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Lawrence J. Parkhurst
University of Nebraska - Lincoln, lparkhurst1@unl.edu

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New and Notable

A Nanosecond ORD Study of Hemoglobin

Lawrence J. Parkhurst
Department of Chemistry, University of Nebraska,
Lincoln, Nebraska 68588-0304 USA

This year marks the one hundredth anniversary of the first observation of CO photodissociation from HbCO, with the correct interpretation and publication following in the next year (Haldane and Smith, 1896). Little of kinetic importance could be learned from this photosensitivity, however, until the development of sensitive photodetectors and short duration high intensity light sources. These allowed Gibson, in pioneering discoveries in the mid to late 1950's (Gibson, 1956, 1959), to discover "Hb*," a rapidly reacting form of hemoglobin, which we now associate with deoxy Hb in the tertiary relaxed "r" state with the protein in the quaternary R state. Because this state was not readily accessible by rapid mixing experiments, photolysis was needed to generate the reactant. It became clear that cooperative binding of CO was largely manifested through changes in the association rate constant. Photolysis also allowed Gibson to make what were probably the first precise measurements of the rate of a conformational change in a native protein, the R→T transition in hemoglobin (Gibson, 1959).

During the past twenty years, new instrumentation and experimental techniques, which derive from the early photolysis studies, have provided for myoglobin and hemoglobin what are arguably the most precise details of the reaction potential energy surface available for any protein. Discoveries made and concepts developed during this period include geminate recombination (Duddell et al., 1979), strongly nonexponential ligand rebinding at low temperatures (leading to the concept of a multitude of conformational substates) (Austin et al., 1975), tertiary relaxations in the heme and heme environment (Scott and Friedman, 1984), and intermediate ligand states within the protein matrix (Jongeward et al., 1988; Murray et al., 1988). The paper by Shapiro et al. (1995) in the previous issue reports both the development of a new experimental technique for measuring optical rotatory dispersion in the nanosecond time regime and its application to the study of geminate rebinding and conformational transitions in hemoglobin.

One of the intriguing questions in hemoglobin kinetics is whether one can detect distinct states between the canonical R and T quaternary structures to provide some "snapshots" of this process, the slowest relaxation after ligand photolysis. Various hybrid hemoglobins have unusual kinetic and spectroscopic features that are suggestive of such intermediate conformations, but these hemoglobins have not been subjected to detailed kinetic studies in the 0.1–20 μs time regime. Recent time-resolved absorbance change studies, after photolysis of MbCO and HbCO, suggest that the various geminate processes and heme relaxations have all occurred at room temperature by 1 μs (Jones et al., 1992). Thereafter, subunit communication results in the R→T conformational change in hemoglobin. These studies have involved the analysis of extensive data sets where photolysis energy, probe wavelength, temperature, and solvent viscosity have been varied, and SVD analyses have been used to derive basis spectra and rates of transitions involving states defined by these difference spectra (Jones et al., 1992; Ansari et al., 1994). There was no evidence for other than a single process connecting R and T states. A recent publication from the Spiro laboratory, however, reports that the W17 resonance Raman difference spectrum of beta-37 Trp, at the alpha$_1$-beta$_2$ interface, shows changes on the 1 μs time scale that might be interpreted as providing evidence for a structure that is intermediate between R and T (Rodgers et al., 1992). Rates of both the tertiary relaxation and the R→T change have been shown (Scott and Friedman, 1984; Parkhurst and Parkhurst, 1990) to be sensitive to solvent composition, and it has been suggested (Parkhurst and Parkhurst, 1990) that the sensitivity to phosphate is evidence for a T-like intermediate in the quaternary transition. The recent report of a new R state for human hemoglobin (Silva et al., 1992) may also bear on the question of conformational intermediates. Intimately linked with the allosteric conformational change is the nature of the so-called "storage" of ligation free energy, whether it is localized or diffuse, and how it is coupled to the quaternary conformational transition.

