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Physicochemical Properties and Biological Activity of the Water Depleted of Heavy Isotopes

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Abstract—Data on physicochemical properties of the light water (deuterium concentration is reduced to the ratio $D/H = 4$ ppm and that of the isotope of oxygen 18—to the ratio $^{18}O/^{16}O = 750$ ppm) have been obtained. They include the melting and boiling points, kinematic viscosity, density, the spin-spin proton relaxation time, self-diffusion coefficients, and the small-angle laser light scattering. It was found out that the biological activity of the light water was determined by the mechanisms different from the ligand-induced toxic effects. An explanation was proposed for the detected significant changes of the light water as compared with the high-resistivity water having geochemically ordinary isotope composition. This explanation was based on the mechanism of formation of supramolecular density inhomogeneities in water representing the deuterium-stabilized heterophase clusters.

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INTRODUCTION

Natural water represents a multicomponent mixture of molecules of different isotope composition (isotopologues). In natural water one million molecules on average includes 997284 molecules of $^1H_2^{16}O$, 311 molecules of $^1HD^{16}O$, 390 molecules of $^1H_2^{17}O$, and about 2005 molecules of $^1H_2^{18}O$ [1]. The concentration of water molecules containing heavy isotopes (D, ^{17}O , and ^{18}O) in natural waters fluctuates within the limits established in two basic international standards for the isotope composition of hydrosphere: VSMOW (Vienna Standard Mean Ocean Water) and SLAP (Standard Light Antarctic Precipitation) introduced by the International Atomic Energy Agency (IAEA) [1, 2]. The VSMOW standard determines the isotope composition of the deep water of the world ocean, while the SLAP standard determines the isotope composition of the natural water from the Antarctic (deuterium concentration varies depending on the depth of ice and its location on the mainland within the limits of ~ 10 ppm) at the current level on the surface equal to ~ 90 ppm (EDC station [3]). In accordance with the VSMOW standard the absolute contents of deuterium $D/^{1}H$ and oxygen-18 ($^{18}O/^{16}O$) in the ocean water amount to 155.76 ± 0.05 and 2005.20 ± 0.45 ppm, respectively, [4]. The content of the lightest isotopologue ($^1H_2^{16}O$) in the water corresponding in terms of the isotope composition to the VSMOW standard and the SLAP standard amount to 99.73 and 99.76 mol. %, respectively. The SLAP standard characterizes the lightest natural water on Earth. Hence, the concentrations of deuterium D/H and oxygen-18 ($^{18}O/^{16}O$) in this water amount to 89 and 1894 ppm, respectively, [5]. The light water can be defined as follows: as a result of special treatment the share of the lightest isotopologue $^1H_2^{16}O$ in this water was increased as compared with standard SLAP.

The reason for designating the light water as deuterium-depleted was, probably, the fact that the most significant reduction of deuterium content was achieved by rectification in the process of removing heavy isotopologues and also due to technological difficulties of determining the content of oxygen isotopes. As shown

in papers [6–8], “deuterium-depleted water” as a particular case of the light water possesses pronounced anti-tumor properties. The light water speeds up the exchange processes (both, physicochemical and biological) resulting in different physiological effects: immunostimulating, reproductive, and also in changes of the receptor sensibility to catecholamine and angiotensin [9].

It can be assumed that such important biological responses registered according to the results of experimental investigations must have at the bottom common reasons, to which primarily we should refer the variation of the physicochemical properties of water. That is why the present paper is devoted to determination of the classical “tabular” physicochemical parameters of the light water and the comparative description of results (for preparations of the light, natural and heavy waters) obtained by new methods of investigating the associated liquids.

EXPERIMENTAL

The deionized (high-resistivity) water (18 M Ω ·cm at 25°C) was prepared by subjecting the pyrogenic distilled water to purification on the Milli-Q system produced by Millipore, Great Britain. The light water was produced at OJSC Almaz by the vacuum rectification method. The content of deuterium (D/H) and oxygen-18 (¹⁸O/¹⁶O) in light water amounted to 4 and 750 ppm, respectively, as compared with D/H = 140 and ¹⁸O/¹⁶O = 1966 ppm for deionized water. The heavy water (99.9%) corresponded to Technical Specifications TU 95-1893-89. As a result of the performed investigation using the atomic absorption spectrometry with electrothermal atomization and Zeeman background correction [10], such elements as Al, V, Cr, Mn, Ni, Fe, Cu, Zn, As, Cd, and Pb were not detected in any preparations of water (concentration < 0.1 nM; the detection limit was 0.1–10 nM).

