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ORIGINAL PAPER

Delayed luminescence: a novel technique to obtain new insights into water structure

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Abstract Fully understanding the structure of water is a crucial point in biophysics because this liquid is essential in the operation of the engines of life. Many of its amazing anomalies seem to be tailored to support biological processes and, during about a century, several models have been developed to describe the water structuring. In particular, a theory assumes that water is a mixture of domains constituted by two distinct and inter-converting structural species, the low-density water (LDW) and the high-density water (HDW). According to this theory, by using some particular solutes or changing the water temperature, it should be possible to modify the equilibrium between the two species, changing in this way the water behavior in specific biological processes, as in governing the shape and stability of the structures of proteins. In this work, we assess the possibility of obtaining information on the structures induced in water by specific salts or by temperature by measuring the delayed luminescence (DL) of some salt solutions and of water in the super-cooled regime. Previous works have demonstrated that the delayed luminescence of a system is correlated with its dynamic ordered structures. The results show significant DL signals only when the formation of LDW domains is expected. The measurement reveals a similar activation energy for the domains both in aqueous salt solutions and super-cooled

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water. It is worth noting that the time trend of DL signals suggests the existence of structures unusually long-lasting in time, up to the microsecond range.

Keywords Water structures · Aqueous salt solutions · Low-density water · Super-cooled water · Delayed luminescence (DL)

1 Introduction

To our knowledge, water is so special that life cannot exist without it. Water is singular as a liquid because of its ability to form three-dimensional networks of molecules, mutually hydrogen bonded, starting form the basic tetrahedral structure that is central to its structural versatility in the condensed state (solid and liquid). This same extended network also supports proton conduction, a flow of positive electricity that occurs much faster than the diffusion of ions.

The ability of water to dissolve an extensive range of compounds makes it a superbly fit environment to support life. All enzyme reactions take place in water at the surfaces of proteins, and the hydrophobic interaction drives protein folding. According to Klotz [1], in accounting for the behavior of proteins in solution, and in the investigation of the chemical nature and molecular structure of macromolecules to interpret thermal unfolding of proteins, one should consider also the dependence of the structure of water itself on temperature. That is, if one wants to understand protein folding, one probably needs explicit water.

The idea that cell water is distinct from bulk liquid water goes back a long way to pioneers like Gilbert Ling and Albert Szent-Györgyi in the 1960s and 70s. Since the 1970s, many physical and physiological techniques have demonstrated that cell water behaves very differently from bulk water. It is dynamically ordered or oriented, and exhibits restricted motion compared to water in the bulk. Similarly, interfacial water is generally recognized as being restricted in motion, relatively ordered, and having somewhat different properties from water existing in the bulk. Moreover, recent papers [2–4] suggested that hydrophilic surfaces could extend their influence over distances from the interface much larger than one or two layers. Water forms a massive ‘exclusion zone’, where solutes are excluded, next to a hydrophilic surface, up to hundreds of microns thick, that is stable if undisturbed, for days and weeks, once it is formed. Most interestingly, the exclusion zone water was found at the air–water interface and NMR measurements confirm that the layer is associated with decreased mobility (increased ordering) relative to the bulk water, while infrared imaging showed it emitted much less than bulk water, again indicative of increased order [4, 5].

To explain the strange properties of water, it was suggested, firstly by Wilhelm Conrad Röntgen, that liquid water exists in two states. At ordinary temperatures, liquid water consists of rapidly inter-converting low-density water (LDW) clusters where the second neighbor intermolecular O—O distance is 0.45 nm, and compact high-density water (HDW) clusters where the corresponding O—O distance is 0.35 nm. In super-cooled water, LDW clusters predominate, and hence its volume shrinks when heated, as more and more HDW clusters form until 4°C, when practically all the clusters are HDW, and the water is at its maximum density [6].

According to Wiggins [7], dense water is characterized by extremely bent and weak hydrogen bonds with many unbounded molecules. This water should be extremely reactive and its viscosity should also be low. LDW, on the other hand, has many ice-like straight hydrogen bonds; it should be inert and its viscosity should be higher.

The presence of ions causes localized water clusters to be stabilized over their state in the bulk of the solution as they reduce the hydrogen bonding exchanges of the affected water molecules. All environments accessible to a solute are equivalent in single-state water, whereas in two-state water, the solute will partition differently in the two kinds of domains according to its preference for the different domains. There are two classes of solutes, those that prefer LDW and others that prefer HDW. Small, highly hydrated cations tend to accumulate in HDW, while larger, singly charged cations prefer LDW. Highly charged anions also prefer the denser of the two regions, while singly charged anions, which are rather large, accumulate in LDW. The rank order of ions corresponds to the Hofmeister series [8]. Such sorting of ions between regions of different water densities is reminiscent of the more familiar sorting of ions across biological membranes. There is no doubt that membranes have active-transport devices, such as ATP-driven cation pumps, which contribute to the extreme concentration gradients of ions across membranes, but, to some degree, this mechanism of selection of ions by populations of water molecules of differing structure might also contribute to ion sorting [7]. Moreover, populations of LDW may have a role in the hydrophobic interaction, which drives protein folding. Since neutral salts, which stabilize LDW, also stabilize the folded conformation of proteins, it seems probable that the folded protein needs the presence of LDW, and that unfolding follows its destruction [9].

