Abstract

During cryopreservation, cryoprotectants are used to protect cells from cryoinjury. Currently, 10% dimethyl sulfoxide (DMSO) is commonly used as a cryoprotectant. However, since DMSO is highly cytotoxic and membrane-permeable, it must be removed promptly after freezing and thawing. Polyampholytes have high cryoprotective effects, but the cryoprotective mechanism is poorly understood. In this thesis, I focused on elucidating the cryoprotective mechanism of polyampholytes and their application to cryopreservation using intracellular permeation.

In Chapter 2, in addition to the previously reported poly(2-(dimethyl amino)ethyl methacrylate/methacrylic acid) (pD), I synthesized poly(vinyl acetate/acrylic acid (AA)/2-(dimethyl amino)ethyl acrylate (DMAEA)) (pV) and poly(methyl vinyl ether/AA/DMAEA) (pM) with alternating cations and anions, and poly(2-acrylamido-2-methyl propanesulfonic acid/(3-acrylamidopropyl)trimethyl ammonium chloride) (pA) with a higher degree of dissociation, and correlated their cryoprotective effects and polymer structures. The cell recovery rate of the alternating array pV was the highest and comparable to that of carboxylated ε-poly-L-lysine (PLL). Cryoprotective effect of pA was the lowest, and measurements of residual water volume by temperature variable solid state magic angle spinning NMR showed that freezing of bulk water was significantly higher. In addition, the tendency of vitrification and trapping of Na ions was observed in the higher temperature range of pV and PLL, which showed high cryoprotective effect, suggesting that the ability to adjust salt concentration and vitrification during freezing by trapping salt or water is important for the cryoprotective effect of amphoteric electrolyte polymers.

In Chapter 3, we attempted to enhance the cryoprotective effect by utilizing the permeation of the zwitterionic polymer poly(sulfobtaine) into cells. Furthermore, we synthesized a copolymer of DMSO, a cell-permeable cryoprotectant, and 2-(methyl sulfinyl)ethyl methacrylate (MSEMA), which has the same methyl sulfinyl group, and confirmed its cryoprotective effect. Intracellular permeation was confirmed by the fluorescently modified polymer, and the polymer was found to easily counter diffuse by incubating at 37 ° C for about 30 minutes after permeation. Poly(SPB) and poly(SPB/MSEMA10) improved the cell recovery rate by about 20 or 30% through intracellular permeation. Cell recovery rate after counter diffusion of the polymer at 37 ° C was comparable to that before polymer permeation, and the cell pellets alone can be cryopreserved after polymer permeation, indicating that poly(SPB) can improve cryoprotection by intracellular permeation.

This study revealed that vitrification and salt/water trapping are important for cryoprotection of polyampholytes. This understanding of the cryoprotection mechanism will provide important guidelines for the molecular design of cryoprotectants in the future. Furthermore, the ability of zwitterionic polymers to reversibly permeate into cells and enhance their cryoprotective effects offers the potential for cryopreservation of more complex three-dimensional cellular structures such as spheroids, and the ability of artificial polymers to It is expected that this will open up new avenues for development.

[keyword] Cryopreservation, Polyampholytes, solid-state MAS NMR, Intracellular permeation