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Возможные процессы возникновения жизни и живой материи в обогащенной дейтерием горячей минеральной воде

Possible processes for origin of life and living matter in deuterium enriched hot mineral water

Аннотация: Обсуждается изотопный состав воды и ее температура в процессе эволюционного происхождения жизни. Высказано предположение, что в условиях первичное атмосферы, лишенной защитного кислородного слоя под воздействием коротковолнового солнечного излучения, геотермальной активности и электрических разрядов в гидросфере мог накапливаться дейтерий в форме HDO, физико-химические свойства которой отличаются от H_2O. В ходе эксперимента были получены адаптированные к максимальным концентрациям D_2O клетки различных микроорганизмов, реализующие метилотрофный, хемогетеротрофный, фотоорганогетеротрофный и фотосинтетический пути ассимиляции углеродных субстратов, весь биологический материал которых вместо атомов водорода содержит дейтерий. Их дальнейшее исследование позволит дать ответ на вопрос, как функционируют дейтерированные макромолекулы в условиях первичной гидросферы и горячих D_2O-растворов. Также методом ИК-спектроскопии были исследованы образцы горячей минеральной, морской и горной воды из Болгарии.

The Abstract: In the present paper the isotopic composition of water and its temperature in the process of evolution of life is analysed. It was proposed an assumption, that under conditions of the primary O_2 free atmosphere, under influence of short-wave solar radiation, geothermal energy and powerful spark discharges, deuterium in form of HDO could be collected in hydrosphere, which physical-chemical properties differ from those of H_2O. There were obtained adapted to the maximal concentration D_2O cells of various microorganisms realizing methylotrophic, chemoheterotrophic,

photoorganotrophic, and photosynthetic pathways of assimilation of carbon substrata, all biological material of which instead of hydrogen contains deuterium. Their studying will allow to give the answer as who function the deuterated macromolecules in conditions of primary hydrosphere and hot D_2O-solutions. Also were performed experiments for the research of hot mineral, sea and mountain water from Bulgaria with IR spectroscopy.

Ключевые слова: дейтерий, тяжелая вода, гидросфера, эволюция.

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

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1. Introduction

Natural prevalence of deuterium (^{2}H or D) makes up approximately 0,015 at.% D, and depends strongly on the uniformity of substance and the total amount of matter formed in the course of early Galaxy evolution [1]. Constant sources of deuterium are explosions of nova stars and thermonuclear processes occurring inside the stars. Probably, it could explain a well known fact why the amounts of deuterium are increased slightly during the global changes of climate in worming conditions. Gravitational field of the Earth is insufficiently strong for 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 is capable to be collected by the hydrosphere. Therefore, as a result of this natural process of fractionation of isotopes H/D throughout the process of Earth evolution there should be an accumulation of deuterium in hydrosphere and surface waters, while in atmosphere and in water vapor deuterium contents are lower. Thus, on the planet there is occurring a natural process of separation of H and D isotopes, playing an essential role in 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, compile $D/H = (155{,}76\pm0{,}05)\cdot10^{-6}$ (155,76 ppm) [2]. For the international standard SLAP of natural water of Antarctic Region containing less deuterium, the absolute contents of deuterium compile $D/H = 89\cdot10^{-6}$ (89 ppm). The average ratio of H/D in nature compiles 1:5700. In natural waters the contents of deuterium are distributed non-uniformly: from 0,015 at.% D for water from the Antarctic ice – the most deuterium depleted natural water with deuterium contents being in 1,5 times smaller, than in sea water, up to 0,02-0,03 at.% D for river and sea water. Thawed snow and glacial waters in mountains and some other regions of the Earth usually contain on 3–5 % less deuterium, than drinking water. On the average, 1 ton of river water contains approximately 150–300 g of deuterium. Other natural waters contain varying levels of deuterium from δ = +5,0 D, %, SMOW (Mediterranean Sea) up to δ = -105 D, %, SMOW (Volga River).

