Report for experiment LS 1994: Structural dynamics: time resolved diffraction of photolytic intermediates of myoglobin and hemoglobin mutants.

We are carrying out a structural and functional characterisation of multiple mutants of the distal heme pocket of myoglobin. This mutant, carries two new residues in the pocket distal to the heme (i.e. Leu(B10)Tyr and His(E7)Gln) and it has been fully characterized in their reactivity towards oxygen, carbon monoxide and nitric oxide (1-3). The high-resolution structure of different ligation states of this protein, obtained by us, provided the basis for a consistent picture of the molecular control mechanism of reactivity.

The triple mutant of myoglobin called Mb YQR was investigated previously by time-resolved Laue crystallography. We collected data sets in Dec 2000 and Dec 2001 at ID09. The latter sets of data, covering a time range from 3 nsec to 3 μsec, after reduction, scaling and refinement proved of exceptional value given the very good diffraction quality of the crystals of Mb YQR-CO. An example is shown in Figure 1. The percentage of photolysis was consistent with the amount of CO-bound Mb, and the difference maps clearly show: i) displacement of the Fe from the heme plane, ii) tilting of the heme itself, iii) conformational relaxation of distal side residues, and iv) movements of the helix E and water molecules.

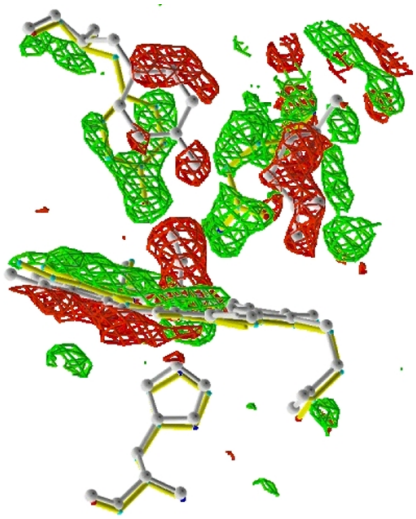

Fig. 1: F_{off}-F_{on} electron density maps of YQR myoglobin 32 ns after the photolysis of CO. The maps, obtained from Laue data collected at ESRF - ID09 on December 2001, are contoured at 3.5 σ.

The final picture emerging from these and other studies (4-5) will be compared with the predicted role of protein cavities and internal packing defects in determining the preferential pathway to and the reactivity of the heme in hemeproteins (6). These findings add to our understanding of the structure-function relationship in proteins at large, but may also be relevant to biotechnology in so far as they may help designing better mutant hemoglobins that do not interfere with nitric oxide physiology and thus may be better candidates as blood substitutes.

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