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Cryoprotective mechanism of using Ficoll for cell cryopreservation at non-cryogenic temperatures: A molecular dynamics study

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ABSTRACT

Molecular dynamics simulations were carried out to investigate the cryoprotective mechanism of using Ficoll, a highly compact spherical polysucrose, as a non-permeating cryoprotectant for practices of cell cryopreservation at temperatures higher than −80 °C. Three types of simulation boxes were prepared for Ficoll-dimethyl sulfoxide (DMSO)-water, sucrose-DMSO-water, and DMSO-water systems, respectively, and depicted with the Optimized Potentials for Liquid Simulations (OPLS-all) as potential function for molecular systems. The entire molecular system for each scenario was firstly fully equilibrated into a state with known concentration, density and temperature, in agreement with the corresponding existing or newly measured phase-diagrams. Thereafter, molecular dynamics simulations were performed to characterize the behavior of liquid water molecules surrounding a pre-sited ice nucleus that was placed at the center of each molecular system by calculating the radial density distribution (RDF) and root-mean-square distance (RMSD) of atomic positions. The results showed that the system with Ficoll molecules present behaved significantly different from the other two systems at various non-cryogenic temperatures (−10 °C, −20.3 °C, −33.9 °C, and −80 °C). The Ficoll molecules obviously prevent the water molecules from approaching the ice nuclei, and simultaneously lower the activities of the water molecules. These results agree well with previous thermal studies that demonstrate the effect of using Ficoll to minimize recrystallization of such solutions, and also provide a qualitative explanation on a molecular level for why Ficoll facilitates long-term storage of cells at non-cryogenic temperatures.

1. Introduction

With recent advances in development of cell-based therapy, tissue engineering and regenerative medicine technologies for biomedical applications, there has always been an urgent need in improving the cryopreservation efficiency for large stocks of cell and tissue [1–3]. Traditional cryopreservation media contain primarily cell membrane permeating cryoprotectants, e.g., dimethyl sulfoxide (DMSO), ethylene glycol, and 1,2-propanediol, plus low concentrations of non-permeating small molecular or polymer cryoprotectants (e.g., sucrose, trehalose, polyvinylpyrrolidone, and polyethylene glycol). These solutions are thermally unstable when frozen at non-cryogenic temperatures, e.g., −80 °C, and the recrystallization temperature of the unfrozen portion of those solutions is generally higher than −100 °C [4–7]. Since recrystallization causes severe mechanical damage to cells [4–7], current long term cryopreservation of cell stocks routinely requires the use of liquid nitrogen and associated facilities to provide a temperature range for safe storage between approximately −120 °C (the temperature of the vapor phase in Dewar or cryogenic freezers) and −196 °C (the saturation temperature) to prevent recrystallization during storage.

However, the frequent use of liquid nitrogen is a heavy financial burden and requires the construction of safe but expensive liquid nitrogen supply systems. The associated operating procedures (e.g., sample loading and collection, liquid nitrogen refilling and storage tank cleaning, handling and shipping) are time consuming and laborious, with numerous safety and maintenance issues also generated (e.g., cold burn and asphyxiation). Based on previous differential scanning calorimetric experimental investigations [8], we discovered that using a low concentration (typically 10% w/w) of a highly compact spherical polysaccharide, Ficoll 70 (also called polysucrose 70 by some manufacturers), significantly increases the recrystallization temperatures of cryopreservation media to a much greater extent than use of normal non-permeating or

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Nomenclature

A	Lennard-Jones parameters, N m^{13}
C	Lennard-Jones parameters, N m^{7}
e	electron charge
E	energy, J
f	fudge factor
K	spring constant, N/m or N/rad
q	charge on each atom, electron
r	position, m
r	distance, m
t	time, s
V	energy, J
x	position, m

Greek symbols

δ	distance, m
ϵ	dielectric constant, F/m
θ	angle, °
Φ	angle, °

polymer cryoprotectants (e.g. sucrose and polyvinylpyrrolidone) of similar concentrations. This invention enables long-term storage of mammalian and insect cells in regular deep freezers operating at −70 °C to −85 °C with the post-thaw viability and functionality comparable to those from the standard storage protocol using liquid nitrogen facilities, and thereby significantly improves the efficacy of cell cryopreservation and biobanking [8,9]. We hypothesized that the highly compact structure and highly hydrophilic surface of Ficoll molecules play critical roles in modification of the recrystallization processes, and subsequently carried out molecular dynamics to validate our hypothesis in this study.