It can be presumed that primary water contained more deuterium at early stages of life evolution, and deuterium was distributed non-uniformly in hydrosphere and atmosphere [3]. As is known, the primary reductive atmosphere of the Earth, consisted basically of gas mixture CO, H_2, N_2, NH_3, CH_4, was lacked O_2–O_3 layer protecting the Earth surface from rigid short-wave solar radiation carrying huge energy capable to cause photolysis and radiolysis of water. The processes accompanying accumulation of deuterium in hydrosphere were solar radiation, volcanic geothermal processes and electric discharges in atmosphere. These natural processes could lead to the enrichment of hydrosphere by deuterium in the form of HDO which evaporates more slowly then H_2O, and condenses faster. The formation of HDO occurs in D_2O–H_2O mixtures via the isotopic exchange: $H_2O + D_2O = 2HDO$, causing deuterium at small amounts to be present in water in form of HDO, and at high amounts – in form of D_2O. The structure of 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,

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freezes at 3,82 ^{0}C, has density at 20 ^{0}C 1,105 г/см^3, and the maximum of density is not on 4 ^{0}C, as for H_2O, but on 11,2 ^{0}C (1,106 г/см^3). These effects are reflected in energy of a chemical bond, kinetics and chemical reactions rates in D_2O-H_2O mixtures. Enzymic reactions in D_2O are considerably slowed down. However, there are also such reactions which rates in D_2O are higher, than in H_2O. Basically, they are reactions catalyzing by D_3O^+ or H_3O^+ ions or OD^- and OH^- ions. 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 constant of ionization of H_2O [4]. The maximum kinetic isotopic effect at ordinary temperatures in a chemical reaction leading to rupture of bonds involving H and D was calculated, and the maximum ratio k_h/k_d in macromolecules is varied from 6 to 8 for C–H versus C–D, N–H versus N–D, and O–H versus O–D bonds [5].

Deuterated cells of various microorganisms adapted to the maximal concentration of D_2O in growth media (95–98 at.% D) are convenient objects for evolutional and adaptation studies as well as structural-functional studies. During the cellular growth on D_2O media there are synthesized macromolecules in which hydrogen atoms in carbon skeletons are almost completely replaced on deuterium. Such deuterated macromolecules undergo the structural-adaptive modificational changes necessary for normal functioning of cells in the presence of D_2O [6].

Practical interest to further applying of deuterated cells of various microorganisms in the research on their basis mechanisms of cellular adaptation to D_2O and molecular evolution has predetermined a direction of our studies. The purpose of the present reseach was studying of isotope effects of deuterium and conditions of primary hydrosphere (temperature, value pH, isotopic composition). In frames of the research were studied various samples of water from Bulgaria.

2. Experimental part
Materials and methods

The research was carried out with using of microorganisms, realising 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);

3 – sea water (a resort Varna, Bulgaria);

4 – mountain water (Teteven, Bulgaria).

Also cactus juice of *Echinopsis pachanoi* was investigated by the IR-spectroscopy method.