In addition, the presence of impurity particles of the dispersed phase was not registered in the specified preparations of water by using the methods described in paper [11].

Determination of the isotope composition. The methods used for measuring the deuterium concentration included the mass-spectrometry method [12] and the method of multiple-pass laser absorption spectroscopy using an LWIA-24d instrument produced by Los Gatos Research, Inc. [13]. The content of oxygen-18 was also controlled by using the multiple-pass laser absorption spectroscopy.

Density of water was measured by a digital densimeter of model LiquiPhysics Excellence DM45DR produced by Mettler Toledo, and also by using a PZh-2 picnometer in accordance with GOST 18995.1-73. The relative error of the method was 0.016%; P = 0.95.

Viscosity of water was determined by using a VPZh-2 capillary glass viscosimeter (capillary internal diameter of 0.56 mm) with an additional thermostating device [14]. The relative error of the method was 0.89%.

Boiling point of water was measured on the Sventaslavskii instrument [14].

Melting point of water was determined by the method of differential scanning calorimetry (DSC). The scanning speed was 4 deg/min. The solution under investigation in the amount of 0.0010–0.0100 g was pressed into container and subjected to cooling with liquid nitrogen for one–two minutes to a temperature of 223 K. It was assumed that at such high speeds of cooling the changes of its phase state will be minimum. Next, the system was held during several minutes at temperature 223 K for the establishment of equilibrium. Thermograms were registered in the interval 223–293 K. In order to determine the reproducibility of results of DSC and their dispersions, 10 measurements were performed for each water sample. The calculated sample variance amounted to 0.012.

Spin-spin relaxation time of water protons was determined by the NMR spin-echo method [15].

Molecular dynamics characteristics of water were determined by the method of monoenergetic neutron scattering (13.289 meV) [16]. In order to calculate the self-diffusion coefficients, the results were processed by using the Oskotskii-Ivanov model.

Variance analysis of water samples and nanoparticles was conducted by using the method of low-angle laser light scattering (LALLS) and the dynamic light scattering (DLS) (photon correlation spectroscopy) on instruments MasterSizer 2000 and ZetdSizer Nano ZS (Malvern Instruments) [10].

Values of the pH were measured on a PP-20 pH-meter produced by Sartorius and equipped with a thermal sensor and by using the spectrophotometry with an acid–base indicator of phenol red (pK_a = 7.4) at the concentration 30 μ M [17].

Biological activity of water samples was investigated on the cellular biosensor *Spirostomum ambigua*. The setup for investigating the behavior of infusorium *S. ambigua* incubated into water samples consisted of a water thermostat, 5-well plate, and binocular MBS-10 [18]. Low-power luminescent lamps (~10 W) were used for the irradiation.

RESULTS AND DISCUSSION

Biological activity of the light water. The information about the biological and therapeutic effects of light water requires an answer about the mechanism of this activity. In the case of the high-resistivity water depletion of heavy isotopes by rectification, the highest reduction (by more than an order of magnitude) sustains the deuterium concentration. It is well-known that the kinetic isotope effect is most pronounced for the protium–deuterium pair. Biological effects during the transition from the deionized natural water to the light one can be determined by the variation of kinetics of speed-limiting stages of metabolic chains at the expense of reduction of the $^1\text{H}^1\text{D}^{16}\text{O}$ concentration from 15.57 to 0.44 mM (light water with ratio H/D = 4 ppm).

Table 1 shows that the life time of cellular biosensor is reduced more than 10 times in the water both, depleted of heavy isotopes and enriched in terms of the deuterium content. The cellular biosensor reacts to any chemical substances appearing in aqueous solution by the indicator of variation of the activation energy of the cellular transition process, for example on D-mono- and disaccharides [18].

