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Abstract Xenon is a perspective gas for creation of oxygen free environment for different applications of biomaterials. To use xenon in suspensions and products properly it is necessary to know the molecular mechanisms of its interactions with water and cells. This work reports the study of bacterial suspensions of *Escherichia coli* in the presence of xenon using nuclear magnetic resonance (NMR). The work studied how the spin-lattice relaxation times of water protons in suspension change under xenon conditions. Xenon is able to form clathrate hydrates with water molecules at a temperature above the melting point of ice. The work studied NMR relaxation times which reflect the rotation freedom of water molecules in suspension. Lower relaxation times indicate reduced rotational freedom of water. Single exponential behavior of spin-lattice relaxation of protons in the suspensions of microorganisms has been registered. A recovery of longitudinal magnetization in cell suspensions with xenon clathrates has been characterized by two peaks in T_1-distribution. Fast relaxing T_1-component was related to the intracellular water and depended on the amount of xenon clathrates. The obtained results elucidate how the NMR method can monitor the process of clathrate formation and how the xenon atoms and hydrates interact with cells.

Keywords Bacterial Suspensions, Water, Xenon, Clathrates, Nuclear Magnetic Resonance

1. Introduction

Despite their inertness, noble gases possess a number of interesting properties, including their anaesthetic effect [1–4]. They are very soluble in lipids and may bind to both membrane lipids and proteins [3–5]. Because of the high correlation between lipid solubility and anaesthetic potency (known as the Meyer-Overton correlation) it was suggested that the molecular mechanisms of general anaesthetics are associated with the interactions between anaesthetic agents and a hydrophobic interior of the lipid bilayers [2, 4, 5]. Although different theories have been discussed in the literature for long time, the exact mechanism of anaesthetic action of noble gases is still a mystery [5, 6]. It is not clear which of the different mechanisms suggested is most likely to be the main mechanism of action of anaesthetic agents.

Xenon is the most potent anaesthetic of all the noble gases. The high polarizability of xenon compared to the other noble gases increases its affinity to hydrophobic environments. Thus xenon can be substantially partitioned into the hydrophobic core of the membrane bilayer. The solubility of xenon in human serum albumin solutions, hemoglobin solutions, human blood, and other biological solutions and homogenates was studied in the Ref. [7]. For example, at 298 K the amount of xenon dissolved in 1 g human albumin at 0.1013 MPa of total pressure was found to be 0.2382 ml. According to the data [3] a blood-gas partition coefficient for xenon is 0.115 whereas the solubility in water measured identically was 0.096.

The anaesthetic gas xenon is attractive for many applications in biotechnology and medicine [8–10]. Xenon is also the perspective gas for preservation of biological materials including different enzymes and cells [11–16]. This noble gas is able to replace lighter gases of air, e.g. oxygen and nitrogen [17, 18]. This inert gas was already applied for many biological and medical systems in creation of oxygen free environment, in particular, for bacterial cell suspensions and erythrocytes of blood [9, 10, 19–24]. The authors of Refs. [19, 20] developed the method of platelet storage in the presence of xenon. In order to use the xenon in the storage of biological suspensions and biotechnological products properly it is necessary to know the molecular mechanisms of its interactions with cell membrane and cell components [5, 11, 13, 21]. Nowadays many questions are still opened.

One important feature of xenon is its ability to create clathrate hydrates from water molecules at relatively "soft" conditions when the pressure is still about 0.4–0.7 MPa and temperature circa 276–278 K [12–14, 21]. When forming hydrates, lower hydrate formation pressures are desirable in practice [21, 25, 26]. It is known that crystalline hydrates are formed by many simple gases that do not interact strongly with water, and mostly the gas molecules or atoms occupy "cages" formed by the network of water molecules. The majority of these gas hydrates adopt one of two possible cubic cage structures and therefore are called clathrate hydrates [21, 25, 26]. Although high pressure is often needed to hold gas atoms inside the water "cages", xenon gas is an exception from the rule. Xenon hydrates are the clathrates of low pressure. They can be stabilized at about 276–280 K [10, 12, 25].

Clathrate hypothesis of anaesthesia of *L.Pauling* [27, 28] suggested the existence of clathrate microcrystals formed by anaesthetic gas and water molecules even at physiological conditions (at least momentarily): normal atmospheric pressure and temperature of cells. Some suggestions of clathrate theory of *L.Pauling* have been checked later in Refs. [27–33]. First studies on formation of xenon clathrates in cellular suspensions of microorganisms have been initiated in Refs. [9, 13, 14, 34, 35]. Those publications showed how the methods of magnetic resonance can produce valuable information about the changes in bacterial medium under gas pressure. Later publications [21, 36–40] reported more NMR results on xenon clathrates. The use of paramagnetic additives expanded the possibilities of NMR relaxation in studying cell suspensions with gas hydrates [21, 36]. NMR was also applied for studying clathrate formation in two-component and complex systems [9, 37–40]. Nevertheless many questions about the role of xenon and its clathrates in cell suspensions still remain open.

The most intensively investigated prokaryotic model organism in biophysics is the *Escherichia coli* (*E.coli*), K-12 strain [41, 42]. It is considered as very important representative of cellular suspensions in biotechnology. *E.coli* is a common gram-negative rod-shaped bacterium, found in normal human (and other animals) bacterial flora. It is about 2.0 µm in length and its diameter is 0.25–1.0 µm [41]. These microorganisms are facultative anaerobic bacteria which can be easily grown in a laboratory.