The two classes of solutes also happen to correspond to the traditional distinction between chaotropes that exhibit weaker interactions with water than water with itself and thus interfere little in the hydrogen bonding of the surrounding water, and kosmotropes that exhibit stronger interactions with water molecules than water with itself and therefore are capable of breaking water–water hydrogen bonds. The terms ‘kosmotrope’ (order-maker) and ‘chaotrope’ (disorder-maker) originally denoted solutes that stabilized or destabilized, respectively, proteins, and membranes. Later these terms referred to the apparently correlating property of increasing, or decreasing, respectively, the structuring of water. The solute actions are additive: the destabilizing effect of one solute can be balanced by the stabilizing effect of another; two destabilizing solutes are more destabilizing than each of them separately. The rank order of effectiveness of anions in stabilizing proteins is:

$$\text{citrate} > \text{acetate} > \text{sulphate} > \text{fluoride} > \text{chloride} > \text{bromide} > \text{iodide}.$$

The rank order of effectiveness of cations in stabilizing proteins is:

$$N(CH_3)_4^+ > NH_4^+ > Cs^+ > Rb^+ > K^+ > Na^+ > Li^+ > Ca^{2+} > Mg^{2+}.$$

These rank orders of cations and anions also follow observed patterns of partitioning between LDW and normal water. Anions are increasingly accumulated into LDW, going from left to right. Cations are increasingly excluded from LDW going from left to right.

In practice, of course, properties of anions and cations are never measured singly, but only in neutral combinations. A neutral salt that stabilizes LDW must be one that does not create steep micro-osmotic gradients in either direction. It must be composed of one ion that partitions preferentially into low-density water and one that partitions preferentially into normal water. The overriding requirement of macroscopic electroneutrality then prevents generation of large local water activity gradients in either direction.

Due to the relevant role of water in biological systems, it is interesting to explore new experimental techniques that can enable a deeper observation of water structures.

In this paper, we have explored the possibility of revealing the ordered structure of water by measuring delayed luminescence (DL) of salt solutions in which water structuring is anticipated as well. DL has been successfully used to investigate the role of water in collagen [10, 11] where water appears to be structured in regular chains along the collagen fibrils [12], which would indeed facilitate the proton jump-conduction that could be used by molecules, cells, and tissues to intercommunicate. Such collagen water chains are reminiscent of those seen in carbon nanotubes [13], which are thought to represent an entirely new phase of water.

In a first attempt, we choose to use saturated solutions of sodium salts due to the fact that Na^+ specificity is determined predominantly by the properties of the anion of the neutral salt [9]. In this regard, the effects of the kosmotropic ion SO_4^{2-} and chaotropic ion ClO_4^- have been compared with the fairly neutral ion Cl^-. After the experimental results, which gave good response in the case of sodium perchlorate, $NaClO_4$, which partitions into LDW, a second set of measurements have been performed by using solutions of ammonium salts, more precisely ammonium sulfate $(NH_4)_2SO_4$, which stabilizes LDW, and ammonium nitrate, which has the opposite effect.

Tests have been performed at different dilutions to investigate the role that each salt can have on the number of water molecules it coordinates. Moreover, the effect of the temperature has been considered. There are a number of changes to the structure of water that occur with increasing temperature. The water molecules gain energy, which is used to bend and break the hydrogen bonds. Due to the multiple nature of the hydrogen bonding around water molecules, central molecules in clusters are likely to resume unchanged hydrogen bonding after such breakage, but peripheral molecules will be preferentially lost to other clusters, less structured environments, and interstitial sites. On raising the temperature, the size of ordered clusters decreases, the number of smaller clusters increases, the number of hydrogen bonds decreases, and the average distance between the water molecules increases.

Measurements have also been performed on samples of pure water cooled below its freezing point; without freezing, at ambient pressure, as in super-cooled water LDW clusters predominate.

2 The delayed luminescence phenomenon

2.1 Some experimental results

Several materials, after optical stimulation, exhibit luminescence characterized by low intensity and relatively long duration (up to seconds or minutes). This phenomenon, usually termed delayed luminescence (DL), is presented by solid-state [14] and various biological [15–17] systems. Because experiments performed on several biological systems have shown that DL is a sensitive indicator of their biological state [18–21], a large number of experimental works have been performed to investigate the characteristics and the origin of DL.

DL is, in many aspects, different from the better-known fluorescence, currently used as diagnostic tool in biology. Indeed, DL has intensity 10^3–10^5 times weaker than fluorescence and, where ordinary fluorescence attenuates in an extremely short period of time ranging from picoseconds up to dozen of nanoseconds, DL is a phenomena that attenuates more slowly, over several seconds to several tens of seconds.

