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.

ESRF	**Experiment title:** *Deinococcus radiodurans* cells irradiation for proteomics experiments	**Experiment number**: SC-1589
Beamline:	**Date of experiment**: from: 15/09/2004 to: 17/09/2004	**Date of report**: 01/03/2005
Shifts:	**Local contact(s)**: Elke BRAEUER-KRISCH	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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ESRF

Report:

Aims

The aim of this experiment was to measure the viability curve of *Deinococcus radiodurans* (DEIRA) cells under our conditions going to higher doses than before. As for the previous experiment we used a dye, trypan blue, that estimates the proportion of viable cells in a population of cells.
We also wanted to explore the effects of different dose-rates on the cells.

Requirements of the BL

The experiment required:

1. a high dose-rate so that the considerable dose of 15kGy could be delivered in a negligible time compared to the cell division time of DEIRA (~1h), so to be in the same conditions as experiment SC-1457
2. a set-up able to deliver the desired dose homogeneously on the sample
3. an accurate estimate of the absorbed dose
4. possibility of changing the dose-rate by attenuating the beam precisely
5. proximity to biochemistry laboratory to allow prompt manipulation of the sample after irradiation

ID17, in principle, fulfilled all these requirements.

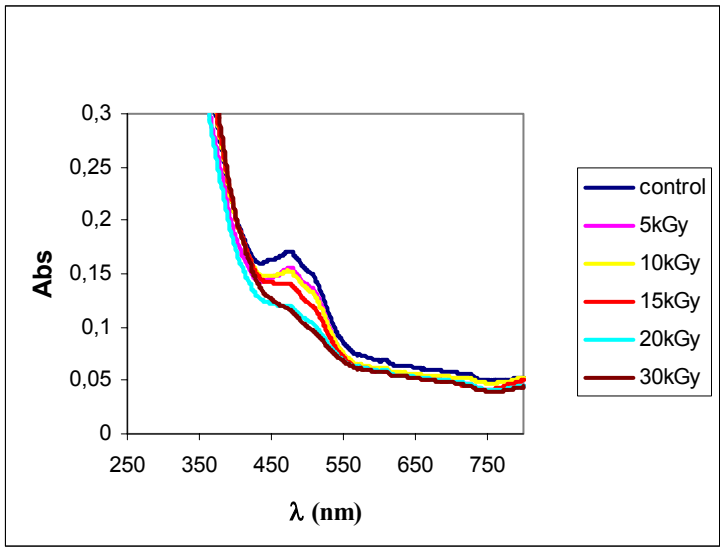
Experimental method

We wanted to reproduce the experiment SC-1457 adding two more doses (20kGy and 30kGy) and using the cells to perform viability tests with trypan blue. The reactivity of this dye is based on the fact that the chromophore is negatively charged and does not react with the cell unless the membrane is damaged.
For each dose the experiment was repeated three times to check for reproducibility. Therefore twenty four DEIRA cultures were grown at 30ºC in 240ml of medium 53 to an OD_{600}=1.0, corresponding to the mid-log phase in the growth curve. Cells were harvested by centrifugation in two batches of 120ml, one to be irradiated and one to keep as control, resuspended in 2ml of fresh medium, stored in 2ml cryo-tubes (Ø = 0.8 cm) to fit to the beamsize (0.5x1.2cm^2), fast frozen in liquid nitrogen and stored at -80ºC until the irradiation experiment could take place.
Another set of samples was produced to perform the irradiation experiment reducing the dose-rate ten and one hundred times.
Just before irradiation the two corresponding tubes were de-frozen and put on ice to avoid any further cell growth. One of the tubes was then mounted on the ID17 using an especially engineered sample holder, kept at 4ºC and irradiated with the requested dose of x-ray radiation, at first, at a dose-rate of 17,200 Gy/s. The tube was rotated and translated into the beam in order to irradiate homogeneously the sample.
Soon after the irradiation, back in the biochemistry laboratory, both the irradiated and non-irradiated cells were diluted to the original volume (120ml) with fresh medium 53 and incubated at 30ºC .
Samples were taken to perform the viability tests. The occasion was exploited also to produce more samples to use for 2Dgel electrophoresis using the same procedure described in the report of experiment SC-1457.
We then tried to repeat the experiment using two different dose-rates (1/10 and 1/100 of the original one) in order to explore the effects of this parameter on the DEIRA proteome.

Results

The first part of the experiment, which consisted on irradiation of cells cultures with different doses using the same dose-rate as experiment SC-1457, was successful. We managed to obtained all the cell samples required. The viability test, though, was not effective (*) since DEIRA's dead cells seem impermeable to trypan blue and no difference were observed between the viable and non-viable cells. This might be due to the fact that DEIRA's cell wall thickens when cells are exposed to radiation and therefore it is not damaged sufficiently when the cells are dead to let the dye in. This experiment needs to be repeated using a different type of viability test which is being tested now.
After irradiation from low to extremely high doses we observed a change of colour on the DEIRA's cells, going from dark pink to yellow (Fig. 1A). The non-irradiated cells have a pink colour, which is due to the presence of the carotenoid deinoxanthin which has an antioxidant action. To understand the reason for a changing of colour with irradiated cells, a process of organic extraction was performed in the cell extract, after extraction an absorption spectrum between 250 and 750 nm was measured for each sample (Fig. 1B). The spectrum peak at about 480nm decreases with increasing dose indicating a progressive reduction of deinoxanthin. This is probably due to its reaction with free radicals produced by radiation. Further characterization of this pigment in the different cell samples is under way.
The investigation of the reactions of DEIRA's cells to different dose-rates could not be performed since the longer exposure time necessary to reach the same absorbed doses was causing an unforeseen increase of heat load on some elements of the beam line, up-stream of the attenuators, which would have caused serious problems to the beam line.

(*) Previously (Experiment SC-1457) the viability test was performed by a stagier who probably mistaken the different colour of cells in different orientations for dead or live cells. How she managed to have consistent results is a mystery, but this has hidden the fact that this dye did not work for DEIRA and mislead us for the following experiment.



A B

Fig.1 A) Tubes with irradiated samples: from left to right, control, 5kGy, 10kGy, 15kGy, 20kGy, 30kGy. It can be noticed that the original dark pink/red colour is turning yellow when cells are irradiated.
B) The corresponding absorption spectra obtained from the deinoxanthin extracts of the samples. The peak at 480nm is progressively decreasing with increasing dose indicating a progressive reduction of the carotenoid.