1262; -0.1287 and -0.1387 eV, accordingly. 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. 2, *curve 5*) was substantially different from the spectra of sea 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 to be compiled at 0.1067±0.0011 eV. When the water temperature is changed, the average energy of the hydrogen H...O bonds alternates. There is a restructuring of energies among H_2O molecules with a statistically reliable increase of local maximums in DNES-spectra.

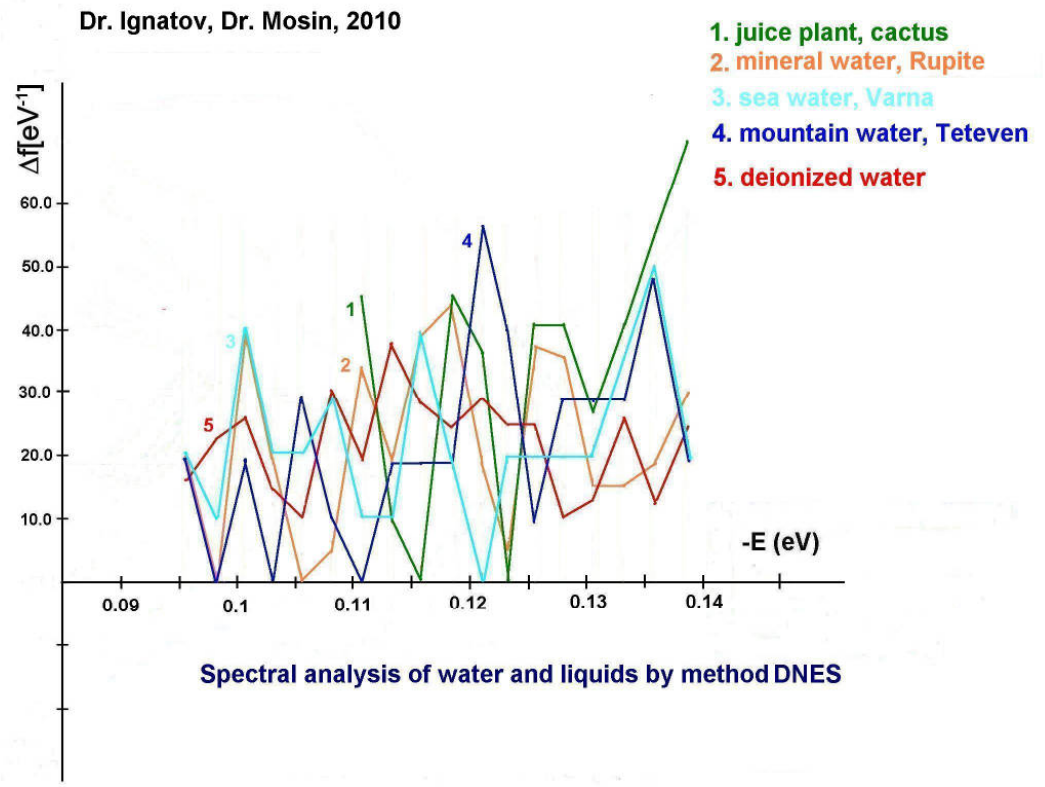


Fig. 2. DNES spectra of water samples of various origin: 1 – cactus juice; 2 – mineral water from Rupite village (Bulgaria); 3 – seawater (Varna, Bulgaria); 4 – mountain water (Teteven, Bulgaria); 5 – deionized water (the control).

As shown from these data, the closest to the IR spectrum of cactus juice was the mineral water from Rupite Village, which DNES and IR spectrum is shown in Fig. 2 and Fig. 3 (Thermo Nicolet Avatar 360 Fourier-transform IR). IR spectra of the 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 the plant 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.

