EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

	Experiment title: Toward an Understanding of the Mechanism of X-ray Radiation Damage in Macromolecular Crystals	Experiment number: ls1624
Beamline: id14-2/4	**Date of experiment**: 2,3 March, 12,15,16 June from: to:	**Date of report**:
Shifts: 15	**Local contact(s)**: Dr. Sean McSweeney	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(*) Dr. S. McSweeney, ESRF

(*) Dr. R. Ravelli, EMBL-Grenoble

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Dates:

02 (7am)-04 (7am) March 2000, ID14-4, 6 shifts
12 (7am)-13 (7am) June 2000, ID14-2, 3 shifts
15 (7am)-17 (7am) June 2000, ID14-4, 6 shifts

Report:

Our long-term-project proposal aimed at understanding the very nature of radiation damage and at devising means for minimizing it. During the first experiments in March and June 2000, we tried both to develop the experimental setup needed for systematic future studies, and to evaluate quantitatively the effect of temperature on radiation damage. Furthermore, the effect of radiation damage on the phasing power of data collected on a crystal of a SeMet containing protein was investigated.

A. Experimental Setup

*) A Helium cryostat was developed at the EMBL-outstation Grenoble, and tested on the beamline. The initial setup gave problems with ice-formation around the spindle axis that eventually (1-2 hour's) let to ice growth on the crystal-sample. By accurately controlling the temperature and flow of "warm" Helium gas stream that surround the "cold" Helium gas stream, we could obtain a setup that was stable both on ID14-2 and ID14-4 for at least 48 hours.
*) Accurate dose measurements are essential for most systematic radiation damage studies. Photodiodes were tested for linearity of response, and a completely light tight housing was made with maximum shielding against electrical noise.
*) Partly based on our studies with temporary attenuators, professional attenuator boxes were developed for all of the ID14 beamlines with aluminum attenuators of known thickness and polished surfaces.

B. The effect of temperature

*) **100 versus 150K**. We used the relative increase of unit cell volume as a measure for radiation damage (Ravelli & McSweeney, 2000). It proved that the increase of cell volume versus exposed dose could vary between crystals of the same protein, but remained very constant for a given crystal as long as the diffractive power remained acceptable. We could use this parameter for systematic studies on the effect of temperature on the process of radiation damage. It proved that, for the sample investigated, the increase in cell volume versus exposed dose was rather constant between 100K and 140K. However, a different behaviour was observed at higher temperatures. At 180K and 200K, the unit cell volume did not expand or even decreased, whereas the formation of ice became apperant from the diffraction patterns. At 160K, we observed a sharp increase in the rate of cell volume growth versus exposed dose. We conclude that larger radicals that can be built up at temperatures below 150K, can start to migrate and even recombine at temperatures of 180K or higher. The experiments were repeated on crystals of another protein, and the precise temperatures of these transitions seem to be sample and crystal-form dependent.
*) **100 versus 40K**. Different data sets were collected on similar samples at 100K (cold N_2-gas stream) and 40K (cold He-gas stream). No obvious improvements could been observed sofar, although some of the 40K data sets were really nice, probably because of the reduced background scatter in the He-cooling setup. More systematic experiments are needed before we could make any statistically correct conclusions.

C. Radiation damage and anomalous diffraction

We have performed the first systematic radiation damage studies on a SeMet containing protein. Seven complete data sets were collected on one crystal at the peak of the fluorescent signal, using the unattenuated beam of ID14-4. The crystal was exposed to additional X-ray photons between some of the data sets in order to enhance the process of radiation damage. All data sets were individually examined for their anomalous signals. Although the crystal hardly showed any signs of decay during the first few data sets, clear differences were found in the quality of the anomalous signals between these data sets. The later data sets, especially the ones after the burn, still showed reasonable Rmerges and diffraction resolution, but were basically unusable for phasing because of severe detoriation of the anomalous differences.

Future prospects

The developments that were made for the experimental setup will not only be useful for our own future experiments, but have proven to be useful for a part of the user community of ID14 as well. The calibrated photo-diode has been used on id14-4 in order to provide accurate dose numbers to users. The same system can be used on other X-ray sources as well. The helium-cooling system has been used for some user groups, and could in principle form a reliable alternative for the cold N_2 gas stream systems. The attenuator boxes that we will use for future dose-rate studies are of direct use to users for, *e.g.*, the collection of low-resolution data.

Our temperature studies show that it could be dangerous to collect data at or above the transition temperature at which larger radicals become mobile. We have developed an accurate and elegant way for the determination of this temperature. Our studies also show that if heating of the crystal plays an important role for radiation damage, one should prevent the local temperatures within the crystal to pass this transition temperature. In future studies, we will investigate the effect of protein, crystal form and additives on the transition temperatures, and determine on a structural level what the differences in specific radiation damage at different temperatures are.

The observations made in the radiation-damage experiments on the SeMet containing protein are of high relevance to all 3rd generation synchrotron MAD undulator beamlines. We aim to do additional systematic studies for other SeMet containing proteins, in order to device some general rules on how to collect the best anomalous signal on SeMet samples on these beamlines.

References

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