The current work reports NMR-study of bacterial suspensions of *E.coli* under xenon pressure addressing how the xenon and its hydrates participate in the interactions with cells and affect the state of intra- and intercellular water. The biomaterials with crystals of xenon clathrates are "transparent" for radio waves. This allows using the methods of radio spectroscopy (i.e., NMR) to analyze the structural and phase state and molecular-dynamic properties of water in the cell suspensions in the presence of a clathrate phase. The paper presents how NMR water proton spin-lattice relaxation times in *E.coli* suspensions vary under xenon conditions.

2. Experimental Materials and Methods

2.1. Materials

The cells of *E.coli* have been grown in flasks with volume of 0.75 liter using beef-extract broth or mineral liquid medium with glucose. A sterilized phosphate buffered saline (PBS) was added. The cells at the stationary phase of growth were used (age of 14–16 hrs). The samples with cells were centrifuged at 3000 rpm for 15 min. The precipitate was washed twice in distilled water, PBS and was used in subsequent experiments as in Refs. [9, 21, 36]. A concentration of the biomaterial (cellular suspensions) under study was expressed in g of dry mass per g H_2O and by a number of cells per 1 ml [21].

2.2. Custom-made Thermostat Chamber with Xenon Kit

We designed and built thermostat chamber with transparent front door from polymethyl methacrylate. Inside the chamber a system to transfer the gas to tubes with samples was built. The gas cylinder (with medical grade xenon) was connected to the special kit with tubes filled by suspensions / biomaterials [21, 27, 36]. The tubes with samples were under gas pressure as described in earlier publications [21, 37]. Mostly the pressure circa 0.8–1.0 MPa or less was enough to produce stable clathrates at 278–280 K. In some experiments "dry" ice (carbon dioxide) was used to touch the bottom of the tubes for the initiation of clathrate formation according to described procedure [21, 36, 43]. After saturation of the samples with gas and xenon clathrate formation the tubes could be detached from the kit in the thermostat chamber and be used in NMR measurements at above melting point of common ice but not exceeded 286 K.

2.3. NMR Methods in Studying Water Clathrates of Xenon

The NMR studies of cell suspensions and suspensions with clathrate crystals were performed at a proton resonance frequency of 90 MHz. Some experiments have been carried out also at 20 MHz. In the single-pulse ^1H NMR experiments, the free induction decay (FID) signals were measured. The FID consists of a 90° pulse which rotates the magnetization onto the transverse plane where it decays with characteristic time. The initial part of the FID is hidden in the dead-time zone of the spectrometer. The length of the 90° pulse was 8 µs. The FIDs on the samples with clathrates have been measured according to the procedure of earlier publications [27, 37, 43]. The signal of total time-domain is the sum of the signals of the water protons and the protons of clathrate crystals: $S = S_{wat} + S_{clath}$. In the study of water dispersions of xenon clathrates [21,37] it was shown that spin-spin relaxation time T_2 of the fast relaxing component (protons of clathrates) did not exceed 25 µs. The structure of clathrate ice in studied solutions and suspensions at above melting point of common hexagonal ice was relatively "softer" than that in common ice with $T_2 \sim 10$ µs. For ice protons the most

part of FID was hidden in the dead-time zone. Only the tail of the transverse magnetization decay might be observable in the experiment. Although the part of FID for the clathrates is a bit longer than the FID for common ice, the situation with FID in clathrates is comparable with that in ice. To monitor the water signal only, the contribution of the protons of clathrate ice should be subtracted. In such a case, normally, the measurements were carried out at experimental conditions when transverse magnetization of clathrate protons decayed very fast to zero (whereas the transverse magnetization of liquid water has not decayed) and only protons of water could be measured in the study [37,43]. On the base of this approach a fraction of water built in clathrate structures was determined using the difference of the proton signals of liquid water in suspensions before and after clathrate formation [21].

The spin-lattice relaxation time T_1 was measured by the inversion recovery (IR) method using the pulse sequence $(180°–t–90°)$ at the pulse sequence repetition time $\geq 5T_1$ [45, 46]. The period of $5T_1$ was waited between the consecutive scans of pulse sequence in order to ensure that the fully recovered signal was acquired. When t is varied then experimental dependence of magnetization $M_t = f(t)$ can be obtained and fitted by the law $M_t = \sum M_{0i} \cdot [1 - 2\exp(-t/T_{1i})]$, where i is the number of relaxation component. The analysis of relaxation data was conducted after transferring the raw data from spectrometer computer to desk computer using in-house *MatLab®* codes. In these NMR studies there was an aim to model experimental data as a sum of several relaxation components or distribution of NMR-relaxation times. To fit the data inverse Laplace transform (ILT) in one direction was commonly applied [47–50]. The kernel function in ILT was associated with M_t data obtained from IR NMR experiments.

3. Results and Discussion

3.1. Spin-lattice Relaxation of Water Protons in Suspensions of *E.coli* before and after Xenon Clathrate Formation

The T_1–experiments discovered that the recovery of longitudinal magnetization of measurable protons in all studied samples of cell suspensions (concentration range of 0.001–0.24 g dry mass /g H_2O) can be fitted by single exponential curve and characterized by a single value of the spin-lattice relaxation time T_1. However a recovery of longitudinal magnetization of protons in bacterial suspensions with clathrates of xenon has bi-exponential behavior. T_1 values of the two exponential components differ by a factor of 6 to 10. Alternatively, the treatment of the recovery of longitudinal magnetization using inverse Laplace transform resulted in a distribution of spin-lattice relaxation times T_1 in the kind of 2 peaks. Fig.1 shows T_1-distributions obtained by one- dimensional ILT applied to the data sets for original bacterial suspension and *E.coli* with xenon clathrates.

