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Freezing-driven ionic charge imbalance leads to pore formation and osmotic injury of lipid membranes

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ABSTRACT

In this work, we reveal the fundamental mechanism controlling osmotic injury of lipid membranes under low-temperature preservation using an in-vitro membrane system under freezing temperature and the molecular dynamic simulations. The freezing-driven ionic charge imbalance is the major factor affecting the membrane conformation and causing the osmotic injury. Under freezing temperature, the ionic charge imbalance, originating from the preferential incorporation of anions into the growing ice crystals, results in membrane poration with the directional penetration of ion molecules. Subsequently, the osmotic efflux of water molecules through the pore causes cell dehydration, eventually leading to the lethal osmotic injury of lipid membranes during freezing. Moreover, we find a stark difference in tolerance to freezing and the times required for pore formation in membranes with different lipid compositions. Membranes enriched with cholesterol and anionic lipids exhibit increased resistance to freezing-induced osmotic injury, as the addition of cholesterol and anionic lipids in membranes delays the pore formation under freezing temperature. These findings advance in depth the molecular-level understanding of freezing injury on lipid membranes and provide an opportunity to develop an alternative strategy to protect diverse cells during preservation at subzero temperatures by regulating the composition of lipid membranes.

1. Introduction

The protection of cells has been a major challenge in cryopreservation. In most cases, cooling the biological samples below the freezing temperature inevitably results in the formation of ice crystals. Subsequently, ice crystals grow into larger crystals at the expense of smaller ones, leading to mechanical damage to cells. Numerous endeavors have been focused on pursuing antifreeze materials such as glycoproteins [1,2], polymers [3–5], supramolecules [6–8], carbon materials [9–11], and metal-based nanoparticles [12,13] to inhibit ice growth for protecting cells. However, the discrepancy between the antifreeze activity and cryoprotective performance of materials indicates that other lethal events besides growing ice crystals can inflict damage to cells. Cells experience damage in a cryopreservation cycle due to (i) the osmotic intolerance on lipid membranes [14], (ii) solute and ion effects during freezing [15], (iii) intracellular ice formation [6,16], and (iv) chilling or cold-shock injury during freezing [17], which are not fully recognized. A fundamental understanding of these mechanisms on lipid membranes becomes critical in the development of the cryoprotective agent and implementation of optimized cryopreservation protocols.

As ice forms and grows under the freezing temperature, salt ions and solutes previously dispersed in the bulk solution are excluded from the growing ice crystals and concentrated in the residual liquid phase [18,19]. The partially frozen extracellular solution becomes hypertonic than the supercooled intracellular solution, causing an osmotic gradient across the lipid membrane. It can allow the osmotic efflux of water from cells and cause cells to dehydrate [20,21]. Some cellular dehydration is beneficial for cryopreservation, preventing intracellular ice formation; however, prolonged exposure of cells to the hypertonic solution can induce lethal osmotic shock to membranes [22,23]. In addition to the osmotic gradient across the lipid membrane, ice growth and recrystallization can result in the ionic charge imbalance across the membrane. During the ice growth, a small amount of salt ions can be accommodated in the ice lattice [24–26]. It is revealed that the chloride ions in the

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