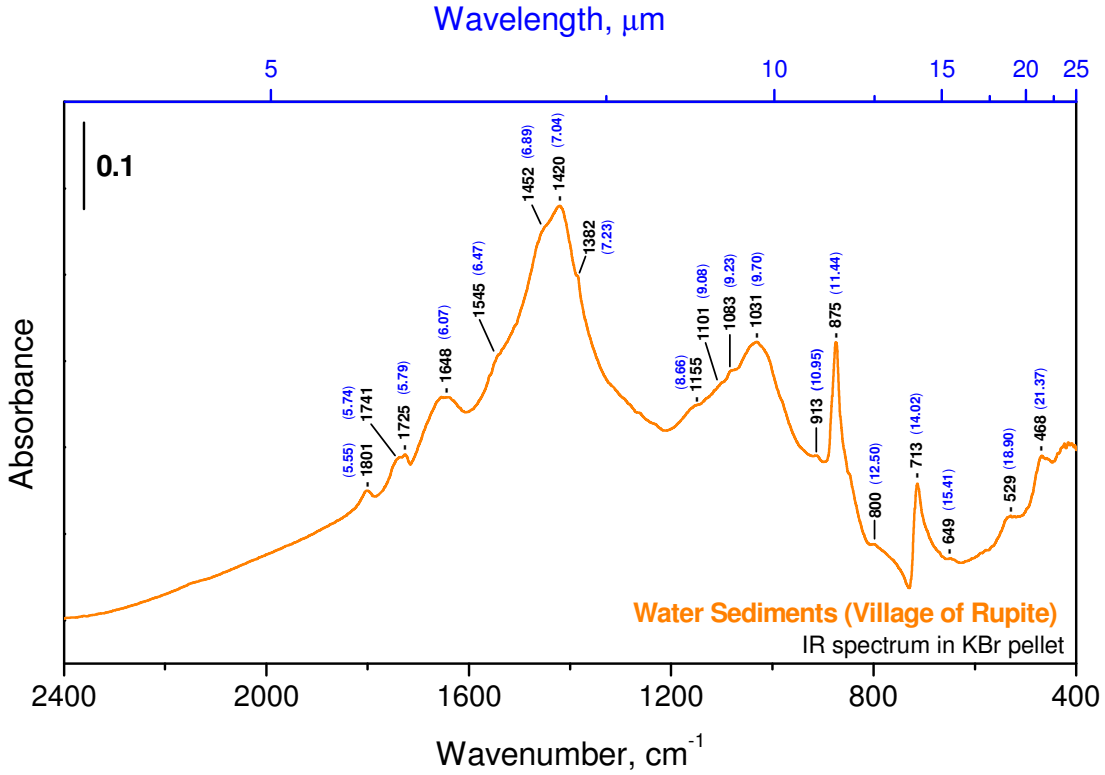


Fig. 3. IR spectrum of water obtained from Rupite Village (Bulgaria)

Table 4. Characteristics of spectra of water of various origin obtained by DNES-method*

-E (eV) Cactus juice	-E (eV) Mineral water from Rupite Village	-E (eV) Seawater	λ (μm)	κ (cm^{-1})
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 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.

3.3. Sea water for origin of life and living matter

The results with jelly fish indicated that jelly fish has local maximums in IR-spectra at 8.98 and 10.18 μm. On comparison seawater has a local maximum at 8.93 μm. These results are obtained with Thermo Nicolet Avatar 360 Fourier-transform IR. With DNES method the local maximums of jellyfish are at 8.95 and 10.21 μm and seawater at 9.10 μm. A differential spectrum was recorded between the jellyfish and the seawater with the Thermo Nicolet Avatar 360 Fourier-transform IR method. In the living organism are observed more pronouncedly expressed local maximums at Thermo Nicolet Avatar 360 Fourier-transform IR and DNES method. Measurements demonstrate that two common local maximums are observed in the jellyfish and seawater. These maximums are not observed in the spectrum of the cactus juice and mineral water from Rupite, Bulgaria. For several days the jellyfish was resorbed in the seawater. It contains 97% water and is a more unstable living species compared to stromatolites. The explanation is the smaller concentration of salts and the smaller number of local maximums in the spectrum in relation to life medium – seawater.

