ID14-EH4, microspec beamtime (experiment MX515)

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Context

DsbA, a thiol-disulfide exchange oxidase, is an enzyme whose function relies on the redox state of its sole disulfide bond, which constitutes its active site. We have characterized structurally several DsbA mutants lacking one of the cysteine, to probe the functional flexibility of this enzyme. Our aim was to check if we could characterize by spectroscopy the extend of disulfide bond breakage upon X-ray irradiation, a characteristic that could be related to its enzymatic reaction. *In fine*, we could use this to trap the structure of an intermediate state.

ECFP (enhanced cyan fluorescent protein) has been shown to be very sensitive to X-rays (unpublished results), and its active site gets degraded. We were eager to follow by spectroscopy the degradation and to try to get the less x-ray damaged structure as possible.

Results

On-line spectrophotometry

DsbA

We have tried to monitor the rise of a peak around 400 nm in the crystal absorbance spectrum while it is irradiated by X-rays, the spectroscopic signature of a radical species on the disulfide bond, leading to breakage. Unfortunately, the apparition of a species absorbing in the near UV (300 nm) has made it very difficult to see. Still, we cannot rule out its presence, because the OD is rising indeed, but we gave up since it will not provide us with a non-ambiguous result complementary to the X-ray data.

ECFP

Previous experiments have shown that it is difficult to monitor photo-degradation of this fluorescent protein upon the X-ray irradiation, because the whole baseline is non-uniformly affected. Hence, together with John McGeehan, we have investigated the possibility of performing steady-state fluorescence measurements using the on-line microspec. We used one objective, with a bifurcated fibre, leading either to a laser source (440 nm) or the spectrophotometer CCD detector. The spectra are of excellent quality. Unfortunately, the laser source participates to the photo-degradation of the protein in a similar way as X-rays, which hampers the use of stead-state fluorescence technique. We can envisage to use a TTL signal that could trigger the laser at the same time the spectrophotometer records a spectrum.

Diffraction experiments

DsbA

We were able to record a few datasets on new crystal forms. On one crystal, we have recorded a few datasets with increasing X-ray dose to check the influence of disulfide bond reduction on the active site configuration. We have processed the diffraction data and are now refining the corresponding structures. Then we will compare the different models and verify if the radiations have damaged the disulfide bonds and induced some conformational changes.