For preparation of growth media it was used D_2O (99,8 at. % D), DCl (95,5 at. % D) and [D]methanol (97,5 at. % D), received from the Russian research centre “Isotope” (St. Petersburg, Russia). Inorganic salts and glucose were preliminary cristallized 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 vol.% D_2O. Cultivation of methylotrophic and chemoheterotrophic 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 with 1–2 vol.% [^{2}H]methanol. Cultivation of chemoheterotrophic bacteria was carried out on FM medium (m/m.%): glucose – 12; yeast extract – 2,5; NH_4NO_3 – 3; $MgSO_4 \cdot 7H_2O$ – 2; chalk – 2. Cultivation of halobacteria was carried out on TS medium (g/l): (*D*,*L*-Ala – 0,43; *L*-Arg – 0,4; *D*,*L*- Asp – 0,45; *L*- Cys – 0,05; *L*- Glu – 1,3; *L*-Gly – 0,06; *D*,*L*-His – 0,3; *DL*-Ileu – 0,44; *L*-Leu – 0,8; *L*-Lys – 0,85; *D*,*L*-Met – 0,37; *D*,*L*-Phe – 0,26; *L*-Pro – 0,05; *D*,*L*-Ser – 0,61; *D*,*L*-Thr – 0,5; *L*-Tyr – 0,2; *D*,*L*-Trp – 0,5; *D*,*L*-Val – 1,0; AMF – 0,1; UMF – 0,1; 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^+-cytrate – 0,5; $MnSO_4 \cdot 2H_2O$ – $3 \cdot 10^{-4}$; $CaCl_2 \cdot 6H_2O$ – 0,065; $ZnSO_4 \cdot 7H_2O$ – $4 \cdot 10^{-5}$; $FeSO_4 \cdot 7H_2O$ – $5 \cdot 10^{-4}$; $CuSO_4 \cdot 5H_2O$ – $5 \cdot 10^{-5}$; glycerine – 1,0; (biotin – $1 \cdot 10^{-4}$; folic acid – $1{,}5 \cdot 10^{-4}$; vitamine B_{12} – $2 \cdot 10^{-5}$). Blue-green algae grew on Tamia growt 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 % agarose media M9 with gradually increasing concentrations of D_2O, combined with the subsequent selection of clones resistent 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). Halobacteria and blue-green algae were grown up 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 % agarose media, and on absorbance of cell suspension measured on spectrophotometer Beckman DU-6 (Beckman Coulter, USA) at λ = 620 nm.

The analysis of protein hydrolisates was carried out using Biotronic LC 50001 chromatograph (“Eppendorf-Nethleler-Hinz”, Germany), 230 × 3.2 mm, working pressure – 50–60 atm, flow-rate – 18,5 ml/h.

The levels of deuterium enrichment were defined 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 by ^{1}H-NMR-spectroscopy on device Brucker WM-250 (“Brucker”, Germany) (working frequency of 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 sm^{-1}; visible – 2500–8000 cm^{-1}; the permission – 0,5 cm^{-1}; accuracy of wave number – 0,1 cm^{-1} on 2000 cm^{-1}).

3. Results and discussion

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) in D_2O with using ^{1}H-NNR-, IR-, and mass-spectrometry technique. The method of step by step adaptation to deuterium was developed for adaptation of cells of various microorganisms consisting in plating initial cells on firm (2 % agarose) growth media with increasing gradient of D_2O concentration (from 0; 24,5; 49,0; 73,5 to 98 % D_2O) and the subsequent selection of clones resistent to deuterium. Cells grown on media with a low gradient of D_2O concentration were transferred on media with big gradient of concentration, up to 98 % D_2O. Degree of cell survive on maximum deuterated media was about 40 %.

Our experiments demonstrated, that the effects observed at the cellular growth on D_2O possess complex multifactorial character connected to changes of morphological, cytologic and physiological parameters – magnitude of the log-period, time of cellular generation, outputs of biomass, a ratio of amino acids, protein, carbohydrates and lipids synthesized in D_2O, and with an evolutionary level of organization of investigated object as well. The general feature of bacterial

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growth in D_2O was the proportional increase in duration of the log-period and time of cellular generation at reduction of outputs of a microbial biomass. The experimental data testify that cells realize 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 atom 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 consider adaptation to D_2O as adaptation to the nonspecific factor effecting simultaneously 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 ration of synthesized metabolites is changing. Furthermore, deuterium induces physiological, morphological and cytological alterations in the cell. This leads to the formation in D_2O of large atypical cells [7–9]. 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. The data obtained confirm that adaptation to D_2O is a phenotypical phenomenon as the adapted cells return back to normal growth after some log–period after their replacement into H_2O. At the same time the effect of convertibility of growth on H_2O/D_2O growth media does not exclude an opportunity that a certain genotype determines displaying of the same phenotypical attribute on growth in D_2O.