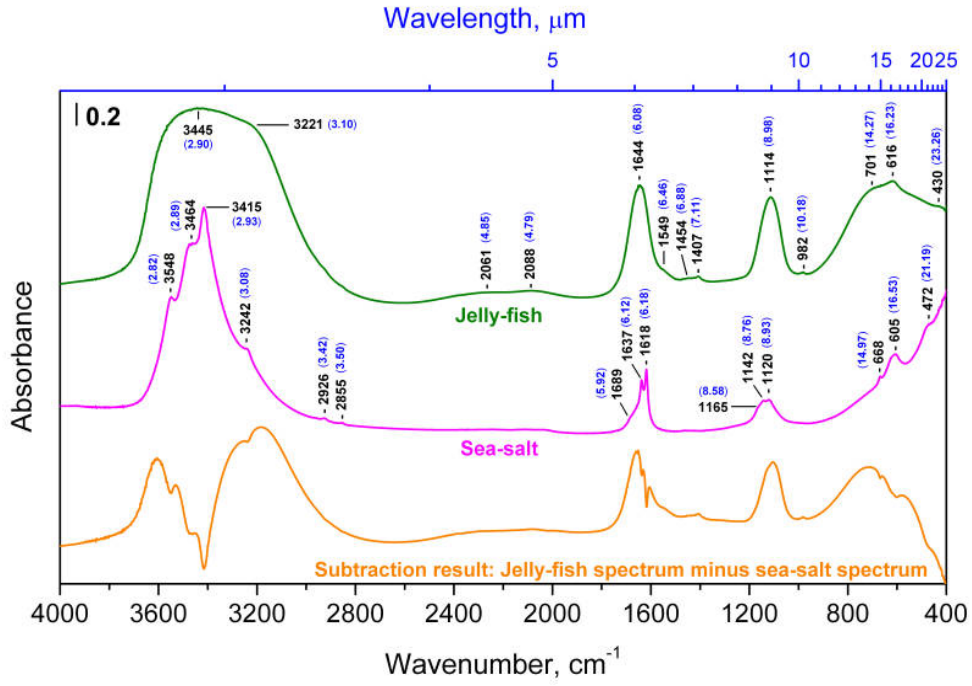


Fig. 4. IR-spectrum of seawater obtained from Varna (Bulgaria) and Jelly fish, Chalkida (Greece), Aegean Sea

3.4. The processes for formation of stromatolites in hot water

Such a character of the IR- and DNES-spectrum 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. In hot mineral waters the local maximums in the IR-spectrum are more manifested compared to the local maximums obtained in 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 is $p < 0.05$. These data indicate that the origination of life and living matter depends on the structure and physical chemical properties of water. The most closed to the IR and DENS-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 model system. The most closed to local maximums of spectrum of cactus juice are IR- local maximums 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:

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

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. It was previously thought that the first living forms evolved in hot geysers and in hot geysers in the ocean (Pons et al., 2011). The 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. These 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 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.