The two peaks registered for the suspension of *E.coli* with

xenon clathrates (Fig.1) had maximums at 0.25 and 1.2 s. The integral intensities of these peaks corresponded to 66% and 34 % of measurable protons. The single peak observed in T_1-distribution in suspension of *E.coli* without clathrates was at $T_1 \approx 0.70$ s (Fig.1). This was in line with previously published NMR relaxation data on cellular suspensions [21, 40] which showed fast exchange between the protons of intra- and intercellular water, i.e., single exponential T_1-relaxation [51–53]. The permeability of the cell membrane to water is quite high (for instance, in red blood cells exchange time of the order of 10 ms [53]). Water proton relaxation times in *E.coli* bacteria showed one exponential behavior, i.e., two sites (two compartments: intracellular and extracellular spaces) are in a fast exchange limit. Thus a researcher needs to reduce one of the relaxation times to less than exchange time in order to remove this condition and to generate two exponential behavior [51, 52]. In the presence of paramagnetic ions of manganese in the extracellular water shortening the relaxation times outside the cells it was possible to record two separate NMR signals in the population of cells with non-damaged cellular membranes [36, 51, 52].

The relaxation rates of the water molecules in cell suspensions are governed by several important factors, in particular, the strength of local magnetic interactions between water nuclei, the molecular motion and proton exchange rates. Nuclear magnetic inter- and intramolecular dipole-dipole coupling describes the interactions between water nuclei [51–53]. The magnetic interactions are partially averaged within the hydration layer by the processes depending on the interactions of water with macromolecules/cell surface/clathrates. These are proton transfer, dynamic orientation and diffusion of water molecules through regions of different orientations [26, 40, 44, 53]. The NMR relaxation times are sensitive to molecular motions in the range of 10^{-8} to 10^{-12} s [45, 46, 53]. In liquid solutions water molecules tumble at a rate of about 10^{-12} s [45]. This motion is considerably slowed down when water molecules interact with biological macromolecules/cell surface. For example, the correlation time is of the order of 10^{-12} s for the water molecules non-involved in the association through hydrogen bonds with surface of cells. The rotational motion of water molecules associated via hydrogen bonds with polar groups of the macromolecules is reduced so that their correlation time is even sometimes of the order of 10^{-6} s [53]. Under the conditions of rapid exchange between the hydration and bulk water in suspensions and macromolecular solutions, single relaxation rate $(1/T_1)$ is observed.

Single exponential relaxation of protons in suspensions of *E.coli* confirmed fast exchange between intracellular and intercellular water on the NMR time scale. Formation of xenon clathrates affected the exchange rate and resulted in the measurement of two separate compartments. The population of fast relaxing T_1-component was associated with the fraction of intracellular water. It depended on the amount of xenon clathrates.

Fig.2 shows how the amount of clathrate ice, i.e. the amount of water molecules bounded within the structure of

xenon clathrates, depends on the concentration of cells in suspension. The fraction of clathrates has been calculated according to the procedure [37–40] which compares the measurements of the FID in original bacterial suspension and suspension with xenon clathrates. The transition of liquid water into solid phase of clathrates resulted in a change of spin-spin relaxation times T_2 of protons. $T_2 \sim 1-2$ s for free mobile water whereas $T_2 \sim 10$ µs for protons of solid phase. Such a difference in T_2-values gives an opportunity to separate proton signals of liquid and solid phases much better. The results of Fig.2 are in line with the previously published data on the xenon clathrates formation in solutions of biomacromolecules [12, 27, 31, 32, 34]. It was shown [31, 32, 34] that a water fraction bound in clathrate structures was due to abundance of hydrophobic groups on the surface of proteins. Probably some groups on the cellular surfaces also participate in the formation of xenon hydrates stabilizing clathrate lattice from water molecules [21,28,31]. As a result the amount of stable clathrate structures was increasing with cell concentration in studied range. One observed that the amplitude of fast relaxing T_1–component (relatively to total water content in cell suspension) increased nonlinearly with an increase in the initial cell concentration. Apparently this is due to the advent of solid phase of water (clathrates) and subsequent displacement of the microorganisms in the free volume of the suspension.