Experiments with D_2O have shown (fig. 1), that micro algae is capable to grow on 70 % D_2O, methylotrophic bacteria – 75 % D_2O, chemoheterotrophic bacteria – 82 % D_2O, and halobacteria – 95 % D_2O.

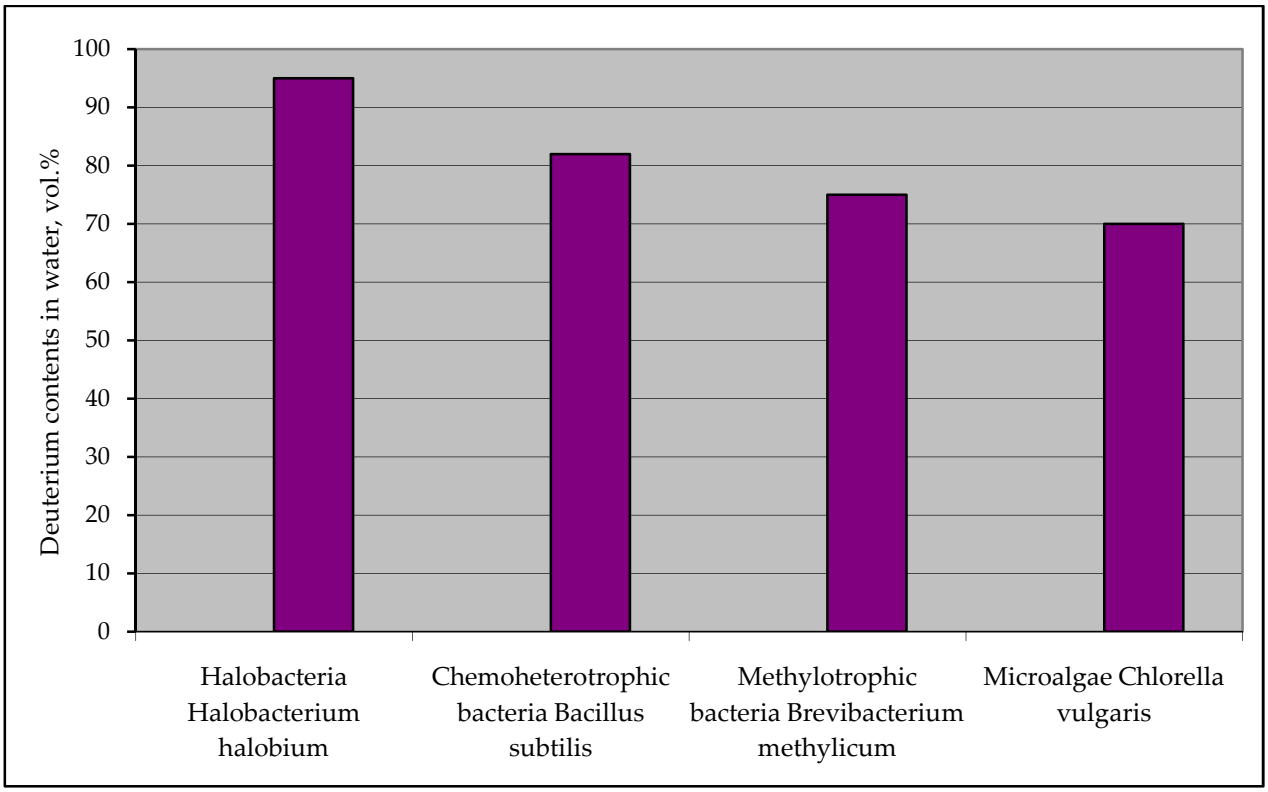

***Fig. 1**. Survival rate of cells of various microorganisms in water with various content of deuterium*

In the process of adaptation to D_2O the most important for macromolecular structure are dynamic short-lived hydrogen (deuterium) bonds formed between the electron-deficient neighbor atoms of H(D) and electron-negative O, C, N, S- hetero atoms, acting as acceptors of H-bond. The hydrogen bond, based on weak electrostatic forces, donor-acceptor interactions with charge-transfer

