High Pressure Cooling of Protein Crystals without Cryoprotectants

Chae Un Kim\textsuperscript{1,2} and Sol M. Gruner\textsuperscript{1,2,3}

1) Field of Biophysics, 2) Cornell High Energy Synchrotron Source (CHESS) and Macromolecular Crystallography at CHESS (MacCHESS), 3) Physics Department, Cornell University, Ithaca, NY 14853, USA

Abstract

We have developed a novel method to cryocool protein crystals without the need for penetrative cryoprotectants. The method involves mounting protein crystals in a cryoloop with a droplet of oil, pressurizing the crystal up to 200 MPa (2000 atm) in He gas, cooling the crystal under pressure, and then releasing the pressure. Results are presented from two representative proteins. Dramatic improvement in diffraction quality in terms of resolution and mosaicity was observed in both cases. A plausible mechanism for the success of the pressure cooling method is proposed involving the dynamics of water at high pressure and high density amorphous (HDA) ice.

Motivation

1. Procedure

a) Pressurization of crystals up to 200 MPa at 10 °C with He gas
b) Cooling crystals under pressure by dropping samples into the LN2 bath
c) Releasing pressure while crystals are kept cooled
Pressure-cooled crystals are handled thereafter just like normal room pressure flash-cooled crystals

2. Apparatus

Sample Pin Pressure Tubing Assembly Pressurization Machine

1. Room Pressure Cooling vs. High Pressure Cooling

So far, high pressure cooling method has been tested with about a dozen different kinds of protein crystals. Excellent diffraction has been observed in many cases. Here are two cases with dramatic improvement in diffraction quality.

1) Glucose Isomerase

Resolution = 1.8 Å, Mosaicity = 1.29°

2) Thaumatin

Resolution = 1.3 Å, Mosaicity = 0.39°

Experimental Results

1. Procedure

a) Pressurization of crystals up to 200 MPa at 10 °C with He gas
b) Cooling crystals under pressure by dropping samples into the LN2 bath
c) Releasing pressure while crystals are kept cooled
Pressure-cooled crystals are handled thereafter just like normal room pressure flash-cooled crystals

2. Apparatus

Sample Pin Pressure Tubing Assembly Pressurization Machine

2. Protein Structure Comparison

The difference (r.m.s. deviation) between the room pressure flash-cooled structure and the pressure-cooled structure is less than 0.5 Å in both cases, which is comparable to the differences seen when changing temperature

3. Study of pressure effects on protein structures

Future Applications of HP Cooling

1. Cryoprotection for protein crystallography
2. Application for phasing with Kr and Xe
3. Study of pressure effects on protein structures

Proposed Mechanism

To date we have successfully pressure-cooled more than a dozen different proteins. We observed in almost all cases that water vitrification in protein crystals was achieved without any penetrative cryoprotectants. Furthermore, in many cases, improvement in diffraction quality was observed. We propose that these effects involve the dynamics of water under pressure and the formation of high density amorphous (HDA) ice. Details are described below.

References


This work was supported by the MacCHESS grant (US NSF grant RR-001646) and by US DOE grant DE-FG02-97ER25643 and CHESS, which is supported by the US NSF and NIH-NIGMS through NIH grant DMR-0225180.