The recent literature includes several experimental studies of DL, measured from both solid-state systems and various kinds of living organisms [10, 11, 14–21]. These experiments show a general characteristic of DL: it appears to be dependent on the order parameters of the system.

For instance, concerning the solid-state systems, a strong correlation has been found between the total number of DL photons emitted in a defined time window (DL yield) and the dimension of the grains of cadmium sulfate [14]. In particular, DL yield increases with the grain dimension. Furthermore, a dramatic decrease in DL emission (down to zero in some cases) has been observed in several solid-state systems when their ordered structure disappears. This occurs, for example, when samples of phenyl salicylate (PhS) pass from the solid (crystalline) to the liquid state, and for 4-methoxybenzylidene-4′-*n*-butylaniline (MBBA) on changing from nematic liquid crystals to isotropic liquid state [14].

Analogous behaviors of DL are shown by simple biological systems. For example, the DL yield of the unicellular algae *Acetabularia acetabulum* increases with the process of polymerization of the monomers constituting the cytoskeleton structures (e.g., microtubules) [14]. In biological tissues, like collagen in tendons, the DL emission is strongly related to the degree of hydration of the sample [10] and consequently to the order of the structures. Moreover, in collagen solutions, a sharp change in DL yield is measured when a sol-gel transition occurs [11].

Taken all together, this experimental evidence from very different samples agrees with the hypothesis that DL is strongly correlated with the ordered structures of the systems and cannot be attributed to the excitation and radiative decay of excited states of single molecules or fluorophores.

2.2 A possible theoretical approach

Despite the many recent experimental studies of DL in various materials, there is no consensus regarding the mechanisms by which DL occurs.

A theoretical model has been developed [22] that is able to reproduce the DL data of the algae *Acetabularia acetabulum*, and it has been successfully applied to the collagen system. This model connects DL with the excitation and subsequent decay of non-linear coherent localized electron states (excitons or solitons), whose presence is favored in low-dimensional macromolecules, for example alpha-helical polypeptide proteins, actin filaments, etc. These structures are represented by arrays of parallel quasi-one-dimensional (quasi-1D) polypeptide chains formed by periodically placed peptide groups along the chain of hydrogen bonds. From the point of view of electronic structure, these macromolecules are semiconductor-like quasi-1D systems with a filled valence band and an empty conduction band separated by a gap of finite width. Illuminating the sample populates molecular solitons or 1D polaron-type states, which are present in the forbidden band and, following the mechanism proposed by Davydov, participate in charge and energy transport during metabolic processes. The radiative decay of these excited states is responsible the DL phenomenon.

This model has been recently applied to the collagen system, starting from the experimental results of DL and dielectric permittivity measurements. It seems that soliton excitation may be typical of native biological systems, and change in the type of collective excited state accompanies denaturation, as the loss of water during the drying process, which in turn affects the DL characteristics.

Fundamental for the model is the existence of quasi-1D structures, whose intra-chain and inter-chain bonds are mediated by H-bonds of water molecules directly bonded to the biological structures under study. Water then seems to play a fundamental role in DL phenomena coming from biological systems, and the molecules directly bonded to the 1D structures are of especial importance. Indeed, a sudden change in DL from collagen appears when the molecules of the inner layers of the collagen hierarchical structures of tendons were removed. This view can be framed in phenomenological models, proposed by cell biologists, which suggest that water inside cells has properties differing from water in the bulk state.

3 Use of DL for analysis of water

The strong dependence of DL on the long-range structures and the evident importance played by water-mediated H-bonds in these structures suggest the possibility of using DL as a tool to investigate the properties of water in general and water role in biology in particular.

Some experimental evidence reported in the literature also supports this hypothesis. Indeed, recently dielectric measurements have evidenced a Debye-like slow relaxation process, which was associated with structural and/or dynamical inhomogeneities on a considerably long length scale of the order of 100 μm [23]. The measured relaxation process can be explained by connecting the distorted hydrogen-bonded structures of water to chainlike structures with slow dynamics, where DL processes can take place [24]. Evidence of the presence of such structural polymorphism of water in the presence of ions or in proximity of various hydrophilic surfaces is also reported by spectroscopic measurements. In particular, the presence of a solute-free zone near hydrophilic surfaces, having a width of 100 μm and remaining stable for days, was reported in the literature [2, 3].

One decade ago, experimental evidence for a weak intrinsic luminescence of water was presented in [25], leading to the conclusion that water should be regarded as a continuous polymorphous self-organizing system containing ordered clusters and disordered regions, like a crystal with defects.

Taking into account the evidence presented here, the study of water structuring under particular conditions by using DL measurements seems to be a viable approach.

