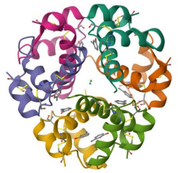
Canadian Light Source
Students on the Beamlines Program – August 2022



Evaluation of the Stability of Zinc-Containing Insulin: An X-Ray Crystallography Study at the Canadian Light Source

Rayan Ramadan, Grace Z. Yu, Peter Yin, Jason J. Lee, Hok Yiu (Jerome) Wong, Tsz Wun Wong, Roy J. Zhao

Abstract

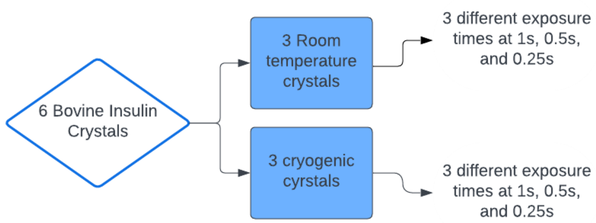
We present results of an x-ray crystallography study of the insulin protein. This study was conducted under the Students on the Beamlines (SotB) program at the Canadian Light Source using the Canadian Macromolecular Crystallography Facility (CMCF) bending-magnet (BM) beamline. We evaluated the stability of zinc-stabilized insulin molecules at constant beam strength and demonstrated that relative radiation damage is linearly proportional to exposure time. Cryogenic conditions lowered the relative radiation damage by a factor of 40. The protein fully denatured at room temperature. The B-factor of protein residues was plotted as a function of radial distance to the zinc ion and was found to increase as a function of radial distance.

Introduction

X-ray crystallography is among one of the most widely used probes for macromolecular structures. Protein molecules are crystallised and an x-ray beam is directed to it while its diffraction pattern is recorded. By exposing the molecule under X-ray radiation, one could analyze the diffraction pattern and deduce the conformation of a molecule. It offers high resolution due to the x-ray wavelength being short enough such that the resolved diffraction pattern can render the inner patterns of atomic structure. A large component of this energy is absorbed by the protein crystal, so radiation damage can cause slight denaturation. We aim to a) analyze the magnitude of radiation damage and quantify it for a specific molecule and b) identify the role metal ions can play in the stability of molecules.

Methods

We crystallized a set of bovine insulin using standard crystallization conditions and the hanging drop method. Crystals were fished out from droplets and placed in pucks for transport to the CMCF-BM beamline. The crystals averaged between 50 to 70 micrometers in width, and the beamline has a 200 micrometer width. Diffraction patterns were recorded and solved using on-site computational software.



Results

Due to limitations on beam time and the severe degradation of the room temperature crystals, we were able to obtain only 6 out of 14 total structures.

The diffraction data was analyzed using the Crystallographic Object-Oriented Toolkit (COOT). The rendered protein structure is compared with the protein database model of Zinc-stabilized insulin.

The main methods of comparison were relative radiation damage, root mean squared deviation, and beam factor. The relative radiation damage was plotted over exposure time, and the B-factor (or temperature factor) was plotted over radial distance from the zinc ion.

Results – Radiation Damage & B-Factor

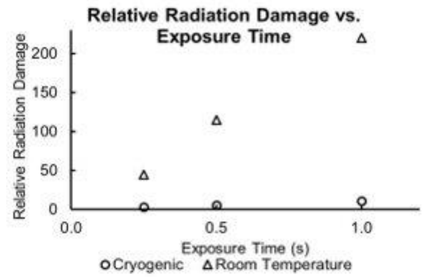


Figure 2: Relative Radiation Damage over Exposure Time for 6 different Bovine Insulin Crystals.

We decided to plot the relative radiation damage over exposure time to gain a measure of the relative efficacy of cryogenic conditions as the exposure time increases. As expected, we observed a linear increase in radiation damage as cystals were exposed for longer periods of time.

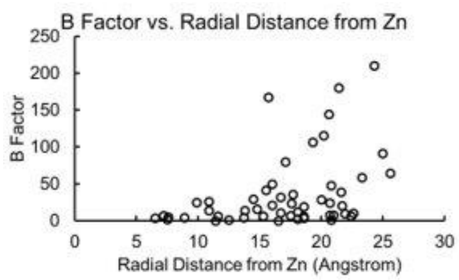


Figure 3: B-Factor over Residues' Absolute Radial Distance from the Zinc Ion.

We additionally evaluated the insulin crystal imaged at cryogenic temperatures and measured the temperature factor, or "instability" of all the residues plotted over each residue's radial distance from the zinc ion. The purpose of this analysis was to see if zinc was playing a stabilizing role in insulin.

Results - Resolved Structures

We include 5 of the 6 resolved structures below, processed using COOT and rendered using PyMol. It is worth noting no significant differences between **RMSD and B-Factor** of these structures, indicating close alignment regardless of the measured relative radiation damage.

Cryogenic Temperature – 0.25 s Exposure | Cryogenic Temperature – 0.50 s Exposure | Cryogenic Temperature – 1.0 s Exposure

Room Temperature – 0.25 s Exposure | No data available | Room Temperature – 1.0 s Exposure

Conclusions & Future Directions

We conclude a possible dependence between the zinc ion and the stability of the Insulin molecule that can be better investigated using molecular dynamics simulations of dimeric structures with vs. without zinc ions. We verified the impact of high exposure time on the relative radiation damage seen by imaged molecules, and hope to continue investigating the stability of insulin in the context of its potential to misfold.

Acknowledgements

The research described in this paper was performed using beamline CMCF-BM at the Canadian Light Source, a national research facility of the University of Saskatchewan, which is supported by the Canada Foundation for Innovation (CFI), the Natural Sciences and Engineering Research Council (NSERC), the National Research Council (NRC), the Canadian Institutes of Health Research (CIHR), the Government of Saskatchewan, and the University of Saskatchewan. The authors of this poster gratefully acknowledge the travel funding provided by the CLS, as well as Cindy Hops, Carrie Mohoruk, Shannon Porter, Charles Schofield, Amanda Pfeiffer, Anna- Maria Boechler, Kurt Nienaber, and Michel Fodje for administrative assistance and helpful conversations. The CLS, in Saskatoon, SK, is located on Treaty Six land in the traditional territories of the Nêhiyawak (Cree), Anishinabek, Lakota, Dakota and Nakota Nations, and the homeland of the Métis.