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and intermolecular van der Waals forces, is of the vital importance in the chemistry of intermolecular interactions and maintenance of spatial structure of macromolecules in aqueous solutions. The substitution of H with D atom affects the stability and geometry of hydrogen bonds in apparently rather complex way and may, through the changes in the hydrogen bond zero-point vibrational energies, alter the conformational dynamics of hydrogen (deuterium)-bonded structures of DNA and protein in D_2O. It may cause disturbances in the DNA-synthesis, leading to permanent changes in DNA structure and consequently in cell genotype. The multiplication, which would occur in macromolecules of even a small difference between the hydrogen and deuterium bond 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. 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. Other important property is defined by the three-dimensional structure of 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 [10]. While placing the cell in D_2O, not only H_2O is removed from a cell due to reaction of D_2O dissociation, but also there is occurred fast isotopic (H–D) exchange in hydroxyl (-OH), ***sulfhydryl*** (-SH) and amino groups ($-NH_2$) of all 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 substances with bonds such as C-D can be synthesized de novo [11–13].

Biological experiments with D_2O and structural-conformational studies enable to modeling conditions under which life might be evolved. The most favorable for maintenance of life seem to be alkaline mineral waters interacting with $CaCO_3$ and then sea waters [14]. Circulating in bowels of cracks, crevices, channels and caves karst waters are enriched with $Ca(HCO_3)_2$, actively cooperating with live matter. Once appeared in these waters 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.

In connection with these data are important the following reactions:

(1) $CO_2 + 4H_2S + O_2 = CH_2O + 4S + 3H_2O$;

(2) $CaCO_3 + HOH + CO_2 = Ca(HCO_3)_2$;

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

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

The equation (1) shows how some chemosynthetic bacteria use energy from the oxidation of H_2S to S. The equation (2) is related to formation of $Ca(HCO_3)_2$ from H_2O, CO_2 and $CaCO_3$. In the presence of hydroxyl OH^- ions CO_2 transforms into HCO_3^- (equation (3). Equation (4) is valid for the process of dolomite formation of stromatolites [15–17].

To test this hypothesis we have carried out the research of various samples of mineral water from karst springs, sea water and mountain water from Bulgaria (fig. 2, curves *1-5*, the table). For this aim it was employed the IR-spectroscopy and differential non-equilibrium energy spectrum (DNES) method relative to the control – deionized water. Also the castus juice was investigated by DNES method (fig. 2, *curve 1*). The cactus was selected as a model system because this plant contains about 90 % water. The closest to the spectrum of castus juice was the spectrum of mineral water contacting with $CaCO_3$ (fig. 2, *curve 2*). DNES-spectra of plant juice, mineral water and water of the karst springs have magnitudes of peaks at -0,1112; -0,1187; -0,1262; -0,1287 and -0,1387 eV, accordingly. Similar peaks in the DNES-spectrum between cactus juice, mountain and sea water

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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 mineral and mountain water.

Another important parameter was measured by the DNES method - the average energy ($\Delta E_{H...O}$) of hydrogen H…O-bonds between 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 changes. There is a restructuring of energies between H_2O molecules with a statistically reliable increase of local maximums in spectra [18–20].