As discussed in the Introduction, among the several theories proposed to explain the peculiar properties of water, the two-state model describes liquid water as consisting of two microdomains of LDW and HDW clusters, in dynamic equilibrium. The equilibrium point can be shifted by the presence of specific ions in water or by extreme temperature conditions, favoring LDW or HDW domains.

Indeed, the presence of ions causes localized water clusters to be stabilized over their state in the bulk of the solution as they reduce the hydrogen bonding exchanges of the affected water molecules. Different ions, even with the same inner hydration level, would cause different degrees of hydrogen bonding in their surroundings, because ions partition differently between contiguous micro-domains, as previously indicated. This rank order of partitioning recalls the traditional distinction between chaotropes and kosmotropes in the Hofmeister series [8]. So, using different neutral salts it should be possible to stabilize LDW or HDW domains, allowing the selective probing of their properties.

Another method that can be used to induce formation of LDW or HDW domains is by varying temperature. Prevalence of LDW structures is foreseen by theoretical calculation when the liquid water is cooled down to the super-cooled regime.

Following all these considerations, DL measurements on aqueous salt solutions and pure water were performed, in order to investigate possible water structuring triggered by ion presence or temperature effects. To choose the salt solution, we started from the consideration that effects of anions are more pronounced than those of cations. Consequently, sodium ions, having a central position in the Hofmeister series, were coupled with anions having extreme and also central positions in the rank order. Thus Na_2SO_4, NaCl, and $NaClO_4$ were selected for their increasing capacity to favor LDW domains formation. Moreover, two ammonium salts were studied, NH_4NO_3 and $(NH_4)_2SO_4$, as the first one should demolish LDW domains owing to the competitive behavior of cation and anion, while the second one should stabilize LDW [9].

4 Materials and methods

4.1 Salt solutions

The salt solutions were prepared in bidistilled water (Carlo Erba) using Na_2SO_4 by Fluka Biochemika, NaCl and $(NH_4)_2SO_4$ by J.T. Baker, $NaClO_4$ and NH_4NO_3 by Sigma-Aldrich. Firstly, saturated solutions were prepared, and then dilutions were obtained by adding the appropriate amount of saturated solution to prefixed volumes of bidistilled water. DL was measured first in aqueous solutions at 9:10 dilution of the saturated solutions at a constant temperature of 20°C. Three sets of different solutions were prepared by different operators to avoid systematic errors. For $NaClO_4$ and $(NH_4)_2SO_4$ salt solutions, different dilutions where performed in order to measure DL as a function of the salt concentration. For two fixed dilutions, behavior on changing temperature was investigated.

4.2 Super-cooled water

Bidistilled water was placed inside the holder used for DL measurements 12 h before the experiment. The holder was completely filled by 177 ml of water, and it was partially submerged in a thermal bath (Haake C25) set at a temperature of 25°C. The temperature inside the holder was measured with a Pt100 temperature sensor (TP472 I.0; type diving; field of use −50°C to +400°C, accuracy ±0.25°C) connected with a thermometer RTD HD2107.1 produced by Delta OHM.

Before the experiment, the thermal bath was brought to the desired temperature. Each measurement of delayed luminescence begins 20 min after the read temperature in bidistilled water has reached the set value. Each set of measurements was performed proceeding from the highest temperature to lowest and then from lowest to highest.

4.3 Delayed luminescence

DL was measured in the time interval from 10 μs to 1 s using an improved version of the ARETUSA setup [26]. A schema of the experimental setup is given in Fig. 1. A high-intensity pulsed nitrogen laser (Laserphotonic LN203C, $\lambda = 337$ nm, pulse width 5 ns, energy per pulse 120 ± 5 μJ) was used to illuminate the sample via a bifurcated optical fiber bundle (Oriel LLB-321). After switching off the laser pulse, the DL signals were collected by the same fiber and revealed by a photomultiplier (Hamamatsu R-7206-1) in

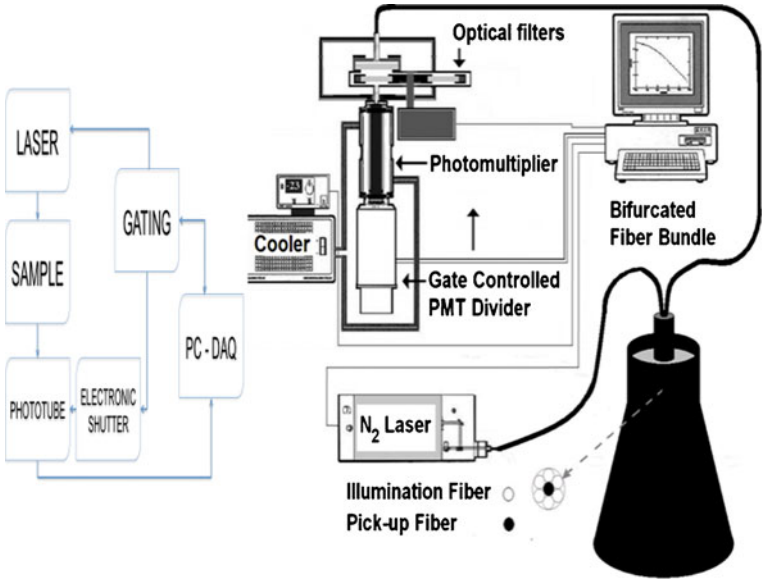

Fig. 1 Schema of the experimental setup used for DL measurements

single photon regime. The photomultiplier was inhibited during the sample illumination by using an expressly designed electronic shutter. A PC implemented with a multi-channel scaler (Ortec MCS PCI) plug-in card was devoted to data acquisition.

