Energy Dependence of Heavy Atom Radiation Damage in Protein Crystals

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Abstract

X-ray crystallography is a technique used to solve the molecular structure of proteins by analyzing the diffraction patterns that result when x-ray light is directed at protein molecules arranged in a crystal structure. Information regarding radiation damage to crystals during data collection is integral to minimizing error in solved structures, and is especially important when considering intricacies in protein function. In this study we attempt to determine if the rate of damage to specific sites in protein crystals frozen at 100K depends on the energy of the x-ray beam. Four crystals grown from a heavy metal derivative of a nucleic acid binding protein containing twelve selenium-methionine and eight cysteine residues were each subjected to 2-7 MGy of cumulative x-ray exposure by collecting eight data sets from each crystal at energies of 14 keV or 9 keV. The integrated electron density surrounding each sulfur and selenium atom was calculated for each data set and the change in electron density around each atom was compared at the two energies. The rate of electron density decrease per cubic angstrom vs. dose was determined to be slightly yet significantly greater at 14 keV than 9 keV for both sulfur and selenium atoms.

Introduction

Protein Structure Determination: Step 1. Crystallization

Crystallization is the rate-limiting step in protein crystallography, as it can take years to discover the necessary conditions for crystal growth. Protein crystals (Fig 1.) are made up of a precise arrangement of protein molecules (Fig 2.). A **unit cell** (Fig 3.) is a three dimensional quadrilateral shape with dimensions (a, b, c) and angles (α, β, γ) that is repeated throughout the entire **crystal structure**. Each crystal shown in Figure 1 below may be composed of approximately 10^9 (one trillion) unit cells arranged in a lattice structure.

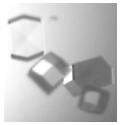


Fig 1. Lysozyme protein crystals, each measuring less than 100 microns, about the width of an average human hair

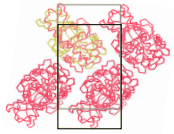


Fig 2. The crystal orientation of four molecules of the nucleotide binding protein used in this study. This protein is composed of two identical subunits, colored yellow and red in the upper left of the image.

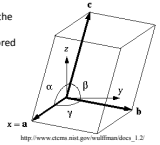
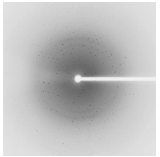


Fig 3. A model of a unit cell, which repeats throughout the crystal. The unit cell is also indicated by the black rectangles in figure b.

Step 2. X-ray Data Collection

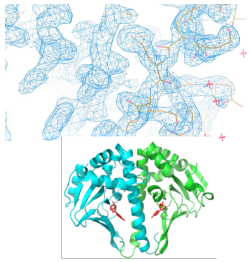


The crystal is frozen in liquid nitrogen and suspended in a cryo-stream during data collection, keeping the crystal at 100K. The image on the left shows the **x-ray diffraction pattern** of one of the crystals analyzed in this experiment.

Photons colliding with atoms in the crystal are refracted at specific angles and the resulting **reflections** (spots) are recorded by the detector. Each reflection is the result of scattered photons striking the detector after passing through the sample and interacting with the regularly spaced atoms of the crystal.

Step 3. Structure Determination

A **data set** consisting of a series of images like the one shown above is collected at a range of angles and processed using computational methods that determine the amplitudes and phases of all the diffraction spots in order to generate an **electron density map**.
The map is analyzed and fitted with a proposed model which is refined multiple times in order to solve the atomic structure. The image on the left shows a split screen of the electron density map alone (above-left) and fitted with a model (above-right), which can be used to construct a ribbon diagram of the protein (below-right). The primary purpose of a model is to gain insight into the function of the protein, it is therefore important to identify the precise location of atoms in the protein.



Radiation Damage

A third generation synchrotron such as SSRL generates beams with a flux in the range of 10^{11} photons $s^{-1}\,\mu m^{-2}$. These high intensity beamlines have significantly decreased the amount of time required to collect data; however, they also increase the rate of photoelectric absorption by the crystal and have raised important questions regarding the impacts of radiation damage on protein structure determination.

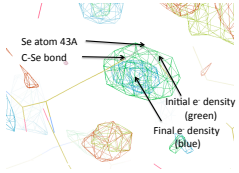
Radiation Damage (continued)

Determining the precise location of atoms in a protein is extremely important, especially when considering intricacies of protein function. It is therefore important to find out if damage can be mitigated under different experimental conditions. This study attempts to ascertain if specific damage is dependent on the incident energy of the beam. Radiation damage is measured in units of Gy (J kg^{-1})

The two types of radiation damage are global and specific. Global damage perturbs the arrangement of molecules in the crystal lattice structure, which can prevent the solution of the structure. **Specific damage** results in chemical changes to the protein molecules, such as disulphide bond-breakage, decarboxylation, and **bond cleavage of heavy atoms**, (Burmeister, 2000) any of which may result in changing the structure from its biological form and lead to erroneous conclusions regarding function. (Holton, 2009 Gonzalez, 2007)

This study focuses on specific radiation damage to the C-Se and C-S bonds of Se-Met and Cys residues by analyzing the electron density around the Se and S atoms of those residues.