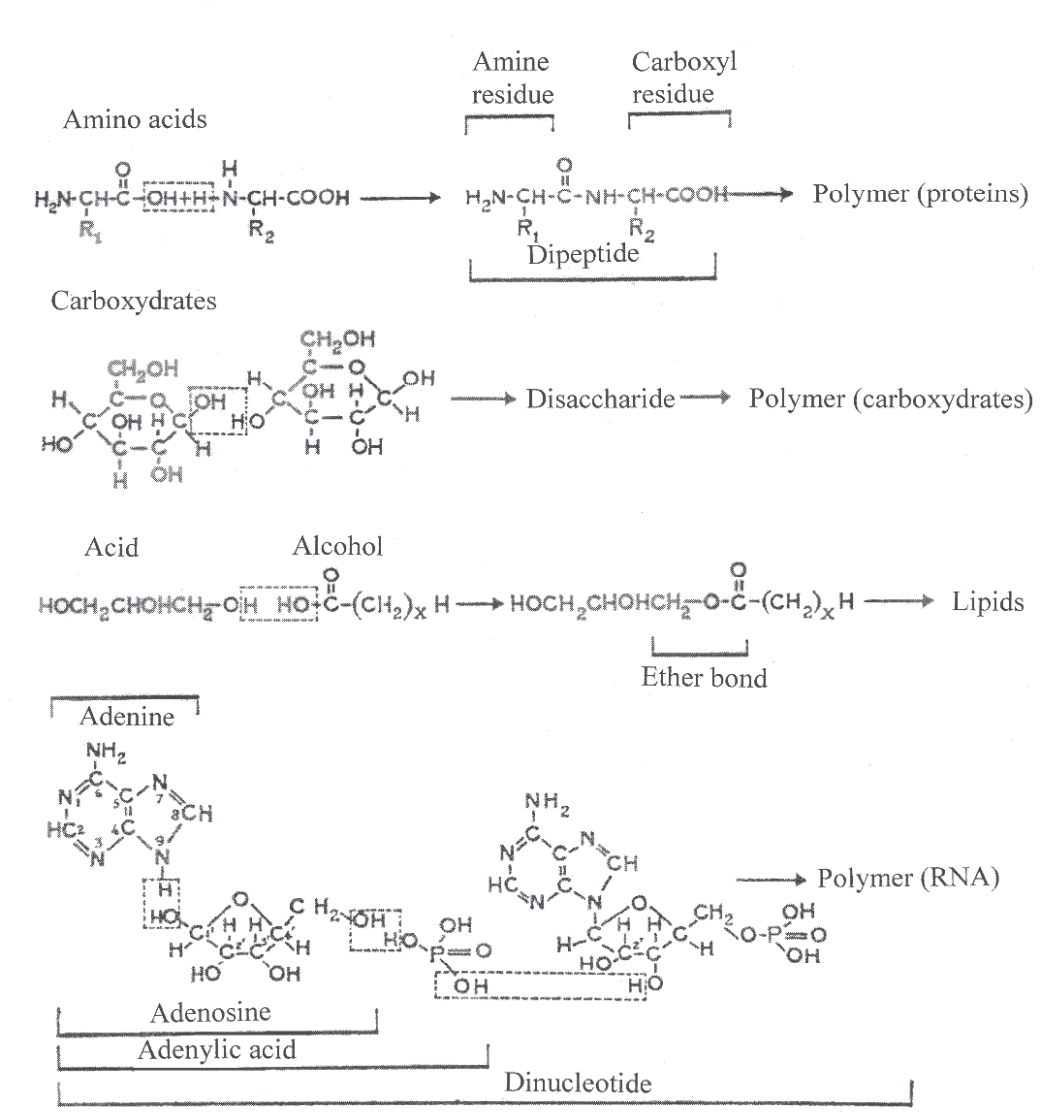
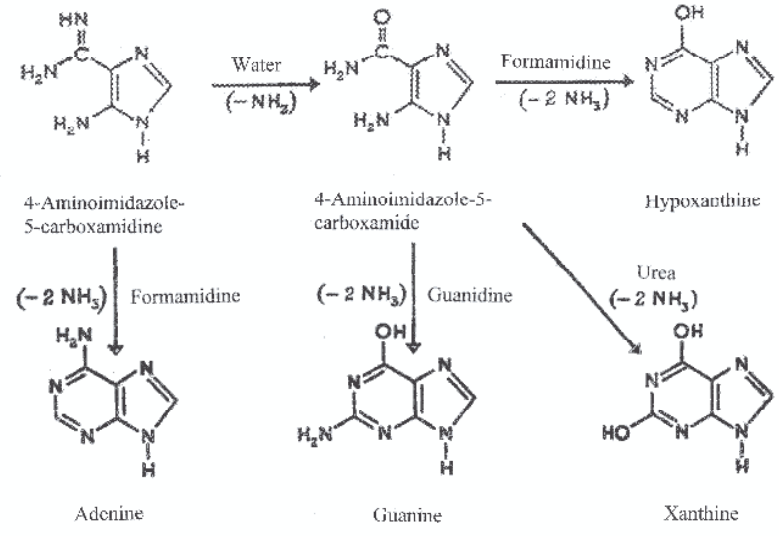