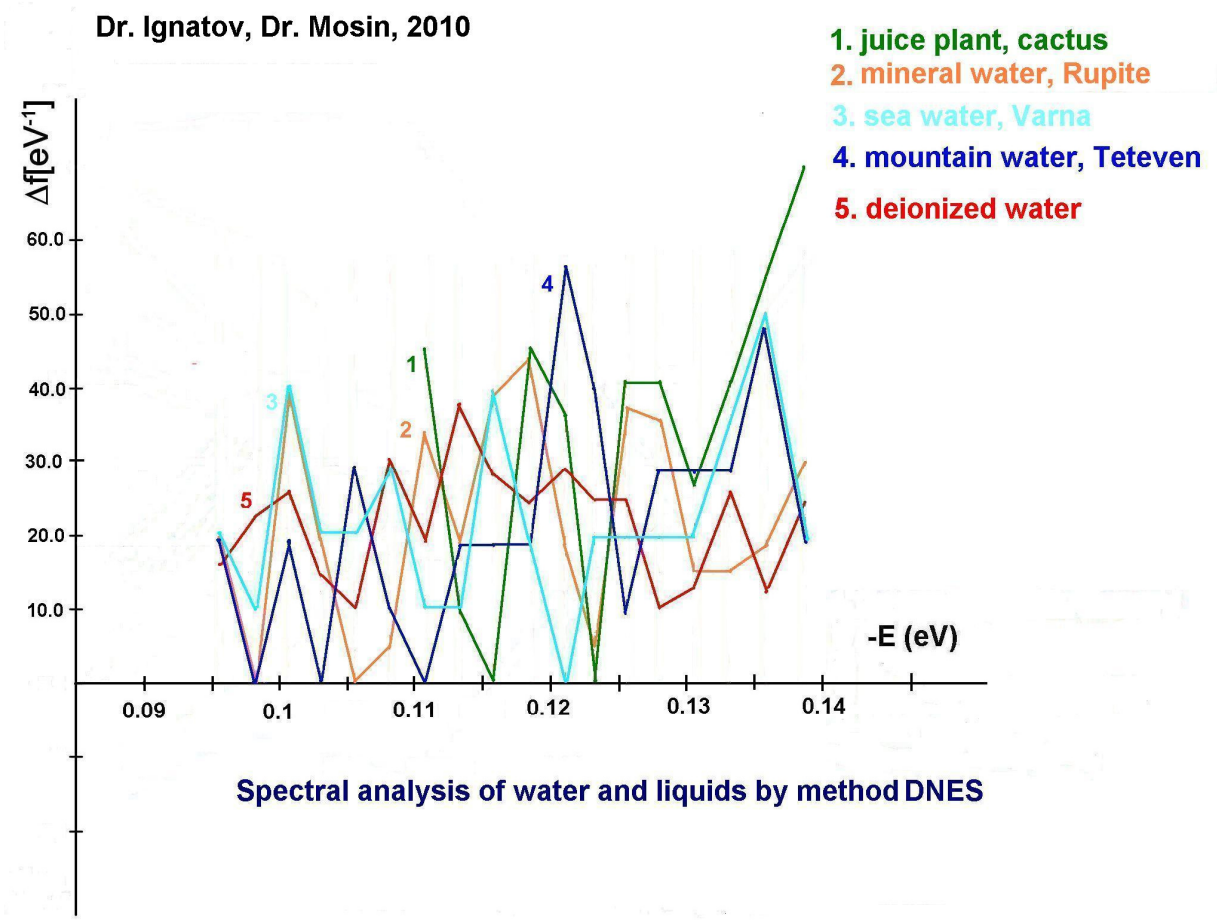

Fig. 2. *DNES spectra of water of various origin: 1 – cactus juice; 2 – mineral water Rupite (Bulgaria); 3 – sea water (Varna, Bulgaria); 4 – mountain water (Teteven, Bulgaria); 5 – deionized water (control).*

The table

Characteristics of spectra of water of various origin obtained by DNES-method

$-E_x$ (eV) Cactus juice	**-E (eV) Mineral water Rupite**	**-E (eV) Sea water**	**μm**	**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.