The image to the right (generated by COOT software) shows the visible difference in electron density around an Se atom of Se-Met residue 43A before and after a dose of 6 MGy was deposited on the crystal.



Instrumentation

The **Stanford Synchrotron Radiation Lightsource (SSRL)** generates intense x-rays by bending the path of high energy electrons traveling in a circular storage ring with undulator and wiggler magnets. These intense streams of x-rays are directed to various beamlines which lie tangent to the ring.

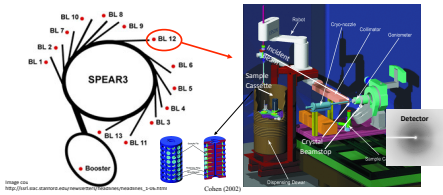


Diagram of instrumentation found at beamlines dedicated to protein crystallography. Crystal samples are stored at 100K in liquid nitrogen in cassettes with magnetic ports. Samples are removed from the cassette by a robot and placed on the goniometer head under a nitrogen or helium cryostream which maintains the low temperature and structural integrity of a sample. The x-rays not absorbed by the crystal are physically blocked from hitting the MARCCD325 area detector in order to protect it.

Methods

Crystals and the structure of the protein studied provided by the Joint Center for Structural Genomics at SSRL. Crystallization details and coordinate files can be found at the Protein Data Bank (PDB code 3gyd).

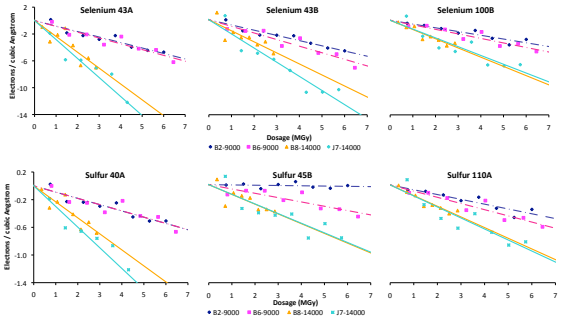
Eight data sets were collected for each crystal in a 100 K nitrogen cryostream. Two crystals were irradiated with 9 keV electron beam (1.38 Å) and two with 14 keV (0.89 Å) using BL 12-2. Dose information was determined for each crystal using RADDOSE.

Crystal ID	Energy (eV)	Wave length (A)	Detector distance (mm)	Exposure time/ Image (s)	Number of images	Irradiated time/data set (s)	Number of data sets	Dose Rate (Gy s^{-1})	Dose per data set (MGy)
B2	9000	1.378	191.5	0.511	99	50.6	8	14825	0.75
B6	9000	1.378	191.6	0.628	143	89.8	8	9011	0.81
B8	14000	0.886	339.2	1.011	121	122.3	8	2943	0.36
J7	14000	0.886	339.2	0.618	152	93.9	8	7664	0.72

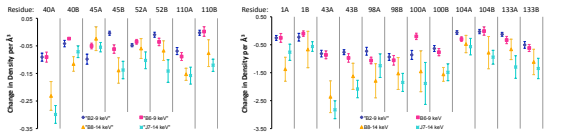
Data sets were integrated using *MOSFLM* (Leslie, 1999; Collaborative Computational Project, Number 4, 1994), and scaled using *SCALA* (Collaborative Computational Project, Number 4, 1994). The electron density of the atoms was integrated at 0.5 Å radius for the 8 S atoms and 1.0 Å radius for the 12 Se atoms using *MAPMAN*. The electron density maps were calculated using *FFT* (Collaborative Computational Project, Number 4, 1994) and visually inspected using *COOT* (Emsley & Cowtan, 2004).

Results

The slopes of the regression lines in the graphs show the rate of decrease in electron density vs. dosage around three Se atoms and three S atoms in each of the four crystals studied. The legend gives the crystal ID followed by the energy (in units of eV) used to collect data for that crystal. These results suggest that higher energy can produce greater damage to C-S bonds in Cys residues and C-Se bonds in Se-Met residues.



The y-intercept of each regression curve was arbitrarily set to 0 for the purpose of comparison. The rate of density loss is indicated by the slope of the regression curves. The steeper slope for the crystals dosed with 14 keV indicates greater movement of the atom. Differences in absorbed dose are due in part to the different locations of each atom in the crystal.



The above graphs compare error in the slope values for each atom studied in each crystal. Note that the error is generally greater for the crystals studied at 14 keV, which is an indication of a greater background noise level. Also, note that whenever there is no overlap between the error bars at the two energies, the rate of density change is greater (more negative) at 14 keV. The most conclusive results are where there is no error overlap: see sulfur atoms 40A, 45B, 110A, 110B and selenium atoms 1A, 43A, 43B, 100B and 104B.

Future Directions

Further studies exploring damage to disulfide bridges in lysozyme crystals are in progress. Preliminary results confirm energy dependence of specific damage to disulfide bridges.

References

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Further Reading

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