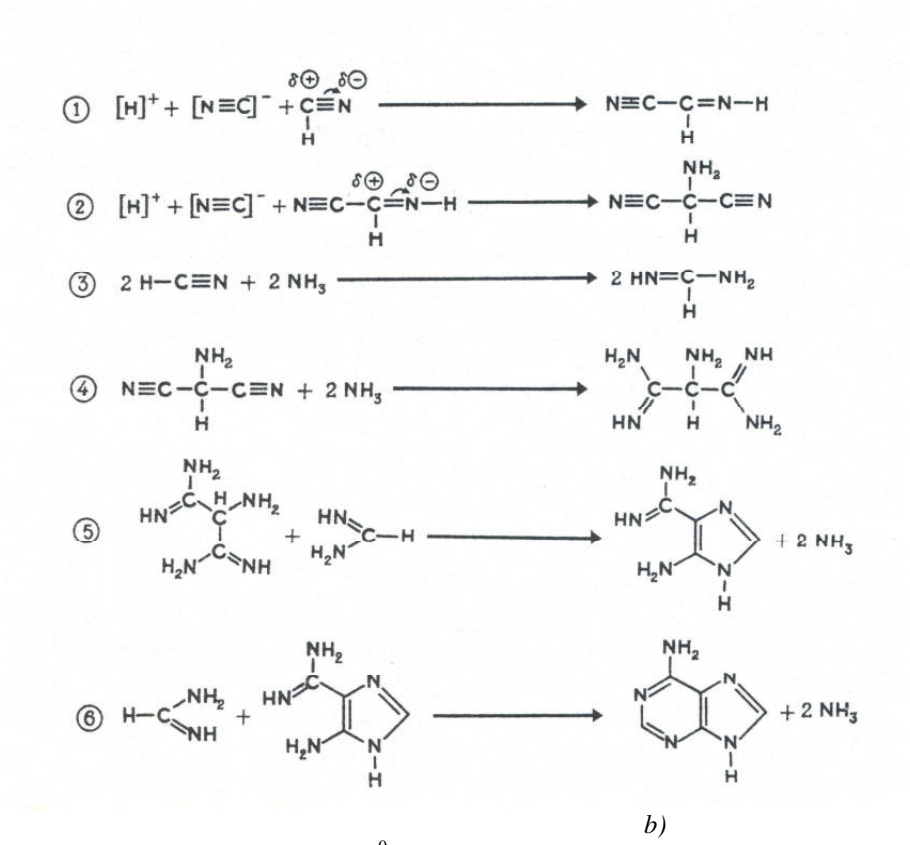
Fig. 5. Reactions of condensation and dehydration in alkaline conditions with pH = 9–10 catalyzed by HCN and its derivatives, resulting in synthesis from separate molecules larger organic molecules of polymers. The top three equations: condensation and the subsequent polymerization of amino acids in 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.

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 and 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 (Fig. 5). 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 and 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 (Fig. 6). 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 (Nakashima, 1987). 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 and Krampitz, 1964) and to have an effect similar to the action of α-melanocyte-stimulating hormone (Fox and 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. 6. 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).

3.5. The processes for origin of life and living matter in gas discharge conditions

Under certain conditions in hot mixture of proteinoids in water solutions are formed elementary structures like proteinoid microspheres with diameter 5–10 μm (Mosin and Ignatov, 2011). Gas electric discharge with color coronal spectral analyses was applied in this type of experiment analogous to S. Miller's experiments (Ignatov, 2007; Ignatov and Mosin, 2012). 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 ions, metal ions (Na, Ca, Mg, Zn, K, etc.) and deuterium molecules (Ignatov and Mosin, 2010). There occurred gas electric discharge (lightning) in the primordial atmosphere close to the water surface. It was used the similar water content on the electrode of the device for gas coronal electric discharge experiment. Water drops were heated to boiling point in an electric field of high frequency and voltage and an electric discharge was applied, like in the primordial atmosphere. As a result, an organized structure with a size of 12–14 mm was formed in interelectrode space (Fig. 7). It was obtained as a result of the self-organization of elementary structures sized 5–10 μm in the biggest structure with size 12–14 mm. There was concentrated in a large structure where the basic electric voltage is applied. On its form it resembles a small jellyfish.

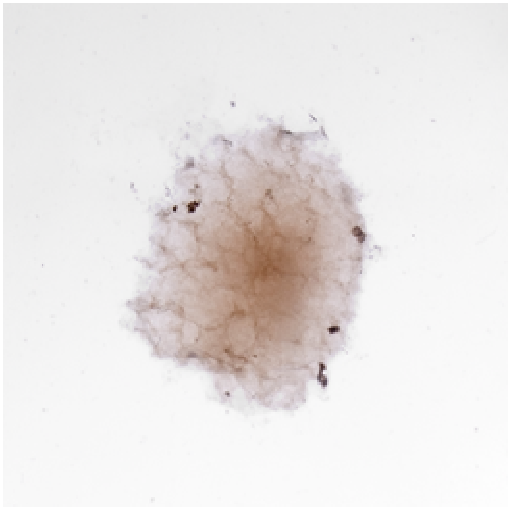


Fig. 7. Organized structure in water on an electrode, which is heated to boiling point in an electric field of high frequency and voltage

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 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. It should be noted that the large structure was preserved with original unchanged size for 2.5 years in the absence of electric discharge.

This experiment shows that the organization of structures in water under certain external conditions as the temperature takes place. Water in natural conditions 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. 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 and Prigozhin, 1979).

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 in a mix of proteinoids in hot water solutions were formed membrane like structures. 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 ^{0}C (Sugawara, 2011). These experiments are excellent confirmation of the possibility that life and living matter originated in hot water.

