EUROPEAN SYNCHROTRON RADIATION FACILITY
ESRF User Office
BP 220, F-38043 GRENOBLE CEDEX, France
Delivery address: 6 rue Jules Horowitz, 38043 GRENOBLE, France
Tel: +33 (0)4 7688 2552; fax: +33 (0)4 7688 2020; email: useroff@esrf.fr; web: http://www.esrf.fr

Experimental Report – MX-1029

"Monitoring an X-ray beam induced conversion of the Dewar valence isomer of the (6-4) photoproduct during data collection using the online microscope"

UV radiation causes the formation of highly mutagenic DNA lesions such as cis-syn cyclobutane pyrimidine dimers (CPD photoproducts), pyrimidine(6-4)pyrimidone photoproducts ((6-4) photoproducts) and their Dewar valence isomers.[1,2] DNA photolyases use blue and UV-A light as the energy source to drive the genome repair, utilizing a light-activated flavin adenine diphosphate (FAD) - cofactor to inject a single electron into the 6-4 lesion to trigger its cleavage.[2] Recently, we solved the structure of the 6-4 photolyase of *D. melanogaster*. Together with biochemical data we could show that after electron donation from the light-excited FAD a radical intermediate is formed which rapidly fragments to give the repaired TpT.[3,4] Further irradiation of the (6-4) photoproducts with UV-A/B light results in the generation of the Dewar valence isomers. [2] However the (6-4) photolyase is able to repair the T(Dew)C, but not the T(Dew)T valence isomer, possibly by first rearranging the Dewar valence isomer back to the (6-4) lesion. [5]
In order to shed light onto the mechanism of Dewar repair we wanted to take advantage of the photochemical properties of the lesions: the (6-4) photoproducts have distinct absorbance bands at 325 nm (T(6-4)T)[6,7] and 314 nm (T(6-4)C)[8] and therefore can be distinguished from their respect dewar valence isomers Thus the goal of the experiment was to discriminate the (6-4) photoproducts from their Dewar valence isomers as well as to follow possible X-ray induced changes in the absorbance spectra during irradiation in the photolyase complex crystals.

Quality of measurement/data and status / progress of evaluation

The experiment was well setup by the beamline scientist and data collection was carried out using the online microscope. Unfortunately only the absorption for the FAD, but not for the (6-4) photoproducts could be determined during the experiment due to the weakness of the absorbance signal of the lesion caused by the limited size of the complex crystals.

Results

Albeit the signal of the 6-4 photoproducts was indistinguishable from the background, the absorbance peak of the FAD cofactor could be clearly detected. Figure 1a shows the spectra of the crystal before (black) and after (red) exposure to the X-ray beam. The fully reduced FAD possesses a fluorescence absorption maximum of about 475nm. Upon irradiation of the photolyase crystals the intensity of the observed signal decreases over time (Fig. 1b, green line), indicating reduction of the FAD. Reduction of the FAD by X-rays was reported previously by Kort *et. al*., which results in a 'rippling' or 'butter bend' of the isoalloxazine ring.[9] The previously collected data to 2.0 Å, which allowed us to solve the structure of the photolyase complex, did neither support nor exclude a rippling of the isoalloxazine ring. Thus no prediction on the redox state of the FAD cofactor could be made, which is important for structure-based quantum chemical calculations of the repair mechanism.

a) **b)**

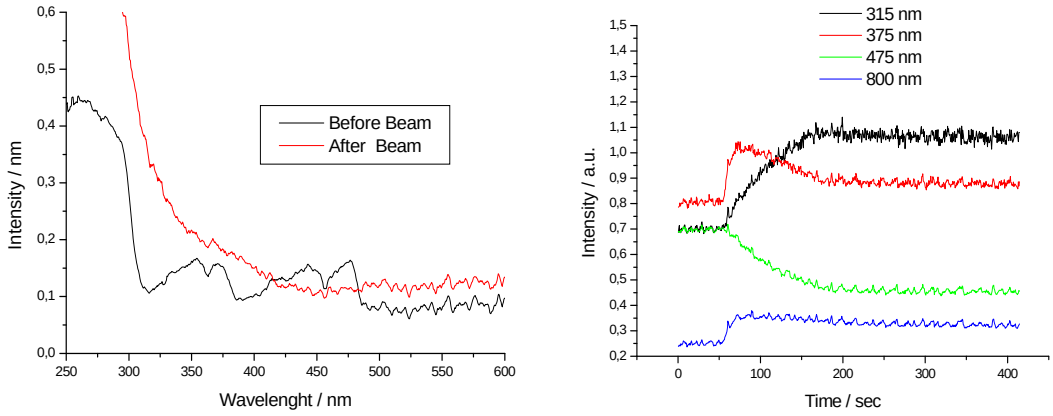

Figure 1 UV/Vis absorption spectra of the (6-4) photolyase in complex with the T(Dew)C. a) Whole spectra before (black line) and after irradiation (red line). (b) Spectral changes over exposure time.

Despite the fact that we were not able to answer the question regarding the fate of the DNA lesions during exposure to the X-ray beam, we can conclude that the previously reported crystal structure, most likely, shows the active site with a reduced FAD. In addition it can be assumed that larger photolyase complex crystals as well further development of the experimental setup, allowing measurements with greater sensitivity could provide a sufficient strong absorbance signal of the 6-4 photoproducts and therefore allow the determination of their photochemical properties and fate during irradiation.

References

1. Kim, S.T., Malhotra, K., Smith, C.A., Taylor, J.S. & Sancar, A. Characterization of (6-4)-Photoproduct DNA Photolyase. *J. Biol. Chem.* **269**, 8535-8540 (1994).
2. Sancar, A. Structure and function of photolyase and in vivo enzymology: 50th anniversary. *J. Biol. Chem.* **283**, 32153-7 (2008).
3. Glas, A.F., Schneider, S., Maul, M.J., Hennecke, U. & Carell, T. Crystal Structure of the T(6-4)C Lesion in Complex with a (6-4) DNA Photolyase and Repair of UV-induced (6-4) and Dewar Photolesions. *Chem. Eur. J.* ***15***, 10387-10396 (2009).
4. Maul, M.J. et al. Crystal structure and mechanism of a DNA (6-4) photolyase. *Angew. Chem., Int. Ed.* **47**, 10076-80 (2008).
5. Glas, A.F. et al. DNA (6-4) photolyases reduce Dewar isomers for isomerization into (6-4) lesions. *J. Am. Chem. Soc.* **132**, 3254-5 (2010).
6. Pearson, M.L., Ottensmeyer, F.P. & Johns, H.E. Properties of an unusual photoproduct of U.V. irradiated thymidylyl-thymidine. *Photochem. Photobiol.* **4**, 739-47 (1965).
7. Rycyna, R.E. & Alderfer, J.L. UV irradiation of nucleic acids: formation, purification and solution conformational analysis of the '6-4 lesion' of dTpdT. *Nucleic Acids Res.* **13**, 5949-63 (1985).
8. Franklin, W.A., Lo, K.M. & Haseltine, W.A. Alkaline lability of fluorescent photoproducts produced in ultraviolet light-irradiated DNA. *J. Biol. Chem.* **257**, 13535-43 (1982).
9. Kort, R., Komori, H., Adachi, S., Miki, K. & Eker, A. DNA apophotolyase from Anacystis nidulans: 1.8 A structure, 8-HDF reconstitution and X-ray-induced FAD reduction. *Acta Crystallogr D* **60**, 1205-13 (2004).