The samples were placed in a special holder specifically designed for each kind of sample. The geometry of the holders and the parameters used during the different samples measurements were optimized to reduce as much as possible the background noise.

More precisely, for the measurements on aqueous salt solutions, as the light absorption coefficient of water at 337 nm is very low ($<10^{-4}$ cm^{-1}), a large volume of solution (330 ml) was illuminated. One hundred measurements of the DL signal were performed for each sample. The sample was kept in a black painted conical holder with a cylindrical neck especially designed to reduce the background signal. The holder was placed in a thermal bath in order to perform the DL measurement at different temperatures. The bifurcated fiber ending was immersed into the solution at the fixed distance of 3 mm from solution–air interface. In these conditions, the DL of bidistilled water is hardly distinguishable from thermal noise of the photomultiplier. Due to the low signal (see below), no reliable spectral analysis of DL signal was performed, so DL data refer to photons revealed in the wavelength range from 350 nm up to 800 nm corresponding to the sensitivity range of the phototube.

To perform measurements on pure water on varying the temperature, the water was placed in a cylindrical stainless-steel beaker (radius 25 mm, a height 90 mm, and a thickness 5 mm) and kept in a thermal bath. The head of the bifurcated optical fiber used for DL measurements is immersed in water for 5 mm, and is tilted at 45° with respect to the lateral surface of the cylinder to avoid the reflected light and to improve the signal-to-noise ratio.

5 DL measurement of aqueous salt solutions and pure water

DL measurements were first performed on aqueous salt solutions at dilution of 9:10 with respect to the saturated ones. Saturated samples were not used for measurements, in order to avoid the possible influence of accidental formation of micro-crystals.

Using the setup with the black holder sketched in Fig. 1, the pure water signal was hardly distinguishable from the background. The total number of counts collected in the time interval explored in the measurement for the different salt solutions after background subtraction is reported in Fig. 2. The background signal is also reported. The error bars in the figure show the standard deviation of repeated measurements. It appears that only for the solutions of $NaClO_4$ and $(NH_4)_2SO_4$, DL measurements gave a signal well above the pure water one. For those solutions, the prevalence of LDW domains over HDW domains was predicted by theory.

For the two salt solutions having high DL signals, further measurements were performed by varying the dilution at ambient temperature and by varying the temperature for a fixed dilution.

In Fig. 3, the temporal trends measured for solutions of $NaClO_4$ (Fig. 3a) and $(NH_4)_2SO_4$ (Fig. 3b) at different dilutions are shown. For comparison, the time trend of pure water is shown in the figure. As expected, for both the salt solutions, the DL signal decreases with decreasing the salt concentration, while the slope of the time decay trend is almost the same for the different dilutions. Moreover, in both cases DL signals are surprisingly prolonged in time, and a well detectable signal is still present 100 ms, for $NaClO_4$, and 10 ms, for $(NH_4)_2SO_4$, after switching off the illumination source (Fig. 3). These trends suggest the existence of structures in water having very long-lasting lifetimes. A detailed analysis of the time trends was performed for the 9:10 dilutions. This analysis, presented in detail in [27], results in a distribution probability of the lifetimes of these structures having a maximum in the microsecond range for both the solutions.

However, the action of the two salts on promoting the LDW domain formation is different, as stressed in Fig. 4, where the total number of counts per mole of solute in the two solutions is reported as a function of the moles of water per mole of solute. The different slope of the points for the two solutions indicates the different behavior of the

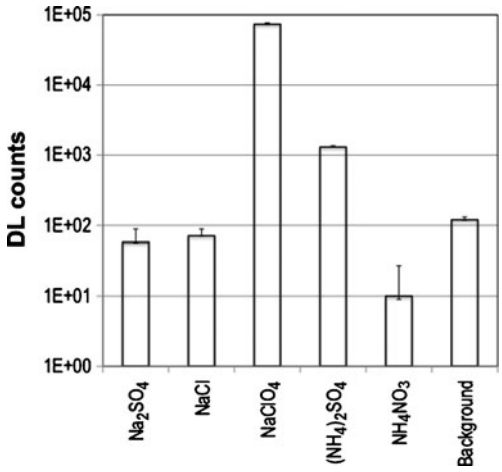

Fig. 2 DL counts (average value and standard deviation of different measurements) after background subtraction, emitted in the time interval 10 μs − 1 s by five different salt solutions at dilution 9:10 starting from saturation. The background signal is also reported for comparison