4. Conclusions

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. It was shown by using different methods (DNES-method, Brucker Vertex Fourier-IR and Thermo Nicolet Avatar 360 Fourier-transform IR), that 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 the 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. These are solar radiation, causing radiolysis and photolysis of water, geothermal activity and electrical discharges in the atmosphere devoid of the protective ozone layer. 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. We examined the possibility that life originated initially in seawater because the first organism lived in the primordial ocean and then on dry land. The

data presented in the report show that the origination of living matter most probably occurred in hot mineral water. This occurred in ponds and hydrothermal vents in seawater. An indisputable proof of our supposition is the presence of stromatolites fossils. They lived in warm and hot water in zones of volcanic activity, which could be heated by magma more stable than the first sea organisms.

Acknowledgements

Authors wish to thank Kristina Chakarova from Bulgarian Academy of Sciences (Sofia, Bulgaria) for registering of IR-spectra and Chavdar Stoyanov for collaboration for the device for color coronal electric discharge.

References

Abelson, P. (1966) Chemical events on the "primitive" earth. *Proc. Natl. Acad. Sci. U.S.* **55**:1365–1372.
Bild, W., Nastasa, V., & Haulica, I. (2004) *In vivo* and *in vitro* research on the biological effects of deuterium-depleted water: influence of deuterium-depleted water on cultured cell growth. *Rom J. Physiol*., **41**(1–2), 53–67.
Calvin, M. (1969) *Chemical Evolution*. Oxford: Clarendon, 278.
Cleland, W.N., O'Leary, M.H., & Northrop, D.D., eds. (1976) *Isotope effects on enzyme-catalyzed reactions*. Baltimore, London, Tokyo: University Park Press, 303.
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., Antonov, A., Galabova, T., & Stoyanov, S. (2001) Self-organization and "informationability" of Water.. Their importance for the possible processes of structuring of the living matter. In: Seminar "*Man and Nature*", (SRCMB), Sofia, Teteven, 63–65.
Ignatov, I. (2010) Which water is optimal for the origin (generation) of life? *Euromedica*, Hanover, 34–37.
Ignatov, I. (2011) Entropy and time in living organisms. *Euromedica*, Hanover, 60–62.
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, 32–35.
Ignatov, I., & Mosin, O.V. (2013) Method for Color Coronal (Kirlian) Spectral Analysis. *Biomedical Radio electronics, Biomedical Technologies and Radio Electronics*, **1**, 38–47 [in Russian].
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, 67.
Ignatov, I., & Mosin, O.V. (2012) Isotopic composition of water and its temperature in modeling of primordial hydrosphere experiments, VIII Intern. Conference Perspectives of the Development of Science and Technique, *Biochemistry and Biophysics*, **15**, 41–49.
Ignatov, I., & Mosin, O.V. (2012) Isotopic composition of water and its temperature in modeling of primordial hydrosphere experiments. *Euro-Eco*, Hanover, 62.
Ignatov, I., & Mosin, O.V. (2013) Color Coronal (Kirlian) Spectral Analysis in Modeling of Nonequilibrioum Conditions with the Gas Electric Discharges Simulating Primary Atmosphere. S. Miller's Experiments. *Naukovedenie*, **16**(3), 1–15 [in Russian] [Online] Available: URL: http://naukovedenie.ru/PDF/05tvn313.pdf (May 10, 2013).
Ignatov, I. & Mosin, O.V. (2013) 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) Structural Mathematical Models Describing Water Clusters. *Journal of Mathematical Theory and Modeling*, **3** (11), 72–87.
Kurihara, K., Tamura, M., Shohda, K., Toyota, T., Suzuki, K., & Sugawara, T. (2011) Self-Reproduction of supramolecular giant vesicles combined with the amplification of encapsulated DNA. *Nature Chemistry*, **4**(10), 775–781.
Kushner, D.J., Baker, A., & Dunstall, T.G. (1999) Pharmacological uses and perspectives of heavy water and deuterated compounds. *Can. J. Physiol. Pharmacol*., **77**(2), 79–88.
Linsky, J. L. (2007) D/H and nearby interstellar cloud structures, *Space Science Reviews*, NY: Springer Science, Business Media, **130**, 367.
Lis, G., Wassenaar, L.I., & Hendry, M.J. (2008) High-precision laser spectroscopy D/H and $^{18}O/^{16}O$ measurements of microliter natural water samples. *Anal. Chem*., **80**(1), 287–293.