Fig.3 shows calculated fraction of intracellular water (open circles) and the dependence of the population of the fast relaxing T_1-component on cell concentration recalculated to the signal of liquid water. This recalculation shows the true concentration of the cells remaining in the liquid phase of the suspension after the formation of clathrates. The dependence of the population of this T_1-component looks almost linear, and this assumes that the fast component of T_1 is due to the protons of intracellular water. The slow T_1-component can be attributed then to the extracellular water protons. This assignment is further supported by a decrease of the population of the slow component with increasing concentration of cells. A fraction of the extracellular water protons which are not in clathrate structures decreased. According to this analysis, the cell concentration increased relatively to volume of liquid phase after clathrate formation. A fraction of intracellular water (Fig. 3, open circles) was calculated on the base of summary volume of cells and had a linear dependence on concentration of cells.
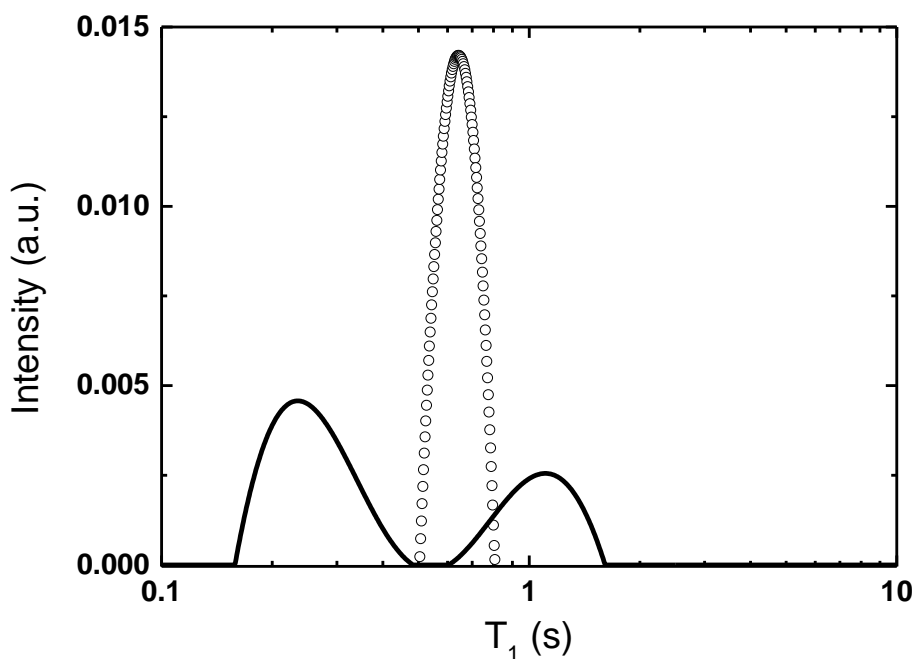
Figure 1. T_1-distribution of ^{1}H obtained by one-dimensional ILT on the recovery of longitudinal magnetization data set measured in the suspension of *E.coli* with concentration of 0.07 g dry mass/g H_2O (open circles) and in the same sample after xenon clathrate formation at 1.2 MPa (solid line). T=280 K. Frequency is 90 MHz.
The integral intensities of 2 peaks in the suspension of *E.coli* with xenon clathrates (*from left to right*) are 66% and 34 % of measurable protons. The signals in each T_1-experiment have been normalized per maximal signal registered after total recovery of longitudinal magnetization.
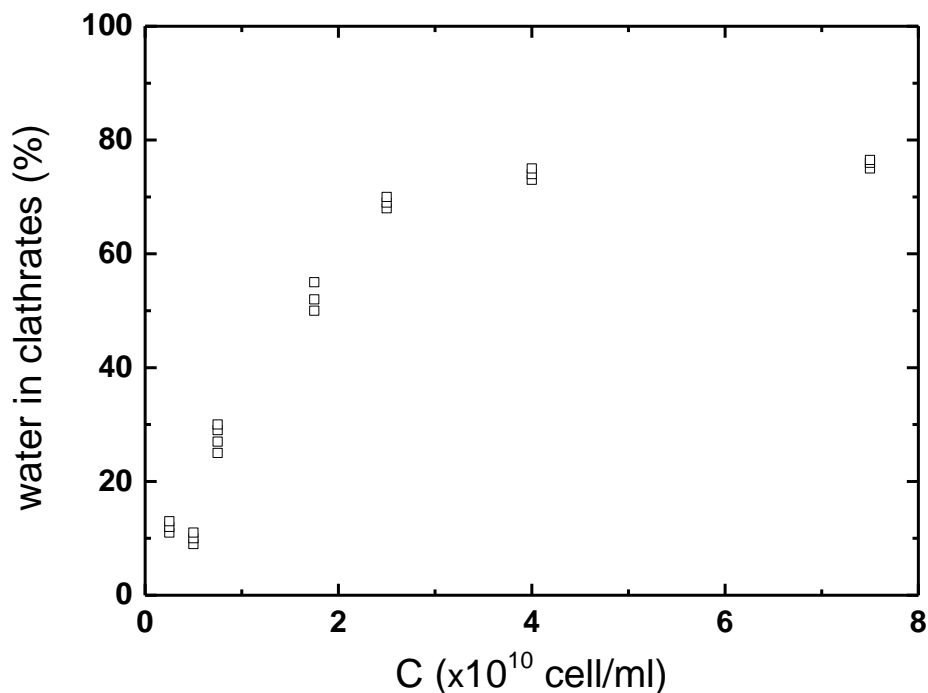
Figure 2. The dependence of the amount of H_2O molecules bounded within the clathrate structures (i.e., protons of solid phase with $T_2 \sim 10$ μs) on the concentration of cells in the suspensions of *E.coli* with xenon clathrates. The results were obtained from NMR (FID) measurements taking into account the procedure of Refs. [37, 38]. T = 280 K. Pressure is 1.2 MPa. Frequency is 20 MHz.
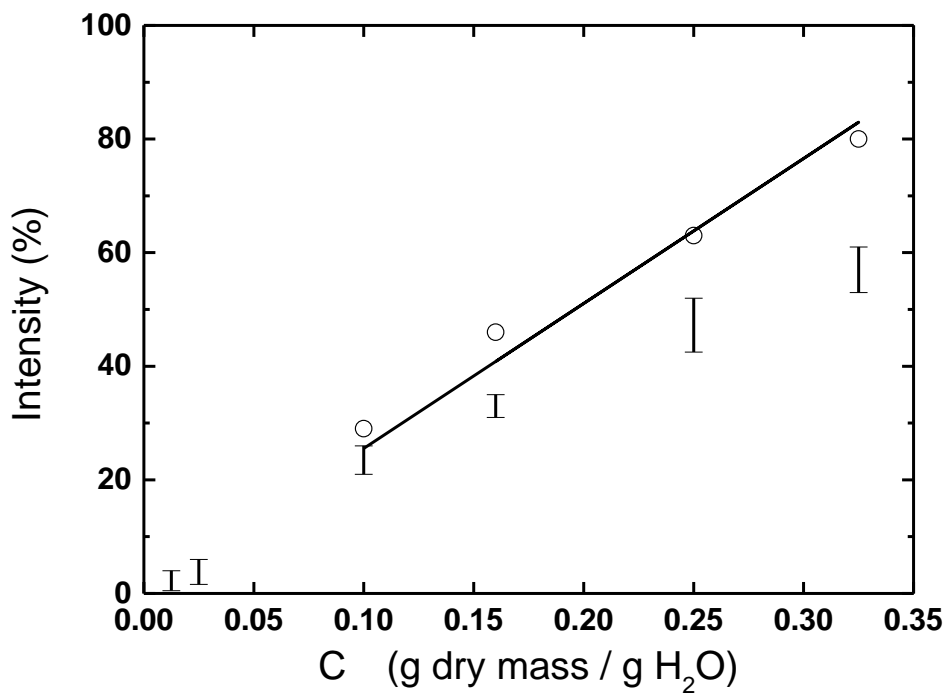
Figure 3. The relative amplitude of fast relaxing T_1-component (vertical line segments) and calculated fraction of intracellular water (open circles) in the liquid phase of *E.coli* suspensions with xenon clathrates as a function of cell concentration (C). Solid line is the linear fit to the data (open circles) calculated on the base of summary volume of cells.
T = 280 K. Pressure is 1.2 MPa. Frequency is 90 MHz.

