Time-Resolved Three-Dimensional Study of Ligand Rebinding in Carbonmonoxy Myoglobin

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The time course of Bragg reflections from MbCO before and after photodissociation of the ligand by a laser pulse has been measured at room temperature with a time resolution of 0.5 msec using synchrotron radiation. Reflection data were collected with a linear PSD (A. Gabriel) and accumulated over about 1000 repetitions (flashes) in 100 time frames. The timing unit triggered also the laser (Xe^*Cl excimer laser pumping a Rhodamin-6G dye laser). The laser repetition rate was 3-5 sec^{-1}, the wavelength 590 nm, the pulse duration 10 nsec.

Intensity changes (either increase or decrease) upon laser excitation have been observed for several (out of in total a dozen) reflections. One example is shown in Figure 1a-c. The reflection intensity relaxes exponentially after the flash with a lifetime of about 3 msec. We may assume that all ligands have been debound by the 500 μJ laser pulse. The intensity observed immediately after the flash, I_o (Fig. 1b), will then correspond to a transient deoxy form of Mb with a different structure factor and slightly different cell dimensions. The relaxation to MbCO (intensity I_1) follows the exponential rebinding of the ligand with a rate constant which is in qualitative agreement with optical results obtained by Frauenfelder and coworkers for MbCO in solution at room temperature (Austin, Beeson, Eisenstein, Frauenfelder and Gunsalus, Biochem.(1975)14, 5355).

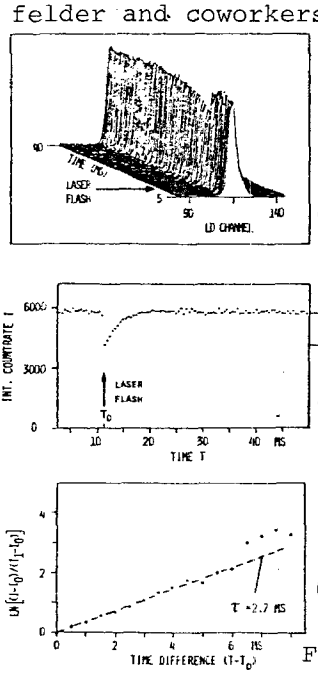

Fig.1

The present study proves the feasibility of time-resolved three-dimensional structure determination on a submillisecond time scale. Further work is underway including use of an area detector (C. Boulin and A. Gabriel) and crystal cooling to subzero temperatures. Even higher time resolution, on a submicrosecond scale, appears to be within reach. This might lead to three-dimensional information on intramolecular motions as a function of time from analysis of temperature factors (Frauenfelder, Petsko and Tsernoglou, Nature (1979)280,558). In theory, a subnanosecond range might be reached in such investigations by exploiting the pulsed time structure of synchrotron radiation in "stroboscopic" data collection.