Lobishev, V.N., & Kalinichenko, L.P. (1978) *Isotopic* Effects of D_2O. in: Biological Systems. Moscow: Nauka, 215 p [in Russian].
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.
Mosin, O.V, Skladnev, D.A, & Shvets, V.I. (1998) Biosynthesis of ^{2}H-labeled phenylalanine by a new methylotrophic mutant *Brevibacterium methylicum*. *Biosci Biotechnol Biochem.*, **62**(2), 225–229.
Mosin, O.V., Skladnev, D.A., & Shvets, V.I. (1999) Biosynthesis of ^{2}H-labelled inosine by bacterium *Bacillus subtilis*. *Izv. RAN. Ser. biologicheskaja*, **4**, 396–402 [in Russian].
Mosin, O.V., Skladnev, D.A., & Shvets, V.I. (2000) Studying of adaptation to heavy water. *Biotechnologija*, **10**, 16–23 [in Russian].
Mosin, O.V, & Ignatov I. (2012) Isotope effects of deuterium in bacterial and microalgae cells at growth on heavy water (D_2O). *Water: Chemistry and Ecology*, **3**, 83–94 [in Russian].
Mosin, O.V., Shvets, V.I., Skladnev, D.A., & Ignatov, I. (2012) Studying of microbic synthesis of deuterium labeled L-phenylalanine by methylotrophic bacterium *Brevibacterium Methylicum* on media with different content of heavy water. *Russian Journal of Biopharmaceuticals*, **4** (1), 11–22 [in Russian].
Mosin, O.V., & I. Ignatov, I. (2013) Microbiological synthesis of ^{2}H-labeled phenylalanine, alanine, valine, and leucine/isoleucine with different degrees of deuterium enrichment by the Gram-positive facultative methylotrophic bacterium *Brevibacterium methylicum*. *International Journal of BioMedicine*, **3**(2), 132–138.
Mosin, O.V., Shvez, V.I, Skladnev, D.A., & Ignatov, I. (2013) Microbiological synthesis of [^{2}H]inosine with high degree of isotopic enrichment by Gram-positive chemoheterotrophic bacterium *Bacillus subtilis*. *Applied Biochemistry and Microbiology*, **49**(3), 255–266.
Mosin, O.V., & Ignatov, I. (2013) Studying of the Biosynthesis of ^{2}H-Labeled Inosine by a Gram-positive Chemoheterotrofic Bacterium *Bacillus Subtilis* B-3157 on Heavy Water (^{2}H2O) Medium. *Chemical and Process Engineering Research*, **15**, 32–45.
Mosin O.V., Shvets V.I., Skladnev D.A. & Ignatov I. (2013) Microbial synthesis of ^{2}H-labelled L-phenylalanine with different levels of isotopic enrichment by a facultive methylotrophic bacterium *Brevibacterium methylicum* with RuMP assimilation of carbon. *Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry*, **7**(3), 249–260.
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.
Nakashima, T. (1987) *Metabolism of proteinoid microspheres*. In: Origin of Life and Evolution of Biosphere, **20**(3-4), 269–277.
Nikolis, P., & Prigozhin, I. (1979) *Self-Organization in Non-Equilibrium systems*. Moscow: Mir, 1–512 [in Russian].
Pons, M.L., Quitte, G., Fujii, T., et al. (2011) Early archean serpentine mud volcanoes at Isua, Greenland, as a niche for early life. *PNAS*, **108**(43), 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).
Szostak, J.W. (2011) An optimal degree of physical and chemical heterogeneity for the origin of life? *Philos. Trans. R. Soc. Lond. Biol. Sci.*, **366**(1580), 2894-901.
Sugawara, T. (2011) Self-reproduction of supramolecular giant vesicles combined with the amplification of encapsulated DNA. *Nature Chemistry*, **1127**, 775–780.
Thomson, J.F. (1960) Physiological effects of D_2O in mammals. Deuterium Isotope Effects in Chemistry and Biology. *Annals of the New York Academy of Sciences*, **84**, 736–744.
Trevors, J.I., & Pollack, G.H. (2005) Hypothesis: origin of live in hydrogel environment. *Progress in Biophysics and Molecular Biology*, **89**(1), 1–8.
Vertes, A. (2003) *Physiological effects of heavy water. Elements and isotopes: formation, transformation, distribution*. Dordrecht: Kluwer Acad. Publ., 112 p.