Table 1. Biological effect of the light water, high-resistivity water having an ordinary isotope composition, and the diluted heavy water on the cellular biosensor ($n = 9$)

Indicator	D/H, ppm		
	4	140	265
Life time of the cellular biosensor (at $28 \pm 0.1^\circ\text{C}$), min	5.6 ± 0.4	64 ± 10	4.3 ± 0.3
Activation energy of the transition process from the active into stationary state without reflection to stimuli (26°C – 36°C), kJ/mol	139 ± 11	138 ± 15	104 ± 17

Cellular biosensor was applied by the authors as one of the variety of physicochemical sensors. In studying the effect of water preparations with a modified isotope composition the activation energy of the process of cellular transition remains practically invariable. The adaptive effect in case of the organism getting into a new medium is usually related to the speeding up of metabolic processes that can result in the form of different physiological manifestations [19]. Apparently, the free-living oligotrophic infusorium *S. ambigua* proves to be adapted just to the natural (for fresh waters of the East European Plain) isotope ratio of hydrogen and oxygen of water. This phenomenon indicates that the biological effect of the variation of the isotope composition of water is related to the water properties as a solvent rather than to the toxic effect of heavy isotopes.

Physicochemical properties of light water. It occurred that the “classical” physicochemical properties of the light water differ from the deionized water having natural isotope composition (Table 2). The differences in surface tension (at the water–air interface) for the light water–deionized water pair are comparable with such for the deionized water–heavy water pair. In terms of the parameter of kinematic viscosity the light water is closer to the deionized water of natural isotope composition than the deionized water to the heavy one. It should be stressed that the density of the light and deionized waters to a great extent is determined by the concentration of heavy isotopes of oxygen (see Table 2, line 3).

The temperatures of phase transitions in the light water are significantly reduced. The reduction of the freezing point of light water ($< -1^\circ\text{C}$) exceeds the similar value for a 100 mM sodium chloride or saccharose solution. This apparent violation of the Raoult law as also the probable variation of the ebullioscopic constant of the light water (the boiling point is reduced by 7°C) make it possible to suggest that the light water represents a new associated liquid that have not been earlier scientifically described in literature. It should be noted that the modern quantum mechanical models function by adopting during the simulation other physicochemical constants obtained for a real liquid heterogenetic in terms of isotope composition [20, 21].

The variation of properties of liquid during the reduction of concentration of heavy isotopes testifies the presence of a “control” function of water molecules containing heavy isotopes: one molecule of $^1\text{H}_2^{16}\text{O}$, one molecule of $^1\text{H}_2^{17}\text{O}$ and 5–7 molecules of $^1\text{H}_2^{18}\text{O}$ fall on ~ 3000 molecules of $^1\text{H}_2^{16}\text{O}$ [1]. Such realization of the isotope effect is possible via the cluster organization of associated liquid. The growing number of tie molecules in light water and its higher “homogeneity” in terms of the structure is corroborated by the variation of its molecular dynamics properties (see Table 2, lines 6–8). The diffusion coefficient increases in the light water reflecting the contribution from collective movements ($D_l = 0.63 \times 10^{-9} \text{ m s}^{-1}$) as compared with the deionized water ($D_l = 0.46 \times 10^{-9} \text{ m s}^{-1}$). The same tendency is also observed by the replacement of protium with deuterium in the preparation of heavy water.

Table 2. Physical properties of preparations having different isotope composition ($T = 20 \pm 0.04^\circ\text{C}$)

Item No.	Parameter	Light water D/H = 4	Deionized water having natural isotope composition D/H = 140	Heavy water 99% of D ₂ O
1	Kinematic viscosity, mm ² /s	0.987	1.012	1.274
2	Surface tension, mN/m	75.172	72.860	67.800
3	Density, g/cm ³	0.99692*	0.99820** 0.99691*	1.1042
4	Boiling point, °C	93.7	99.9	101.4
5	Melting point, °C	-1.5	0	3.8
6	Time of spin-spin proton relaxation (T ₂), s	0.347 ± 0.024	2.000 ± 0.140	—
7	Contribution to the total self-diffusion coefficient from collective movements (D _l × 10 ⁹), m ² s ⁻¹	0.63	0.46	0.52
8	Contribution to the total self-diffusion coefficient from single-particle movements (D _l + D _f = D is the total self-diffusion coefficient), (D _f × 10 ⁹), m ² s ⁻¹	1.60	1.77	1.50
9	Life time of molecules in the oscillating state around the equilibrium center, (τ ₀ × 10 ¹²), s	2.08	2.80	2.79

Notes: *¹⁸O/¹⁶O = 757 ppm; **¹⁸O/¹⁶O = 1966(at $T = \pm 25^\circ\text{C}$).

