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Microscopic diffusion in hydrated encysted eggs of brine shrimp☆

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ABSTRACT

Background: We have studied microscopic diffusion of water in fully hydrated encysted eggs of brine shrimp (*Artemia*).
Methods: We have utilized quasielastic neutron scattering.
Results: Dry eggs of brine shrimp were rehydrated using (1) water without additives, (2) eutectic mixture of water and dimethyl sulfoxide, and (3) a concentrated aqueous solution of lithium chloride. Despite the complexity of the hydrated multicellular organism, measurable microscopic diffusivity of water is rather well defined. Pure hydration water in eggs exhibits freezing temperature depression, whereas hydration water in eggs mixed with dimethyl sulfoxide or lithium chloride does not crystallize at all.
Conclusions: The characteristic size of the voids occupied by water or aqueous solvents in hydrated brine shrimp eggs is between 2 and 10 nm. Those voids are accessible to co-solvents such as dimethyl sulfoxide and lithium chloride. There is no evidence of intracellular water in the hydrated eggs.
General significance: The lack of intracellular water in the fully hydrated (but still under arrested development) state must be linked to the unique resilience against adverse environmental factors documented not only for the anhydrous, but also hydrated encysted eggs of brine shrimp.

1. Introduction

Depending on the environmental conditions, females of brine shrimp (genus *Artemia*) produce either free swimming nauplii, or embryos at gastrula stage. In the latter case, the embryos enclosed in a protective shell are known as cysts. The encysted embryos enter diapause, which is a state of greatly reduced metabolism. Possible role of sugars, lipids, and stress resistant proteins in diapause of *Artemia* eggs has been recognized [1]. What follows diapause depends on the environmental conditions at the time of the diapause termination. Under favorable conditions embryo development resumes, whereas under environmental stress cryptobiotic dormancy (quiescence) follows. Diapause can be terminated, e.g., by dehydration, but dried *Artemia* eggs in the state of quiescence can eventually resume their development under favorable conditions of appropriate hydration, temperature, and salinity.

Artemia occupies a truly special place among multicellular organisms that may undergo cryptobiosis at some development stage [2,3]. While anhydrobiosis in dried encysted brine shrimp eggs has been widely recognized and is important in commercial aquaculture, it is anoxybiosis in hydrated eggs that is rather unique. Hydrated eggs could survive years of complete anoxia, apparently slowing down their metabolism to the values too low to be measured experimentally [4,5].

The kinetics of hydration-dehydration in brine shrimp eggs has been studied experimentally [6], but, to our knowledge, the microscopic dynamics of water in *Artemia* embryos has not been investigated. Such dynamics, primarily of diffusive character, should be indicative of the state of hydration water, which is likely important in molecular transport and metabolic properties at the cellular and intra-cellular level. Here we report a study of microscopic dynamics of aqueous solvents (water with and without co-solvents) in *Artemia* eggs using quasielastic neutron scattering (QENS).

The choice of QENS as a suitable probe of water dynamics in a multicellular organism is far from intuitive. The incoherent neutron scattering cross-section of hydrogen exceeds the scattering cross-sections of most other elements, including deuterium, at least by a factor of 40, making QENS a technique of choice for studies of confined water, but usually in rather simple matrices. Unlike NMR, which can be sensitive to the atomic positions as manifested through the specific chemical shift, QENS measures hydrogen-dominated scattering signal

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averaged over the entire sample. This lack of selectivity is balanced in part by the sensitivity of QENS not only to the energy, but also momentum transfer, which advantageously allows analysis of time-spatial characteristics of molecular-level diffusion processes. A variation of QENS technique, temperature-dependent elastic neutron scattering with zero energy transfer, gives information similar to that provided by differential scanning calorimetry, but often with enhanced sensitivity to the confined water dynamics and phase state, due to the high scattering cross-section of hydrogen. QENS studies of water in the living cells [7–12] gradually gain acceptance, but feasibility of QENS for probing entire multicellular organisms remains to be explored. Here we attempt such a study with encouraging results that shed a light on the state of water in hydrated *Artemia* embryos.