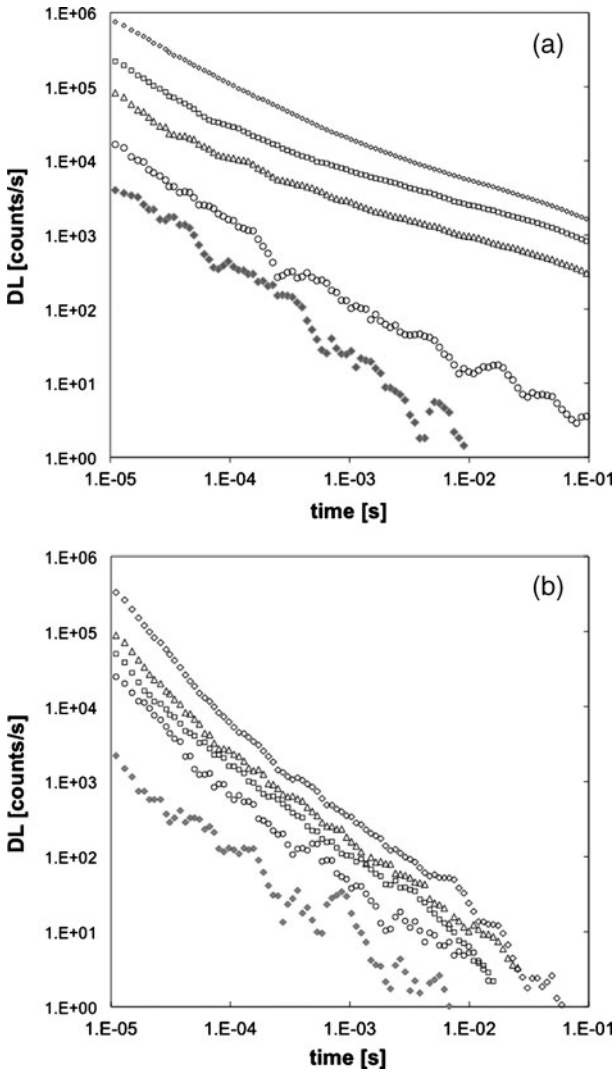

Fig. 3 Time trend of the DL signals for the solutions of $NaClO_4$ (**a**) and $(NH_4)_2SO_4$ (**b**) at different values of dilution. **a** (◇) solution at 9:10, (□) 7.5:10, (△) 5:10, (○) 0.5:10, in **b** (◇) solution at 9:10, (△) 5:10, (□) 1:10 (○) 0.5:10. The time trend measured for water is also reported for comparison (solid triangles)

two salts. In particular, the $(NH_4)_2SO_4$ salt has an anti-cooperative effect on LDW domain formation, i.e., on increasing the moles of solute per moles of water up to a certain limit the number and/or the size of the ordered water domains responsible for the DL signals decreases. On the contrary, the $NaClO_4$ salt presents a strong cooperative action in the water domain formation. This result supports the idea that ions influence the water structuring [28], in contrast with the claim that salts have little or no impact on water hydrogen bonding [29]. The difference in DL behavior between the two salts could reflect the strong variation in thermodynamic parameters as evaluated on analogous salt solutions [26], suggesting different mechanisms in water structuring.

For the two salt solutions, DL was measured for fixed dilution on varying the temperature from 18°C up to 50°C. In particular, in order to prevent saturation at the lower temperatures and to have a good signal-to-noise ratio, the solutions were diluted at 4:10 for $NaClO_4$ and

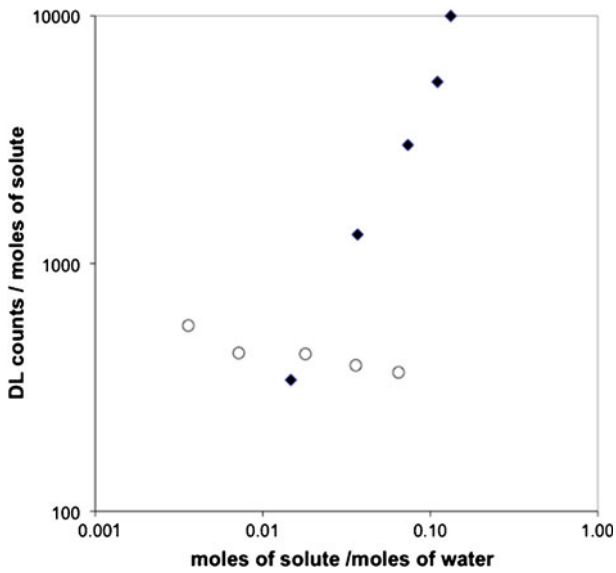

Fig. 4 Total number of photons DL counts emitted in the time interval 10 μs − 1 s, normalized to the moles of solute, as a function of salt concentration for $(NH_4)_2SO_4$ (♦) and $NaClO_4$ (○), expressed as ratio between the moles of water and of solute

at 5:10 for $(NH_4)_2SO_4$ with respect to the saturated solution. The different dilution for the two salts allows us to obtain the same ratio of water to salt molecules. Even in this case, the temporal trends of DL counts do not change with temperature for both solutions, while the total number of counts decreases as a function of temperature following an Arrhenius trend. The temporal trends of the DL signals measured for two temperatures and for the two salt solutions are reported in Fig. 5a for $NaClO_4$ and in Fig. 5b for $(NH_4)_2SO_4$.

