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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noise level is equivalent to a change in concentration of a spin half species of 7×10^{-7} M(Hz)$^{-1/2}$. Both higher sensitivity and 1-μs time resolution are possible with existing technology. Furthermore, in the near future magnetometers with nanosecond time resolution are expected. Such improvements should greatly extend the utility of this method for studies on biochemical systems.

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CARBOXYLATION KINETICS OF HEMOGLOBIN AND MYOGLOBIN

LINEAR TRANSIENT RESPONSE TO STEP PERTURBATION BY LASER PHOTOLYSIS

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The photochemical kinetics of the reactions of myoglobin and hemoglobin with carbon monoxide, in the time domain 10^{-4}–10^{2} s, have been measured with high precision using a step-modulated continuous wave argon ion laser as the photolytic source. A steady state of the chemical system is fixed by the DC component of the amplitude-modulated laser beam; the oscillatory (square wave) component of the beam induces small perturbations of this steady state. The system's CO binding response is followed by monitoring optical absorbance at 435.6 nm. Digital transient recording on a quasi-logarithmic time scale enables single-sweep measurement of a decay with (typically) 10 decades of rate resolution; transient averaging provides the desired signal enhancement. Preparation of controlled protein-CO solutions was carried out in a closed mixing cell integrally connected with an optical measurement cell, designed to overcome sample heating and convection.

The linear kinetic response to small perturbations consists of a superposition of a set of eigenmodes, each an exponential relaxation. The CO-myoglobin response transients are single-mode (single exponential) in character; the rate constants vary linearly with DC laser intensity and free CO concentration, yielding at 20°C $k_F = 2.12 \times 10^5 M^{-1}s^{-1}$ for the combination kinetic constant, and $Q = 0.97 \pm 0.04$ for the photolytic quantum yield. The hemoglobin kinetic response transients are multi-component in character. They have been adequately fitted, using nonlinear least squares fitting methods, by a response function consisting of the sum of three exponentials.[1] The two

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[1] Additional eigenmodes which contribute less than 5% of the total transient amplitude are not detectable by our analysis.