ORD was originally the more popular technique for studying conformational changes in biomolecules or models of biomolecules because of the difficulty of making measurements far into the UV where CD could be observed and because of the difficulty of collimating the light adequately for Pockels cells. The advent of photoelastic modulators (which have large acceptance angles) led to the development of relatively inexpensive CD spectrophotometers. CD measurements showed that the Soret bands of many hemoglobins were optically active, with CD spectra that changed when the subunits of hemoglobin were combined to form the native hemoglobin, as well as when various changes were made in the globin (Geraci and Parkhurst, 1981; Zentz et al., 1994). Theoretical calculations suggested that the Soret CD spectrum should be sensitive to changes in the relative orientation of the heme with respect to aromatic residues in the heme cavity and even to changes in the subunit interfaces (Hsu and Woody, 1971).

It is difficult to make photoelastic modulators that will function well for times below a few microseconds. The Kliger laboratory recently developed

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transient CD on the nanosecond time scale (Lewis et al., 1985), making careful use of the optical calculus developed by R. C. Jones. In the latest paper (Shapiro et al., 1995), the optical calculus of Mueller was used to develop the dispersive counterpart of the transient CD, here known as transient ORD, or TRORD. There appear to be several advantages to the new technique. First, it has a much higher S/N ratio than TRCD. Second, the instrumentation appears simpler. Third, one can, as with ORD, detect changes in optical activity far from the optically active absorption bands. Finally, one can, if so desired, use complex Hilbert transforms (Kramers-Krönig relations) to obtain the CD spectrum at a given wavelength from the complete ORD spectrum. In one of the first applications of the new method, photolysis of HbCO was studied as a function of wavelength in the nanosecond to millisecond time regime and SVD analyses used to obtain minimal basis spectra and associated rate constants. Of considerable interest is the finding, when TROD and TRORD data were combined in a restricted global analysis, as shown in Table 2 and described in the paper, of a fifth exponential phase with a time constant of about 1 μs. Perhaps this is related to changes found by the Spiro laboratory by time resolved UVRR spectroscopy. Studies on modified hemoglobins and on solutions where the solvent is varied should further elucidate the nature of this phase. We can expect that the high S/N of the TRORD technique will lead to important results and insights by allowing one to follow protein folding and nucleic acid-protein interactions by photolysis and other techniques.

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A Novel Computational Prediction of Ion Effects in Oligocation-Oligonucleotide Equilibria

Pamela Mills

Department of Chemistry, Hunter College, New York, New York USA

The most exciting and dangerous event for a theoretician is the prediction of an unforeseen physical-chemical phenomenon that can be verified experimentally. Olmsted et al. (in this issue) examine the salt dependence of the association of an octacation with nucleic acids of varying lengths using Grand Canonical Monte Carlo simulations. They use the extraordinarily simple cylindrical model to make the novel prediction that the salt dependence of the association of an octavalent cation with the interior region of nucleic acids of varying length achieves a maximum for a finite length oligonucleotide. This prediction, if experimentally verified, has implications with regard to the stability of oligocation-oligonucleotide complexes and may aid in the evaluation of the accuracy of computational models of nucleic acid solutions.

The salt dependence of the equilibrium association constant (obtained from the slope of a $\log K_{obs}$-$\log[Na^+]$ plot and denoted $-S_a K_{obs}$) as a function of oligonucleotide length demonstrates that end effects may significantly influence the physical chemistry of nucleic acids. For the association of an octavalent cation at low salt concentrations, the predicted values of $-S_a K_{obs}$ range from 2.5 for very short oligonucleotides to 7.3 for long oligomers and pass through a maximum of 10.2 for oligomers of intermediate length (36 bp). Thus, the equilibrium constant is predicted to decrease by three orders of magnitude with a twofold increase in salt concentration for intermediate length oligomers. These predicted changes in the sensitivity of the equilibrium association constant to salt con-

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