currently in progress to estimate the effect of the radial distribution of ionization on the fluorescence decay. Monte Carlo techniques are being used to determine the initial spatial distribution of spurs, and the spur overlap is calculated from Eq. 2. The results of these calculations will be compared with measurements of the fluorescence decay with proton and alpha irradiation at the same stopping power. These experiments should provide a crucial test of our intratrack quenching model.

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PICOSECOND PHOTODISSOCIATION AND SUBSEQUENT RECOMBINATION PROCESSES IN CARBOXYHEMOGLOBIN, CARBOXYMYOGLOBIN, AND OXYMYOGLOBIN

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The central problem in the study of hemoglobin is to understand the mechanism of cooperativity among the four subunits of the molecule. This cooperativity is evident in the sigmoidal nature of the oxygen saturation (equilibrium) curve and in the Bohr effect. Paramount in the understanding of cooperativity is the study of the trigger mechanism of ligand release and the tertiary and quaternary protein structural changes subsequent to this. Picosecond time-resolved spectroscopy is a relatively new experimental method capable of critically examining the dynamics of ultrafast molecular events in proteins—processes serving as precursors to other extensive protein structural changes. The application of this method with regard to photodissociation measurements on heme proteins permits one to obtain rate data on such processes in a heretofore inaccessible time region. This information, in turn, allows an analysis of the allosteric mechanism(s) of cooperativity from a totally different experimental perspective.

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Since it is of interest to detect the primary structural events cited above, we felt that a picosecond kinetic study of carboxymyoglobin (MyCO) and oxymyoglobin (MyO_2) would be desirable for several reasons. First, it would provide information on protein structural relaxations immediately after photodissociation (known to be facile [1], $\leq$10 ps) in a picosecond time window where such changes are likely to be seen. Second, the structural motions are limited to those of the tertiary type confined to one heme unit, thereby simplifying the interpretation of the effect. In other words, the interpretation of the relaxation results for tetrameric deoxyhemoglobin is not straightforward because of the necessity of differentiating between tertiary and quaternary protein motions and of accounting for the relaxation of individual subunits. Finally, picosecond photodissociation experiments on MyO_2 and MyCO should prove to be of great value since the electronic destabilization of the heme pocket caused by facile ligand detachment must be followed by the evolution of tertiary structural changes until a stable form of the deoxyhemoprotein results, the extent of and the type of destabilization reflecting the differences in the bonding of O_2 and CO to the heme.

Excitation of MyCO and MyO_2 by a single 530 nm, Nd^{+3}-glass laser, 8-ps pulse results in photodissociation monitored by following absorption changes at 440 nm in the Soret band as a function of time (2). Photodissociation occurs in less than 6 ps for both molecules. MyCO shows an additional decay immediately after photodissociation, having a rate $(6.7 \pm 2.7) \times 10^9\ s^{-1}$ ($\tau = 130 \pm 50$ ps) to a steady state that is the stable form of deoxymyoglobin. The presence of such relaxation in MyCO and the absence of relaxation in MyO_2 is a strong indication that the electronic destabilization after ligand detachment is much greater for MyCO than for MyO_2. Based on existing theory (3, 4), we attribute the relaxation in MyCO to tertiary structural changes in the heme pocket.

Because the photodissociation of HbCO provides information on the trigger mechanism for the cooperative binding of ligands to Hb (*vide supra*), we felt that a more complete, wide-range, kinetic investigation of the dissociation and recombination in HbCO would complement the myoglobin study. Excitation of carbon monoxide hemoglobin, HbCO, by a single 530-nm, 6-ps pulse results in photodissociation with a first-order constant of $0.89 \times 10^{11}\ s^{-1}$ ($\tau = 11$ ps). The kinetics of photodissociation, monitored by following absorbance changes in the Soret band at 440 nm, are interpreted as corresponding to predissociation followed by a crossing into a dissociative state. Subsequent recombination of CO with the porphyrin system and protein structural transformations are monitored by use of a continuous He-Cd laser beam spacially coincident with the photolysis and Soret interrogation beams at the sample. We find that the latter events take place in three distinct time regions depending on excitation pulse energy and repetition rate. Excitation of HbCO with a single pulse (0.8–5 mJ) results in a relaxation to the ground state with an associative first-order constant of $5 \times 10^3\ s^{-1}$. With a 100-pulse train ($\sim$7.5 mJ), a new decay grows with a rate constant of $63\ s^{-1}$. For a pulse train energy of 12 mJ or higher, a delay occurs at the onset of the second (slower) recombination. Our pulse train experiments, depending on the

pulse energy, reflect higher degrees of complete photodissociation of the heme sites, followed by possible tertiary and quaternary structural changes on recombination.

An important conclusion is that structural inferences derived from our picosecond experiments on MyCO, and particularly those on HbCO with regard to conformational and tertiary content, may not necessarily be transferrable to the conjugate molecules, MyO_2 and HbO_2, respectively.

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TIME-RESOLVED MAGNETIC SUSCEPTIBILITY

A NEW METHOD FOR FAST REACTIONS IN SOLUTION

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The high sensitivity and rapid response of superconducting magnetometers now make possible the measurement of changes in magnetic susceptibility during fast biochemical reactions in room-temperature samples. This new method has been demonstrated by measuring the recombination kinetics of hemoglobin and carbon monoxide after flash photolysis of HbCO at 20°C. The rate constants so determined are in excellent agreement with those obtained by photometric techniques.

A unique capability of this method is the determination of the magnetic susceptibilities of short-lived reaction intermediates. In partial photolysis experiments the magnetic moment of the intermediate species $Hb_4(CO)_3$ was found to be $4.9 \pm 0.1\ \mu B$ in phosphate buffer. This value compares to $5.3\ \mu B$ per heme for deoxyhemoglobin under these conditions (1), and the difference indicates the change in quaternary structure when three ligands are bound. It is important to note that this method can be used to determine magnetic moments of reaction intermediates such as ferrous heme, which are paramagnetic but show no electron spin resonance signals.

Major improvements in the sensitivity and time resolution of this technique are expected. At present the time resolution is limited by eddy currents to 300 μs, and the

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