It is worth to emphasize that the dependence of intracellular water amount on the concentration of cells in suspension (Fig.3, open circles) ideally should be a straight line coming through the origin of the coordinates and the meaning which can be obtained at tightly packed cells, i.e., when extracellular water would be completely removed. The experimental curve for the population of the fast relaxing T_1-component is positioned lower than that based on the total volume of the cells (Fig.3). This could be explained by the osmotic compression of the cell due to an increase in the concentration of solutes in extracellular space upon xenon clathrate formation and a decrease in the amount of remaining liquid water. This is in line with the published data on paramagnetic doping (when the Mn^{2+} ions are introduced into the extracellular water to separate the proton signals of intracellular and extracellular compartments) in studying the xenon clathrate formation in the extracellular compartments of bacterial suspensions [21, 36].

The behavior of T_1-components in *E.coli* suspensions with xenon hydrates depended on the amount of clathrate microcrystals. This suggests that the proton exchange between the extracellular water and the protons of solid clathrate lattice could also affect T_1-component of the extracellular water. This is in line with some published NMR-data [37, 39, 43, 44, 51]. Ref. [44] showed that the T_1 of protons of ice was increasing with decreasing the temperature from 273 K to 213 K. For example, at T=273 K the T_1 of protons of ice was 0.5 s increasing to 3 s at 250 K. In contrast to ice the T_1 of protons of liquid water decreased with decreasing the temperature of water [43, 44, 51]. According to the published data on dispersions of xenon clathrates in pure water [37, 39], the temperature dependence of T_1 for water protons showed a minimum. A proton exchange between liquid water and solid clathrate phase resulted in relaxation behavior of the protons of water molecules displaced among clathrate microcrystals in dispersion. Upon this influence dependence of T_1 on temperature for the protons of water remaining in liquid state became similar to that for the protons of ice. Thus the protons of liquid water near the crystals of clathrate ice could experience spin-lattice relaxation with increased values of T_1 exceeded typical values for liquid water. However an interpretation of longitudinal relaxation in *E.coli* suspension with xenon hydrates could be much harder than it was for clathrate dispersions of xenon in pure water because of additional relaxation sinks and hydration of cell surfaces. For phase of clathrate crystals at the temperatures above 273 K T_1 is probably of the order of few seconds (the estimate is based on the mobility of water molecules in ice at 213−273 K and in clathrate crystals at 275−280 K [25, 26, 30, 38, 44, 54]). Then for the protons of liquid water remaining in extracellular compartment and dispersed between hydrate crystals and bacterial cells we can expect T_1 to be of the order of 1 s. The amount of water molecules participating in the exchange with clathrate phase was increasing with growth of the amount of hydrate crystals in suspensions as surface of boundaries of water-clathrate ice was growing up. This resulted in changing spin-lattice relaxation time T_1 for slow component of longitudinal magnetization recovery. In one

particular example for *E.coli* suspension with xenon hydrates, T_1 doubled compared to the initial spin-lattice relaxation constant of T_1=0.54 s at a cell concentration of 0.23 g dry mass / g H_2O.