To give deeper insight into the present results, measurements on pure water in supercooled conditions where LDW domain prevalence was foreseen have been performed. To this aim, improvements of the experimental apparatus were carried out, as described in Section 2, so that the pure water signal was well above background. The DL was measured varying the temperature from 18°C down to −6°C and the time trends for these two temperatures are shown in Fig. 6. Even in this case, we found an increment of DL signals when an increased presence of LDW is expected. Also in this case, the total number of photons emitted (N_{tot}) for the data collected below 4°C shows an Arrhenius trend.

Figure 7 reports the logarithm of the total number of counts N_{tot} as a function of the inverse of the temperature for the sample specified above. The set of data relative to the same sample has been rescaled by a constant value, which corresponds, in the semi-log plot, to translating the whole date set upwards or downwards. It appears that the data relative to the three different samples, pure water, $(NH_4)_2SO_4$ and $NaClO_4$ follow to the same linear trend ($R^2 > 0.98$), reported in Fig. 7 as a solid lines. This means that the temperature process analyzed is characterized by the same activation energy $\Delta E = 12.7 \pm 0.4$ kJ mol^{-1}, evaluated from the slope. This energy can be interpreted as the specific amount of energy required to displace the LDW/HDW equilibrium.

All the presented results regarding the measurements performed on aqueous salt solutions can be interpreted in the framework of the two-state model. Indeed, a clear DL signal is present only for the salt solutions where the prevalence of LDW domains is predicted. The temporal trend of the DL signals is different for the two salt solutions, evidencing

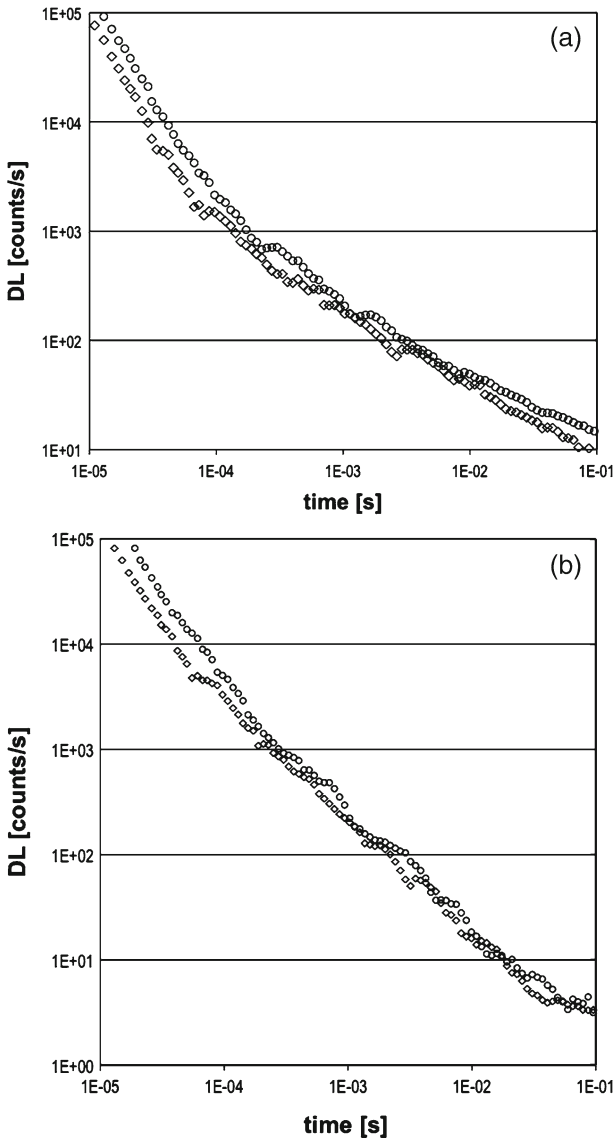

Fig. 5 Time trend of the DL signals from the salt solutions at different temperatures: **a** $NaClO_4$, (◊) 49°C and (○) 23.5°C; **b** $(NH_4)_2SO_4$, (◊) 49°C and (○) 21°C

the specificity of the action of the different salts. In particular, the analysis of DL for different dilution of the two salt solutions shows a cooperative effect of $NaClO_4$ and an anti-cooperative effect of $(NH_4)_2SO_4$ in LDW domain formation. However, once formed, the water structures seem to have analogous characteristics for the two salt solutions. Indeed, in both cases, the distribution of the lifetime of these structures has a maximum in the microsecond range. Moreover, the same energy is required to promote the displacement of the LDW/HDW equilibrium. While the found activation energy value is comparable with the energy values present in the literature (see for example the calculations of the virtual single-particle energy distribution of water hydrogen bonding, giving the values 4.2 and

