Nanosecond Time-resolved Macromolecular Crystallography: Probing Photo-initiated Protein Relaxation

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Structural changes in biological macromolecules are very fast at physiological temperatures and often occur on the sub-microsecond time-scale. Recent technological, instrumentation, and software developments[1] allow investigation of structural changes in protein crystals on the ns (and in the near future, sub-ns) time-scale at the third generation synchrotron sources such as ESRF (France), APS (USA) and SPring8 (Japan). We present here results of ns time-resolved crystallographic experiments conducted at the ID9 beamline of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France on carbonmonoxy complexes of two heme proteins: sperm whale myoglobin (Mb) and dimeric hemoglobin (HbI) from the clam *Scapharca inaequivalvis*. We also report our results on ns structural events in the photocycle of photoactive yellow protein (PYP).[2] The goals of these studies are to understand the evolution of the photo-induced structural changes and their propagation from the active site through the protein; to determine the trajectories and the docking sites of the photo-dissociated ligands in case of heme proteins; and to investigate how these trajectories are affected by mutations of side chains known to affect ligand binding properties. We use intense, focused, white, 150 ps and 1 microsec x-ray pulses in pump-probe type of measurements to investigate structural changes that are photo-initiated by 10 ns laser pulses. The reversibility of the reaction allows us to signal average (typically 10-30 fold) and to obtain complete and even multiple data sets from one crystal. Data are typically 70% complete to 1.7 Å resolution, with Rmerge = 12%. Departure of the CO ligand upon photolysis and its subsequent rebinding and the iron atom displacement from the heme plane are clearly observed in both heme proteins. We identified a possible docking site for the photodissociated CO molecule in the MbCO heme pocket and compared this site with the sites observed at low temperatures[3] and with the sites predicted by computational methods.[4] Our ns PYP investigations yielded the structure of the short-lived, red-shifted intermediate state that develops within 1ns after photoexcitation of the PYP chromophore.[2]

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[1]Bourgeois D. et al., J. Sync. Rad. 3, p. 65 (1996); Srajer V. et al., Science 274, p. 1726 (1996); Chen, Y. et al., Rev. Sci. Instrum. 65, p. 1506 (1994); Ren, Z. and Moffat, K., J. Appl. Cryst. 28, p. 461 (1995), ibid. p. 482 (1995); Ren, Z. and Moffat, K., J. Appl. Cryst.29, p. 246 (1996).

[2]Perman, B. et al., Science 279, p. 1946 (1998).

[3]Teng et al., Nat. Struct. Biol. 1, p. 701 (1994); Teng et al., Biochemistry 36, p. 12087 (1997).

[4]Vitkup et al., Nat. Struct. Biol. 4, p. 202 (1997).