ESRF	**Experiment title:** Time-resolved Laue crystallography of photoactive yellow protein	**Experiment number:** LS-77 1
Beamline: ID9	**Date of experiment:** from: October 8 to: October 10, 1997	**Date of report:** Feb, 28th, 1998
Shifts: 9	**Local contact(s):** Michael Wulff	*Received at ESRF:* 13 MAR. 1998

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Report:

Goals:

The goal of our last experiment was to determine the structure of an early intermediate (I1) in the light cycle of Photoactive Yellow Protein (PYP) (Meyer, 1985). The I1 intermediate forms on the subnanosecond timescale and decays on the microsecond time scale (Hoff et al. 1994). Since the speed of this reaction is faster than typical protein movements the 11 structure is expected to have the isomerized configuration of the chromphore within a protein structure exhibiting only minor differences from the ground state structure. Consequently the questions we are ultimately trying to answer in our experiments are: How can the chromophore isomerize in an essentially static protein environment and how can a protein use the isomerized chromophore to trigger the protein structural changes that were observed in the later stages of the photocyle (Genick et al. 1997)?

Techniques;

During our experiments the ESRF storage ring was operated in single bunch filling. The "Juelich"-shutter was used to isolate the radiation from single bunches of electrons. We collected date using the fast-readout image-intensified CCD camera with a crystal to detector distance of 150 mm. All experiments were carried out at room temperature using wild-type PYP crystals (Borgstahl et al., 1995) mounted in glass capillaries. The reaction was initiated with a laser pulse generated either by pumping a dye laser set at 485 nm with the third harmonic of the beamline's Continuum Nd:YAG laser or by using the 355 nm third harmonic of the laser directly. The intensity of the laser beam was controlled by variation of the laser's Q-switch delay or by inserting neutral density filters between the laser and the optical fiber that was used to deliver the laser pulse to the crystal.

The delivery optics at the end of the fiber were positioned so that the X-ray beam and the laser pulse entered the same of the crystal. This insured maximum overlap between the volumes of the crystal that are light-activated and traversed by the X-ray beam. The laser wavelength, laser power, delay between the laser initiation and X-ray exposure, the number of exposures per image and the size of the crystal were varied for different data sets.

Results and Discussion:

From 15 crystals, we collected 17 data sets consisting of a minimum of 20 Laue diffraction images each before and after light activation. Although data processing and analysis are not yet completed, our experiments have yielded much new information. First, the radiation emitted by a single bunch of electrons passing through undulator 46 and wiggler 70 of beamline ID 9 provides sufficient intensity to generate diffraction patterns even from moderately sized (100x100x500 micron^3) crystals of PYP. However, the quality of the diffraction patterns was substantially increased by accumulating 10 or more individual exposures on the detector before reading them out as a single image. The diffraction data routinely extended to better than 2 Angstrom resolution and were indexed with RMSD deviations between predicted and observed spot positions of less than half the detector's pixel size (i.e. 60 microns). Transient streaking of diffraction spots, which can lead to uninterpretable Laue diffraction patterns, was far less pronounced in images collected with delay times of a few nanoseconds or several millisecond than in images taken with delay times of several microseconds. This confirms that the problem of excessive streaking after laser irradiation can be avoided when the delay time is chosen so that the X-ray exposure preceeds the pressure wave that is caused by the laser heating of the crystal (delay times << microsecond) or so that the X-ray exposure is delayed until the pressure wave has passed through the crystal (delay time >> microsecond). Data sets collected with delay times of less than 10 ns displayed spot shapes which were streaked out to no more than twice the diameter of spots observed from the same crystal in the absence of light initiation. When the poser fo the laser pulse was increase to above 4 mJ the streakiness of the diffraction pattern did not increase substantially, but the crystals often moved in the capillary, producing uninterpretable diffraction patterns. In the future it might be possible to use higher powered initiation pulses and thereby increase the population of light-activated molecules if we are able to prevent the "walking" of the crystals by mechanically restraining them in tight capillaries. Activation of crystals by 355 mn wavelength to prevent secondary light reactions of intermediate I1 showed promising results. A complete data set was collected before a PYP cry&al was damaged by the UV laser light.

Reference: Borgstahl et al. (1995) Biochemistry 34, pp. 6278
Genick et al. (1997) Science 275, pp. 1471
Hoff et al. (1994) Biophys. J. 67, pp. 1691
Meyer (1985) Biochim. Biophys. Acta 806, pp. 175