A significant reduction of the spin-spin relaxation time (0.35 ± 0.02 s versus 2.00 ± 0.10 s for light and deionized water, respectively, (see Table 2, line 6) means a sharp reduction of the degrees of freedom in light water. The removal of deuterium, probably, results in the elimination of geometrical effects in the network of hydrogen bonds. The obtained results well agree with the reduction of the freezing point of the light water.

The possible mechanism of implementation of the isotope effect during the formation of the water having the natural isotope composition. In our view the causes of differences in the properties of the light and deionized water can be explained as follows. In earlier papers [22–27] we put forward a hypothesis on the formation in the water of natural isotope composition of density inhomogeneities of submillimeter range—deuterium-stabilized gigantic heterophase clusters (GHC) with the relaxation time of more than one second. It should be stressed that the long-living supramolecular density inhomogeneities (gigantic in comparison with the well-known nanoclusters) were experimentally observed by other teams of researchers [28, 29]. These density inhomogeneities of water are stabilized by heavy isotopologues at their probable arrangement on the surface of density inhomogeneities that allows them to implement the “control” function.

As was noted in papers [22, 24], the rise of water homogeneity in terms of the isotope composition leads to disintegration of GHC. The opposite statement is also true: formation of GHC in the light water is hampered. This is demonstrated by the performed experiment on induction of the GHC formation by latex nanoparticles. As for any dispersed formation in colloidal solution, a “primer” is required for formation of GHC. In measuring the dimensional spectra of density inhomogeneities by using the LALLS method [11] it is possible to register GHC of the submillimeter range induced by nanoparticles (the same preparation of water, where the solution of nanoparticles is introduced to, is used as a background). Table 3 shows that such induction of GHC formation is only possible in the water having the natural isotope composition.

The analysis of suspensions of the standard latex microspheres of nanometer range by the method of low-angle laser light scattering revealed significant differences in dimensional spectra with respect to standard values. Hence, for hydrophobic nanospheres having the diameter < 100 nm their diameters appear to be exceeded by orders of magnitude that can be explained by the initiation of the formation of water GHC by nanoparticles. The investigation of larger microparticles does not reveal any variations of dimensional spectra: the diameter coincides with the declared value.

In the light water new GHC are not initialized by nanoparticles. Sulfated latex particles having the size >100 nm do not result in formation of GHC. In separate experiments it was revealed that hydrophobic nanoparticles are more effective in respect of GHC induction, i.e. they induce the formation of latter at lower concentrations than the sulfated nanoparticles. The collection of the specified data makes it possible to assume that in addition to our earlier descriptions of the Kolmogorov mechanisms of the white cascade of dissipation

of kinetic energy into thermal one [24], a considerable contribution to the formation of GHC can be made by the dispersion (Deryagin) interactions.

Table 3. Initialization by latex nanoparticles of the formation of density inhomogeneities—gigantic heterophase clusters in water preparations of different isotope composition

True (certified) size of nanoparticles, nm	Measured size of density inhomogeneities in different solvents, nm	
	light water	ordinary water
20	25 ± 5	10000 ± 500
40	50 ± 7	10100 ± 200 26100 ± 1000
80	85 ± 7	30100 ± 900
305	315 ± 5	315 ± 5
1200	1300 ± 50	1400 ± 100

Note. The concentration of sulfated nanoparticles (Invitrogen Corporation) amounts to 0.00005 vol %. All measurements were performed in the circulation mixing mode.

The reduced concentration of GHC in light water [24, 25] results in the formation of a liquid that is closer to the model of “continual” water having the reduced values of the temperature of phase transitions, viscosity, and enhanced surface tension. It is not excluded that the investigation of the properties of samples of the light water with a higher concentration of isotopologue $^1\text{H}_2^{16}\text{O}$ shall reveal further reduction of the melting and boiling points in the direction “specified” by the Mendeleev periodic law for hybrids of elements of the II period and VI group of the main subgroup.

CONCLUSIONS

On the basis of obtained data the following conclusions can be made:

- in terms of a series of physicochemical properties the light water is a new associated liquid that has not been scientifically defined earlier;
- possible mechanism of implementing the “control” function of heavy isotopologues of water molecules is the formation of density inhomogeneities, i.e., deuterium-stabilized gigantic heterophase clusters;
- biological effects of the light water are not related to the ligand-dependent toxic effect, but determined by the adaptive reasons.

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