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.

REFERENCE

1. ALPERT, Y., and R. BANERJEE. 1975. *Biochim. Biophys. Acta.* **405**:144.

CARBOXYLATION KINETICS OF HEMOGLOBIN AND MYOGLOBIN

LINEAR TRANSIENT RESPONSE TO STEP PERTURBATION BY LASER PHOTOLYSIS

D. D. SCHURESKO AND W. W. WEBB, *School of Applied and Engineering Physics, Cornell University, Ithaca, New York 14853 U.S.A.*

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

---

Dr. Schuresko's present address is: Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830.

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

fast relaxations have rate constants which vary approximately linearly with the free CO concentration and the DC laser intensity; the slow relaxation rate constant is independent of both variables. The amplitude of the faster of the two CO-bonding modes predominates at both ligand saturation extremes, as has been previously observed for O_2-hemoglobin (1). Computer eigenmode calculations based on either simple sequential or allosteric schemes fail to reproduce all of this fitted data; hybrid models featuring weakly coupled parallel reaction pathways do reproduce the observed data features.

We wish to thank Professor Q. H. Gibson of Cornell for his suggestions and encouragement.

REFERENCE

1. Ilgenfritz, G., and T. M. Schuster. 1974. *J. Biol. Chem.* **239**:2959–73.