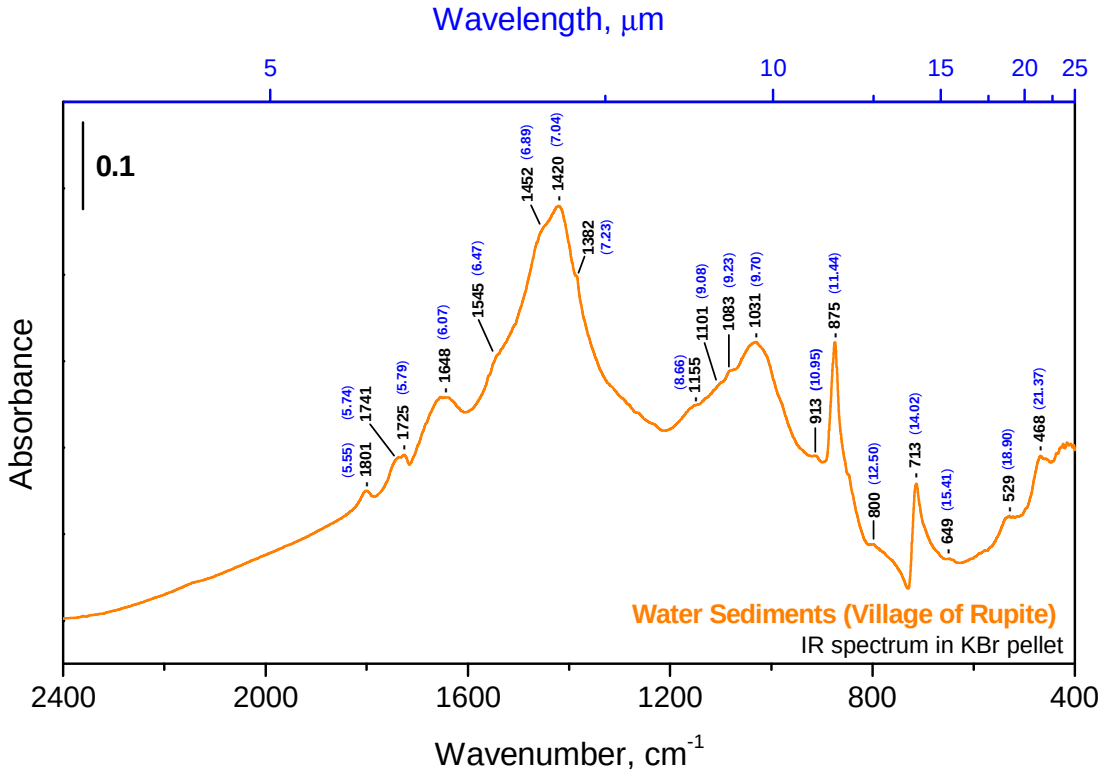

***Fig. 3**. IR-spectrum of water obtained from Rupite Village(Bulgaria)*

The separate spectrum of water from Rupite Village, Bulgaria (fig. 3) was studied with IR-spectroscopy method on a device Thermo Nicolet Avatar 360 Fourier-transform IR. The study was carried out by Christina Chakarova (Bulgarian Academy of Sciences). The value of local maximum was measured at 9,7 μm or -0,1287 eV. A value of local maximum at 9,64 μm or -0,1278 eV was measured with DNES method. The statistical reliability of DNES method makes up ± 0,0011 eV. The local maximums measured by DNES method at 9,83 μm (-0,1262 eV) and 8,95 μm (-0,1387 eV) are located on the spectral curve of the local maximum 9,7 μm (-0,1287 eV). 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. 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 to 80 °C at each 10 °C according to Students' t-criterion – $p < 0,05$. Such a character of the IR- and DNES-spectrum, and distribution of local peaks may prove that hot mineral alkaline water is preferable for maintenance of life than other types of water analyzed by these methods.