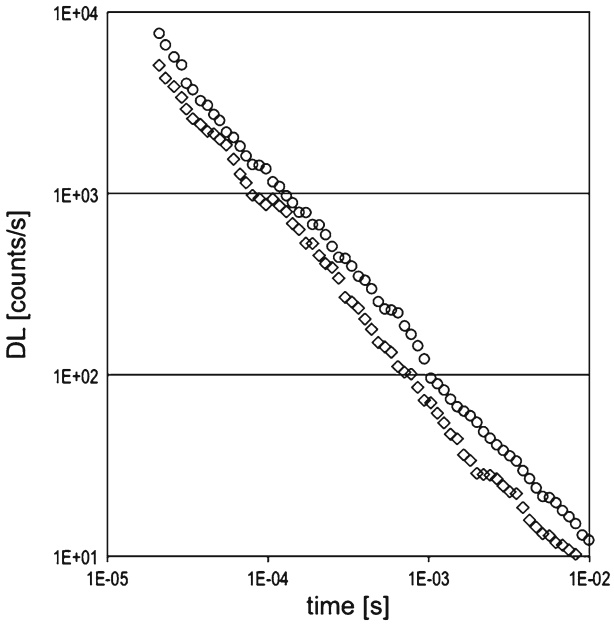

Fig. 6 Time trend of the DL signals from pure water at different temperatures: (◇) 18°C, (○) −6°C

13 kJ mol^{-1} [30], or more simply the energy values required for breaking and completely separating a hydrogen bond, about 23 kJ mol^{-1}), the result regarding the lifetime probability distribution of the water structures is surprisingly higher than previously thought. This long-lasting lifetime of the order of microseconds, as found by DL analysis, leads us to think that water structuring could be used in biological processes as a tool to store and transfer energy.

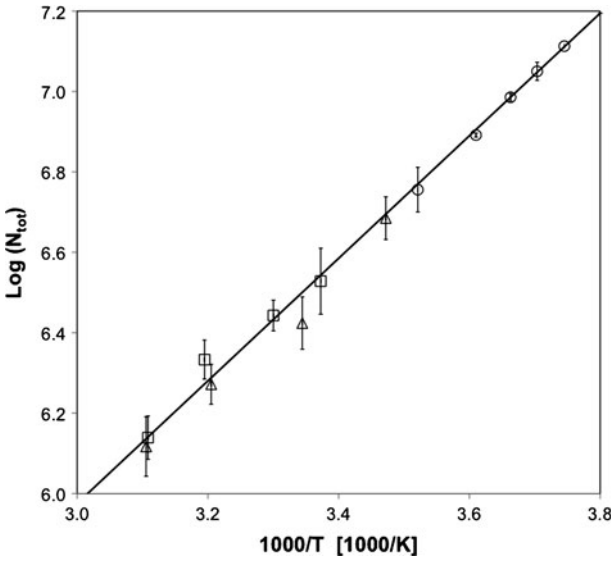

Fig. 7 Arrhenius plot of the total number of emitted photons N_{tot}. (□) $NaClO_4$ aqueous salt solution at dilution 4:10 from saturation, (Δ) $(NH_4)_2SO_4$ aqueous salt solution at dilution 5:10 from saturation, (○) bidistilled water. *Solid line* represents fit according to an Arrhenius trend. *Bars* denote standard deviation from average values

6 Conclusions

The data presented in this paper on the DL of water underline the possibility that electronic collective states could exist in water. These states appear when the two-state theory of water foresees an enhancement of LDW domains respect to HDW ones. In the present work, the LDW/HDW equilibrium was displaced in favor of LDW domains by using specific neutral salt solutions and by cooling pure water down to the supercooled regime. The temperature analysis performed reveals that the same activation energy is necessary, in the studied cases, to change the equilibrium between the two kinds of domain. The calculated values for this energy (12.7 ± 0.4 kJ mol^{-1}) are comparable with the energy values present in the literature, as that obtained from the calculations of the virtual single-particle energy distribution of water hydrogen bonding, which gives the values 4.2 and 13 kJ mol^{-1}. This fact further connects the measured DL to the dynamic structuring of water. Especially important is the fact that the lifetime probability distribution of these water structures is surprisingly higher than previously thought, showing a maximum in the microseconds range. This long-lasting lifetime of water structures, evidenced from DL measurements for the first time, opens new horizons in the role played by water in biology, as water structuring could be used to store and transfer energy and information inside the biological systems.

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