According to the data of Ref. [44], the proton spin-lattice relaxation times in carefully purified ice have been measured for the temperature range of 213−273 K. The authors of this work considered a relationship of T_1 with rotational jump time τ_j as follows: T_1=2.14×10^5×τ_j. The dependence of T_1 against inverse temperature has been characterized by single apparent activation energy of $E_a \approx 59$ kJ/mol [44] (or $E_a \approx 56.3$ kJ/mol according to the data published in Ref. [40]) and T_1 in ice was frequently dependent as $\sim \omega_0^2$ [44]. So the measurements of T_1 at different resonance frequencies could also clarify some details in frequently connecting motion of protons in suspensions with xenon hydrates. From the data of Refs. [37, 43] the temperature dependence of T_1 (an Arrhenius plot [21,40] in the range of 278−286 K) for the protons of water molecules displaced among clathrate microcrystals in dispersion gave the apparent activation energy of E_a =26.4±3.7 kJ/mol. NMR data of Ref. [55] presented apparent activation energy for xenon hydrates as E_a =24.7±3.3 kJ/mol.

In order to estimate correlation times τ_c (as characteristics of molecular motions based on NMR relaxation time measurements) in *E.coli* suspensions with xenon clathrates we applied the approach of earlier works [45, 46, 51, 53, 56] assuming the motions involved in intramolecular relaxation and using the expressions reported for $1/T_1$ in terms of the correlation times:

$$\frac{1}{T_1} = \frac{2}{3} C \{ J(\omega_0, \tau_c) + 4J(2\omega_0, \tau_c) \}$$

$$\text{with} \quad J(\omega, \tau_c) = \frac{\tau_c}{1 + \omega^2 \tau_c^2} \qquad (1)$$

where ω_0 is the *Larmor* angular frequency in a constant magnetic field B_0. It is related to the resonance frequency ν_0 by the relation ω_0=2$\pi\nu_0$. C = 2.5×10^{10} s^{-2} is the constant of dipole binding of water molecules.

The use of Eq.(1) can give first estimation of the relaxation behavior of the protons of remaining water molecules displaced among clathrate microcrystals in bacterial suspensions assuming some values of correlation times. According to published data [56−58] T_1 values for the unfrozen water in some biomaterials (e.g., solutions of agarose or lysozyme) indicate that mobility of water remaining in liquid state can be described by a distribution of correlation times with single peak centered at $\tau_c \sim 10^{-9}$ s. For the conditions (temperature, pressure) when xenon still did not form clathrates in suspension each water molecule spends part of its time as isotropic water with single correlation time. The correlation between motion of protons from the water molecules and from macromolecules in principle gives rise to the process of cross-relaxation or spin-diffusion. The observed relaxation rates of water protons can be influenced by cross-relaxation rates. Spin-diffusion occurs by way of mutual exchange of

spin-magnetization between water protons of the hydration water and protons on macromolecules / cell surface. The contribution of cross-relaxation can become very important under the conditions of slow molecular diffusion.

The mobility of water remaining in suspensions after xenon hydrate formation might be comparable with that for unfrozen water in the solutions of biomolecules. This allows a calculation of T_1 at two experimental resonance frequencies (90 and 20 MHz) at the value of τ_c, for instance, of 2.0×10^{-9} s. The results show reasonable difference (3−4 times) in ratio of T_1-values for 90 and 20 MHz although the value $T_1 \approx 28$ ms (at 90 MHz) looks very small in comparison with experimental T_1-values for extracellular water (0.5−1.2 s) in bacterial suspension with xenon clathrates. For changed value of correlation time (one order) to $\tau_c = 2.0 \times 10^{-10}$ s the calculation of T_1 according to Eq.(1) would result in $T_1 = 60-63$ ms (these simulated values are still small in comparison with T_1 from experiments) showing very small difference between simulated T_1 values for 90 and 20 MHz. The estimation for next change to $\tau_c = 2.0 \times 10^{-11}$ s was also unsuccessful to match the experimental meanings of T_1 for two resonance frequencies by the calculated values. This suggests that the motions with correlation times $\tau_c \sim 10^{-9}$ s (or less for one-two orders) are not likely to be main input in bacterial suspensions with xenon clathrates. So the approach with one correlation time based on literature data should be definitely changed to distribution of τ_c to fit the experiments in *E.coli* suspension with clathrates of xenon.

3.2. Factors Defining Mobility of Water among Xenon Clathrates in Bacterial Suspension

The approach of Ref. [56] which studied bound water in frozen erythrocytes by NMR could be probed on bacterial cells adopting the usual formulations of the relationship between relaxation times and correlation times and assuming as well a log-normal distribution of correlation times with distribution function:

$$P(\tau)d\tau = \frac{1}{\sqrt{\beta\pi}} \exp[-(\frac{Z}{\beta})^2]dZ \qquad (2)$$

Here $Z = \ln(\tau/\tau^*)$ and τ^* is the median of the correlation time distribution and β is the distribution width. The authors of Ref. [56] assumed that β is independent of temperature and τ^* is dependent of temperature following to Arrhenius expression with activation energy for motion corresponding to the correlation times. The spin-lattice relaxation rate $1/T_1$ is then [56]:

$$\frac{1}{T_1} = \frac{2}{3}C[\int_0^\infty \frac{\tau P(\tau)d\tau}{1+(\omega_0\tau)^2} + 2\int_0^\infty \frac{2\tau P(\tau)d\tau}{1+(2\omega_0\tau)^2}] \qquad (3)$$

For the evaluation of the relaxation rates it is necessary to estimate the integrals in Eq.(3). In Ref. [56] this was done numerically using the relation [57]:

$$\int_0^\infty \frac{\omega_0\tau P(\tau)d\tau}{1+(\omega_0\tau)^2} = \frac{1}{2\sqrt{\pi}} \int_{-\infty}^\infty \exp(-\mu^2)[\cosh(X+\beta\mu)]^{-1}d\mu \quad (4)$$

for a log-normal distribution where $X = ln(\omega_0 \cdot \tau^*)$.

