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A first test of the hypothesis of biogenic magnetite-based heterogeneous ice-crystal nucleation in cryopreservation

Atsuko Kobayashi [a, *], Harry N. Golash [b], Joseph L. Kirschvink [a, c]

[a] Earth-Life Science Institute, Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan
[b] Division of Mechanical Engineering, California Institute of Technology, Pasadena, CA 91125, USA
[c] Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA

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ABSTRACT

An outstanding biophysical puzzle is focused on the apparent ability of weak, extremely low-frequency oscillating magnetic fields to enhance cryopreservation of many biological tissues. A recent theory holds that these weak magnetic fields could be inhibiting ice-crystal nucleation on the nanocrystals of biological magnetite (Fe_3O_4, an inverse cubic spinel) that are present in many plant and animal tissues by causing them to oscillate. In this theory, magnetically-induced mechanical oscillations disrupt the ability of water molecules to nucleate on the surface of the magnetite nanocrystals. However, the ability of the magnetite crystal lattice to serve as a template for heterogeneous ice crystal nucleation is as yet unknown, particularly for particles in the 10–100 nm size range. Here we report that the addition of trace-amounts of finely-dispersed magnetite into ultrapure water samples reduces strongly the incidence of supercooling, as measured in experiments conducted using a controlled freezing apparatus with multiple thermocouples. SQUID magnetometry was used to quantify nanogram levels of magnetite in the water samples. We also report a relationship between the volume change of ice, and the degree of supercooling, that may indicate lower degassing during the crystallization of supercooled water. In addition to supporting the role of ice-crystal nucleation by biogenic magnetite in many tissues, magnetite nanocrystals could provide inexpensive, non-toxic, and non-pathogenic ice nucleating agents needed in a variety of industrial processes, as well as influencing the dynamics of ice crystal nucleation in many natural environments.

1. Introduction

An ability to freeze biological tissues without causing ultrastructural damage has been a concern of the Electron Microscopy (EM) community for many years. Extensive work by transmission and scanning (TEM/SEM) electron microscopists has shown that freezing rates of ~10,000 °C/s are needed to prevent cellular damage [6], and this can only be achieved for very thin tissue layers at atmospheric pressure. Using high-pressure techniques that move a sample at room temperature into the ice-stability field, followed by cooling, still only allows a tissue thickness of up to 0.6 mm to be processed.

Conversion of a liquid to a solid during freezing requires structural ordering at the molecular level, which is often inhibited unless seed nuclei or epitaxial surfaces are present to help initiate the crystallization process. Retention of the liquid state at temperatures below the melting point is called *supercooling*, and is a dynamically unstable state in many liquids due to the chance probability of an initial seed crystal nucleating. The rapid cooling techniques for thin samples noted above work by forcing the water to supercool faster than damaging ice crystals are able to nucleate. Supercooling is actually a general phenomenon: standardized tables of physical properties of various compounds will list the melting temperature of a substance, rather than its freezing temperature. Melting is the disruption of a pre-existing, ordered atomic lattice, which occurs when the thermal background energy is high enough to disrupt the atomic ordering of a crystal lattice; this happens at temperatures that are far more reproducible than the 'freezing' temperature.

Supercooling is easily achieved in purified bottled water that has been packaged in amorphous PET [polyethylene terephtalate] bottles, and makes for many impressive video demonstrations that are available on-line. Molecular dynamic studies of the

* Corresponding author.
E-mail address: kobayashi.a.an@m.titech.ac.jp (A. Kobayashi).

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crystallization of ice-I_h (hexagonal ice polymorph I) suggest that upwards of 250–300 discrete water molecules need to assume a transient long-range ordering in the supercooled state in order to initiate crystal nucleation [35]. Once this low-probability, stochastic nucleation is initiated anywhere in the container, it will spread rapidly until the large latent heat of crystallization of water (~80 calories/g) brings the bulk temperature of the crystallizing mush back up to the melting temperature (0 °C), producing a characteristic step function in the time-temperature profile of supercooled water; an example is shown here in Fig. 1A. Subsequent cooling has little additional effect on the temperature curve, until most of the liquid water that has buffered the material at the freezing point has been incorporated into the growing volume of ice. In contrast, water that contains particles capable of rapidly nucleating ice crystals will not show a pronounced supercooling effect, resulting in more conventional cooling curves like those shown in Fig. 1 B & C.