These data can also make suggestions as to the possible way of transition from synthesis of small organic molecules in conditions of increased temperatures to more complex organic molecules as proteins. The important factor in reaction of condensation of two molecules of amino acids into dipeptide is allocation of H_2O molecule when peptide chain is formed. As reaction of polycondensation of amino acids is accompanied by dehydratation, the H_2O removal from reactional mixture speeds up the reaction rates. This testifies that formation of organic forms may occur 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 blocks into larger organic molecules, and

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also polymerization of nucleotides into nucleic acids. Such association is connected with the reaction of condensation, at which from one block removes proton H^+, and from another – hydroxyl group (OH^-) with formation of H_2O molecule [21, 22].

The possibility of existence of condensation-dehydratation reactions under thermal conditions of primary hydrosphere was proven by Calvin in 1965 [23]. From most chemical substances hydrocyanic acid (HCN) and its derivatives – cyanoamid (CN_2H_2) and dicyanoamid ($HN(CN)_2$) possess dehydratation ability and the ability to catalyze the process of linkage of H_2O from primary hydrosphere [24]. The presence of HCN in primary hydrosphere was proven by Miller's early experiments. Chemical reactions with HCN and its derivatives are complex with chemical point of view; in the presence of HCN, CN_2H_2 and $HN(CN)_2$ the condensation of separate blocks of amino acids accompanied by dehydratation, can proceed at normal temperatures in strongly diluted H_2O-solutions. Furthermore, polycondensation of amino acids in the presence of HCN and its derivatives strongly depends on acidity of water solutions in which they proceed [25]. In acid water solutions (pH = 4–6) these reactions do not occur, whereas alkaline conditions (pH = 8–9) promote their course. There has not been unequivocal opinion, whether primary water was alkaline, but it is probable, that such a pH value possessed mineral waters adjoining with basalt, and these reactions could occur at contact of water with basalt rocks.

It should be noted, that geothermal sources might be used in synthesis of various organic molecules. Thus, 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 amino acids were detected [26]. In other experiments reaction products were polimerized into peptide chains that is the important stage towards inorganic synthesis of protein. In a reactionary mixture with a HCN–NH_3 solution in water were formed purines and pyrimidines [27]. 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 [28]. They consisted of 18 of 22 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 [29] and to have an effect similar to the action of α-melanocyte-stimulating hormone [30]. Under certain conditions in hot mixture of proteinoids in water solutions are formed elementary membrane like proteinoid microspheres with diameter 5–10 μm [31, 32]. On morphological features proteinoid microspheres remind a cellular membrane, which may be as well double. The initial stage of evolution, apparently, was connected with formation at high temperature 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 65–100 ^{0}C. Then in a mix of proteinoids in hot water solutions were formed membrane like structures.

In September 2011 a team of scientists led by T. Sugawara (Japan) brought us closer to the fact that life originated in warm or, more likely, hot water. They created proto cells, which were similar to the bubbles [33]. For this purpose, they made an aqueous solution of organic molecules, DNA and synthetic enzymes. The solution was heated to a temperature close to water's boiling point 95 ^{0}C, after that its temperature was lowered to +65 ^{0}C. Under these experimental conditions the formation of proto cells with membrane was observed. They were multiplying that is a further step for creation of synthetic cell. This laboratory experiment is an excellent confirmation of the possibility that life originated in hot water.

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4. The conclusion

The data obtained testify that life maintanence depends on phisical-chemical properties of water and external factors – temperatures, pH. Hot mineral alcaline water interacting with $CaCO_3$ is closest to these conditions. Next in line with regard to quality is sea and mountain water. For chemical reaction of dehydration-condensation to occur in hot mineral water, water is required to be alkaline in the pH range 9–11. In warm and hot mineral waters the peaks in DNES spectra were more expressed in comparison with the peaks received in the same water with lower temperature. The spectral range of DNES was in the middle infrared range from 8 to 14 μm. The content of deuterium in hot mineral water may be increased due to the physico-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 in the primodial hydrosphere was much more deuterium, this is a significant fact regarding the thermal stability of deuterated macromolecules in the preservation of life under thermal conditions.

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