The approach to fit relaxation data in bacterial suspensions with xenon clathrates is based on certain assumptions (water molecules are undergoing to translational and rotational motions governed by a distribution of correlation times) which allow several adjustable parameters for each correlation time: dipole constant C, β, τ^* and activation energy stemming from temperature dependence of τ^*. In principle these parameters could be determined by a computer fit of the data using an iterative method. In reality, the dipole constant of water, in other words, the rigid-lattice second moment is in the range from 1.5×10^{10} s^{-2} for isolated molecule of water to 2.6×10^{10} s^{-2} for crystals of ice [56]. There is indefinite choice for the constant of dipole binding as the relaxation data on T_1 were obtained for the remaining water in *E.coli* suspensions after clathrate formation experiencing an exchange with solid phase of clathrate ice.

Fitting the data to the model with one log-normal distribution of correlation times gives the values for the most of the parameters as widely varied. The authors of Ref. [56] for the remaining liquid water in frozen red blood cell (RBC) suspension proposed a model which describes NMR relaxation data by a distribution with two peaks. Additional parameters for the fraction of water molecules under first peak and for the fraction of water molecules under second peak were introduced in order to fit the distribution of τ_c. Analysing the published model for RBC suspensions we suppose that for clathrates of xenon in cell suspensions more consistent model could be developed further. Some additional experiments should involve relaxation of xenon atoms and introduction of parameters responsible for the presence of xenon in suspensions. An idea to explain a cell metabolism during/after xenon clathrate formation could be developed to add the details to total pattern of cell changes. An increase of salt concentration in extracellular water at xenon clathrate formation causes shrinking the cells by loss of free water. When the cells compressed the anion and cation flux across the membrane can be controlled and coupled to the cell metabolism involving a regulation of water structure. The remaining intracellular water can be more highly structured resulting in decreased relaxation times (i.e., increased rates $R_1 = 1/T_1$) with cell concentration (Fig.4, Fig.5).

For the future modelling the suspensions with xenon it is necessary also to take into account the changes produced by xenon dissolved in cell suspension. It is possible to consider extracellular water as saline with a concentration 0.9% by weight. According to the data [59] the solubility of xenon in saline water is low, in particular, Ostwald coefficient is equal to 0.0926 (at standard temperature and 0.101 MPa of gas pressure). However the T_1 value of xenon is quite long (66 s, when measured at magnetic field $B_0 = 9.4$ T) due to dipolar couplings with proton spins [59].
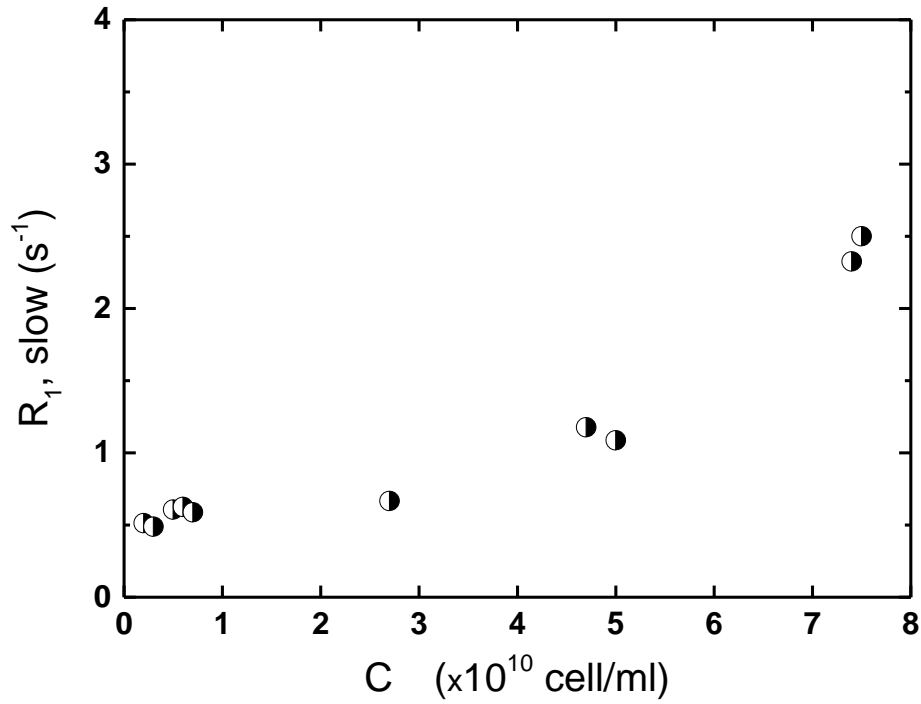
Figure 4. Spin-lattice relaxation rate $R_1 = 1/T_1$ of slow relaxing component of longitudinal magnetization of water protons in liquid phase of *E.coli* suspensions with xenon clathrates as a function of cell concentration. T = 280 K. Pressure is 1.2 MPa. Frequency is 20 MHz
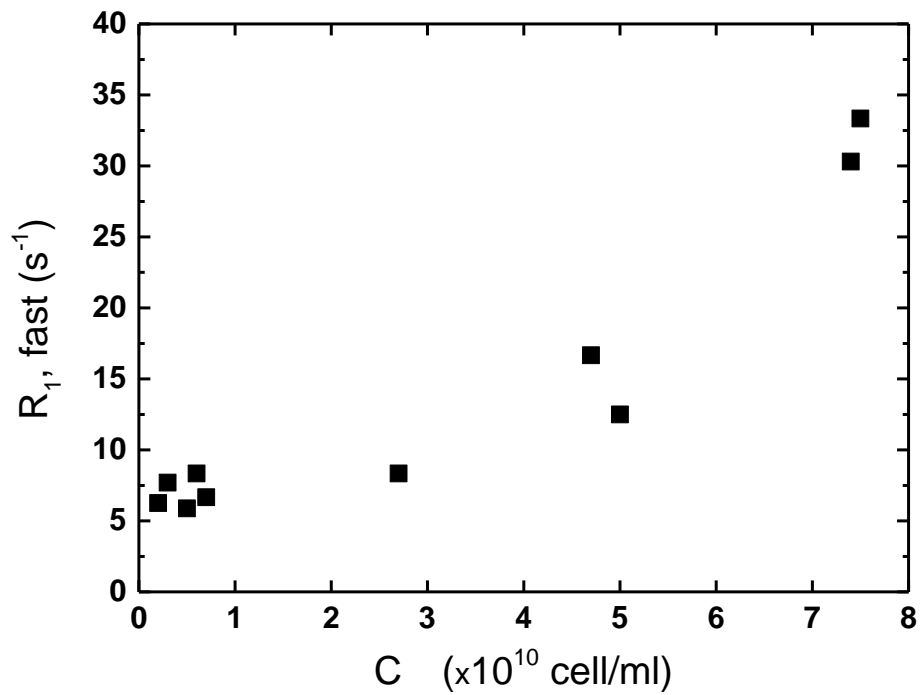
Figure 5. Spin-lattice relaxation rate $R_1 = 1/T_1$ of fast relaxing component of longitudinal magnetization of water protons in liquid phase of *E.coli* suspensions with xenon clathrates as a function of cell concentration. T = 280 K. Pressure is 1.2 MPa. Frequency is 20 MHz