In nature, ice crystals are thought to nucleate on small dust particles made of a variety of common mineral types that are distributed by æolian and fluvial processes, and are present in most natural freshwater and marine bodies of water, as well as forming the dominant aerosol component in the atmosphere. Most of the research has been focused on common minerals found in æolian dust, as well as condensed organic materials. On the other hand, Atkinson et al. [1] have found that the potassium-rich feldspars provide better ice nucleation sites than the other minerals. In any event, mineral dusts that serve as ice nucleation particles may play a significant role in the dynamics of atmospheric convection and radiation, particularly in view of the large latent heat of crystallization.

With these properties in mind, it is important to mention that ice damage to animal and plant membranes during intracellular freezing is a major obstacle to the use of cryopreservation. The damage is two-fold: the ~10% expansion as water freezes changes

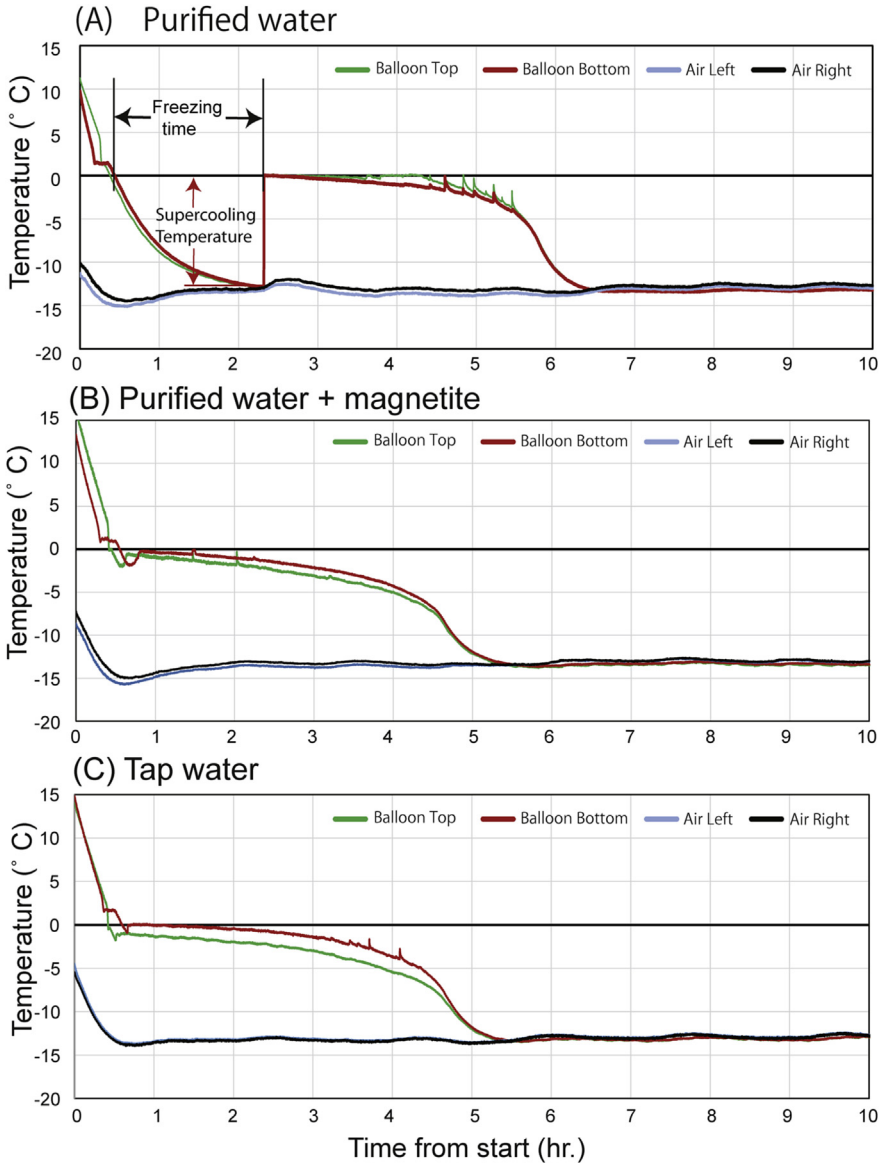

Fig. 1. Examples of time-temperature curves for standard balloon samples, as monitored by the thermocouples placed near the top and bottom of the balloons, and at two points in the cooling chamber. A. Typical example of purified water showing supercooling. Labels show how and where the freezing times and lowest supercooling temperatures were measured from the bottom sensor data, as entered in Table 1. B. Typical example of purified water spiked with ~70 ng/g of standard magnetite powder. C. Cooling curve of laboratory tap water.