The method of molecular dynamics is widely recognized as a powerful and cost-effective computational technique that is capable of providing preliminary views to reveal the mechanism of ice growth from a perspective on the molecular level [10–13]. Hence, this method offered highly valuable information for exploring the potentials of innovative non-permeating cryoprotectants, e.g., xenon gas, trehalose, antifreeze proteins and their analogues, by revealing the associated cryoprotective mechanism [14–19]. However, the recrystallization process of partially frozen solutions generally takes days or weeks at low temperatures, which is far beyond existing computational capabilities if a normal molecular dynamic simulation strategy is applied.

In this study, to overcome these technical challenges, we created a novel model, namely, "inserting" an ice nucleus into each of the simulation boxes for the unfrozen portion of cryoprotectant solutions equilibrating at certain temperatures, and then characterized the water molecule behaviors to predict the recrystallization potentials. We studied and compared the Ficoll-DMSO-water, sucrose-DMSO-water, and DMSO-water systems. To provide the concentration and temperature information for the calculations, we also measured the phase diagram of the Ficoll-DMSO-water system using differential scanning calorimetry, and applied existing phase diagrams of DMSO-water and sucrose-DMSO-water systems.

2. Molecular dynamics simulation

In the present simulations, complex molecular systems, Ficoll-DMSO-water, DMSO-water, and sucrose-DMSO-water system are investigated at given temperatures and concentrations to reveal how Ficoll molecule distinguishes from the others and further improve recrystallization behavior. In order to appropriately describe the molecular system, a standard and optimized potential (OPLS-aa) [20,21] was directly adopted by the liquid simulation force field to describe the molecular systems. Namely, the energy of the system was described as,

$$E(\mathbf{r}) = E_{bonds} + E_{angles} + E_{dihedrals} + E_{non\text{-}bonded} \tag{1}$$

where the energy contribution to the bonds was

$$E_{bonds} = \sum_{bonds} K_r (r - r_0)^2 \tag{2}$$

The energy contribution to the angles was

$$E_{angles} = \sum_{angles} K_\theta (\theta - \theta_0)^2 \tag{3}$$

The energy contribution to the dihedrals was

$$\begin{aligned} E_{dihedrals} = {} & \sum_{dihedrals} \left(\frac{V_1}{2}[1 + \cos(\phi - \phi_0)] + \frac{V_2}{2}[1 - \cos 2(\phi - \phi_0)] \right) \\ & + \sum_{dihedrals} \left(\frac{V_3}{2}[1 + \cos 3(\phi - \phi_0)] + \frac{V_4}{2}[1 - \cos 4(\phi - \phi_0)] \right) \end{aligned} \tag{4}$$

And the energy contribution to the non-bonded was

$$E_{non\text{-}bonded} = \sum_{i>j} f_{ij} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{C_{ij}}{r_{ij}^{6}} + \frac{q_i q_j e^2}{4\pi\varepsilon_0 r_{ij}} \right) \tag{5}$$

According to the methodology described in [21,22], the non-bonded energy was counted for atoms with three or more bonds apart, and the 1, 4 interactions was scaled down by the fudge factor f_{ij} of 0.5 for the intramolecular non-bonded interactions. The geometric rule was assumed for the pair-wise interaction between different atoms. The structures of different molecules are illustrated in Fig. 1(a)–(d), for DMSO, Sucrose, Ficoll 70 and water, respectively. In the previous studies [8], we found that Ficoll 70 is more efficient in prevention of recrystallization than Ficoll 400, because the solutions of Ficoll 70 recrystallize at higher temperature than those of Ficoll 400 when their concentrations are the same, so we chose Ficoll 70 in all cases. Since the Ficoll 70 molecule is neutral, highly branched and compact, and also a spherical polymer of sucrose molecules, then the Ficoll structure in the current model was approximated by organizing a linear chain of sucrose molecules to form a neutrally charged and compact spherical structure with a diameter of approximately 5 nm [23]. The molecular weight (MW) of each constructed Ficoll molecule approaches 70 kg/mole, i.e. the mean MW of mass produced Ficoll 70. The parameters of non-bonded interaction for these molecules are given in the Table 1 [24]. The parameters for the bond/angle/dihedral of each molecule structure were determined based on the types of atoms and through the use of an existing tool, moltemplate [25]. Three types of simulation boxes for molecular systems, namely, Ficoll 70-DMSO-water, Sucrose-DMSO-water, and DMSO-water, were prepared for the molecular dynamics simulations. It is worthwhile pointing out that SPC water model is applied rather than TIP4P/Ice in the consideration of the objective of present study that is investigating the water molecule's activities surrounding the ice nucleus. The conclusion of the study stay valid as long as the same water model is employed for all molecular systems. In addition, three-site model demands less computational resources than that of four-, five-, or six-site models due to its geometrical simplicity. To enable molecular dynamics to study the time-consuming recrystallization process, we deployed the following strategy.