A novel high-pressure cryocooling technique for macromolecular crystallography has been developed and explored at the Macromolecular Diffraction Facility at the Cornell High Energy Synchrotron Source (MacCHESS). The method involves cooling macromolecular crystals to cryogenic temperatures (~100 K) in high-pressure helium gas (up to 200 MPa). Several different kinds of macromolecular crystals have been successfully high-pressure cryocooled and excellent crystal diffraction has been obtained without adding any penetrating cryoprotectants. This new method has great potential for structural biology and high-throughput crystallography. This presentation details technical aspects of high pressure cryocooling. Recent experimental results are presented, including crystal cryoprotection, extension of the method to Krypton/Xenon single-wavelength anomalous dispersion (SAD) phasing, native sulfur SAD phasing, and the use of samples in capillaries, both crystals and solutions. Finally, a mechanism involving high-density amorphous (HDA) ice is proposed as to why the method works. The high pressure cryocooling method is now available to CHESS users on request.

1. Abstract

2. High-pressure Cryocooling

1. Procedure

- a) Pressurization of crystals up to 200 MPa at 10°C with helium gas
- b) Cooling crystals under pressure to the LN2 temperature (77 K)
- c) Releasing pressure while crystals are kept cryocooled

2. Apparatus

3. Applications

1. Crystal cryoprotection

- Water-soluble protein - Glucose Isomerase

- Membrane protein - Kv 1.2 K+ ion channel

2. Extension to diffraction phasing and capillary sample cryoprotection

- Elastase - Krypton SAD phasing
- Thaumatin - Sulfur SAD phasing

3. Pressure effect study on proteins - Citrine

High pressure cryocooling explains why Citrine changes its color under pressure!

4. Mechanism

1. Water vitrification

As shown in the H2O phase diagram, the freezing point of liquid water (Tm) decreases up to 210 MPa. Furthermore, the nucleation and growth rates of ice I & ice II become slower under pressure. As a result, water vitrification can be easily achieved under pressure even at moderate cooling rates (~100 K)

2. Formation of high-density amorphous ice

When protein crystals are frozen, the unit-cell volume usually shrinks by ~5%. But liquid water inside the crystals expands by ~6.7% when crystalline or amorphous ice form. This volume discrepancy causes disorder in the crystal. Furthermore, it is likely that the crystalline ice formation leads to significant damage to the crystal. When rapidly cryocooled at high pressure, however, liquid water freezes into HDA (high-density amorphous) ice and, once formed, it stays metastably in the HDA state when the pressure is removed, as long as the temperature is kept below ~120 K. HDA ice has higher density (1.17 g/cm3) than liquid water and it is in noncrystalline state. The result mitigates crystal disruption at ambient pressure.

3. Evidence for HDA ice by high-pressure cryocooling

X-ray diffraction of solution sample Primary peak position of water diffuse scattering

4. User Support at MacCHESS

The high pressure cryocooling method is now available at MacCHESS.

6. References


7. Acknowledgements

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