2. Materials and methods

2.1. Samples

Artemia eggs were purchased from Carolina Biological Supply Co. The as received eggs were in quiescent dry state, yet they exhibited further water loss of *ca.* 8 wt% in a test when placed in a 10^{-3} mbar vacuum at ambient temperature for 24 h. The as received eggs were hydrated using (1) deionized water, (2) an aqueous solution of deuterated dimethyl sulfoxide (DMSO) of eutectic composition [13], $(H_2O)_{0.67}(C_2D_6OS)_{0.33}$, and (3) an aqueous solution of lithium chloride, $(H_2O)_{0.86}(LiCl)_{0.14}$. For brevity, we may refer to these aqueous solvents as water-DMSO and water-LiCl. As described in the next sections, we have found that quasielastic scattering signal from the hydrated eggs was dominated by scattering from the hydration water, more specifically, the incoherent scattering by protons of the water molecules; hence, we probed single-particle water diffusion dynamics in the samples (besides the scattering signal from the eggs themselves, which was predominantly elastic). Dimethyl sulfoxide is a common cryoprotector utilized to prevent freezing and thawing damage to living systems [14,15], which is known for easy penetration through various tissues. Aqueous solutions of lithium chloride are remarkably close to pure water in their glass-forming properties [16], but allow bypassing [17–19] homogenous nucleation temperature of water to enable low-temperature studies of bulk-like aqueous solutions of biomolecules without resorting to nanoscopic confinement [20–22]. The slightly off-eutectic composition of $(H_2O)_6(LiCl)$ was chosen for its superior glass-forming properties and nanoscopic homogeneity [23]. Although hydration from water vapor could be done in a more controlled manner while monitoring the weight uptake, such a vapor hydration protocol would be impossible to replicate with aqueous solutions as solvents. Therefore, *Artemia* eggs were instead fully hydrated by submerging into the respective aqueous solvent at ambient temperature for 24 h following by drying in open air for 96 h. Exposure to an aqueous solvent not containing sodium chloride is not expected to terminate quiescence. Following air drying, eggs exposed to pure water looked morphologically similar to the as received dry eggs, having appearance of dry powder. They showed water loss of *ca.* 56 wt% in a test when placed in a 10^{-3} mbar vacuum at ambient temperature for 24 h, in agreement with earlier studies [6]. Eggs exposed to water-DMSO and water-LiCl had appearance of a powder (not a paste or a slurry), but retained some minimal residual dampness even at the completion of the air drying, which precluded meaningful water loss measurements. Four powder-like samples (as received and hydrated with H_2O, $(H_2O)_{0.67}(C_2D_6OS)_{0.33}$, and $(H_2O)_{0.86}(LiCl)_{0.14}$ were loaded in flat-plate aluminum sample holders (30 mm wide, 50 mm tall, and 1 mm thick) vacuum-sealed with indium gaskets. The mass of each of the four samples was 1 g. Three reference samples of liquid aqueous solvents, H_2O, $(H_2O)_{0.67}(C_2D_6OS)_{0.33}$, and $(H_2O)_{0.86}(LiCl)_{0.14}$) were loaded in similar indium-sealed flat-plate aluminum sample holders, but only 0.1 mm thick, to minimize multiple neutron scattering effects. Besides, we used a 1 mm thick sample holder to measure a control D_2O-loaded sample prepared as follows: 0.44 g of eggs was loaded with 0.50 g of D_2O, to match the hydration level of the H_2O-loaded sample, which had 0.44 g of eggs and 0.56 g of H_2O. Prior to hydration with D_2O, the eggs were rinsed with D_2O and dried three times to remove labile protons, which otherwise could contaminate the D_2O solvent in the finally measured sample.

2.2. Neutron scattering experiment

Quasielastic and elastic neutron scattering measurements were carried out on a backscattering spectrometer BASIS [24], which has an energy resolution of 3.4 μeV (full-width at half-maximum for the *Q*-averaged resolution value). That is, microscopic dynamics much slower than *ca.* 0.4 ns would not be measurable on BASIS (the corresponding scattering signal would appear completely elastic). With the chosen incident neutron bandwidth center of 6.15 Å and neutron bandwidth choppers frequency of 30 Hz, the dynamic range of accessible neutron energy transfer of -100 μeV to $+500$ μeV could be utilized. The incident neutron beam size is 30 mm by 30 mm. The flat plate sample holders were positioned normal to the incident beam direction. QENS data were collected at 300 K. The elastic intensity temperature scan data were collected from the as received eggs and the three samples hydrated with protonated solvents on cooling down with a ramp rate of 1 K/min and were used to calculate the atomic mean-squared displacement (MSD), $< u^2(T) >$, using a Gaussian approximation, $I_{elastic}(Q,T) = I_{elastic}(Q,T_0)\exp(-Q^2 < u^2(T) > /3)$, where T_0 is the lowest measurement temperature, over the range of $0.2\ Å^{-1} < Q < 1.0\ Å^{-1}$. Additional QENS data were collected at the baseline temperature of 10 K at the completion of the measurement in order to obtain the resolution function under conditions when the measurable dynamics has ceased and the scattering signal has become purely elastic. Besides, the reference liquid solvent samples were measured at 300 K in the same sample geometry for comparison with the hydrated *Artemia* eggs samples.

3. Results

The temperature dependence of the mean-squared atomic displacement measured in *Artemia* eggs is presented in Fig. 1. The data is characteristic of confined aqueous solutions [25]. At sufficiently low temperatures, where there is no measurable diffusion mobility, the temperature dependence of the elastic scattering intensity is controlled

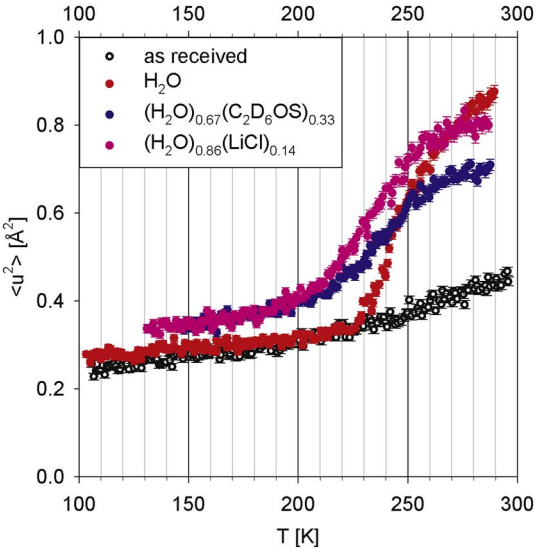

Fig. 1. Mean-squared atomic displacement of hydrogen atoms in brine shrimp eggs, dry (as received) and hydrated with protonated aqueous solvents, as measured by elastic neutron scattering.