An increase of the cell concentration in *E.coli* suspensions with xenon clathrates resulted in decrease of slow (and fast) components of longitudinal relaxation time T_1 (Figs.4, 5). This could be associated with increasing viscosity because of the concentration of solutes (inside and outside cells) increased. This is also could be considered as a result of increasing exchange between the protons of liquid water and the protons of macromolecular surface structures of cell in the case of spin-diffusion. Last suggestion is rather unlikely as published data indicated that spin-diffusion affects the relaxation of protons at high concentration of proteins and macromolecules [45, 60−62]. A temperature dependence of slow T_1 component in the range of 277−289 K (Arrhenius plot) resulted in apparent activation energy E_a=27.2±2.0 kJ/mol which exceeds E_a-value for pure water (~21 kJ/mol [40]). Probably, a mobility of water molecules in liquid phase of cell suspensions at xenon clathrate formation decreased not only due to increasing cell concentration and compress of cytoplasm but significantly due to dissolution of xenon gas in cells in the amount enough to create separate clathrate cages but not enough to build up a space network of completed clathrate structures. According to the NMR data on [129]Xe dissolved in physiological saline at 0.1 MPa and injected into RBC suspension gas-exchange processes between extra- and intracellular compartments occur on the time scale of a few tens milliseconds [59,63]. The mixing of xenon between physiological saline and RBCs resulted in accumulation of [129]Xe NMR signals inside cells with intensity comparable to that in extracellular compartment for just the period of ~1 s [59]. However an affinity of xenon to build up the clathrate structures in extra- and intracellular spaces at the temperatures above melting point of ice can be strongly depending on increased gas pressure and other conditions in cells and outside cells including changed metabolism (flux of water and ions via membrane), availability of free liquid water and accumulation of xenon in hydrophobic part of membrane.

4. Conclusions

Structural-dynamic changes in bacterial cell properties induced by xenon have been studied by proton spin-lattice relaxation measurements. The inert gas xenon is able to form clathrate hydrates from water molecules at pressure circa 0.4–0.7 MPa and temperature about 276–278 K. An amount of water bound in solid phase of xenon clathrates can be estimated from NMR data. The results clarified how NMR method can monitor a process of clathrate formation and how the xenon atoms and its hydrates interact with cells. The formation of xenon hydrates affected exchange dynamics of water between two compartments and resulted in apparent two T_1-components.The results of T_1-measurements showed that the mobility of intra- and intercellular water can be changed at above melting point of common ice with the aid of xenon hydrates. The ability of xenon hydrates to lower water proton relaxation time inside the cells may reflect their propensity to structure and influence on exchange between extracellular and intracellular water.

Detailed interpretation of correlation times as the characteristics of molecular motions in compartments of bacterial suspension in the presence of clathrate ice is not easy because of the lack of suitable mathematical models. However, the results showed that the surface of cells participates in clathrate formation. It affects the dynamics of the water molecules and determines their correlation times.

The changes in mobility of extracellular water at xenon hydrate formation result in changes in cell properties. Molecular mechanisms of these changes (affecting membrane permeability and activity of proteins and lipids) must be further explored involving different NMR facilities. There are many NMR methods for probing the compartments of heterogeneous, in particular, cellular systems. NMR has a useful role in characterizing the molecular properties of separate spaces of cellular suspensions giving a possibility to probe water, solutes and surfaces non-invasively. This allows keeping the biomaterials under xenon conditions further without distortions.

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