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New and Notable

Analysis of Relaxation Processes Helps to Define Molecular States in Biological Systems

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Molecular interactions between small molecules and macromolecules are key to understanding biological control mechanisms in living systems. One such system, the recombination of carbon monoxide with myoglobin following photodissociation, has been studied exhaustively in many laboratories, but we are only beginning to understand the detailed physics and chemistry of the intermediate steps that make up this process. It is hoped that a thorough understanding of this relatively simple process can help us to understand more complex reactions in living systems, and indeed this hope appears to be justified. Early studies (Austin et al., 1975) demonstrated that a simple first order process described by an exponential function yields a poor fit to observed relaxation data. When sufficiently precise observations are made over a very wide range of time and temperature, the data can be described by a power function, and interpreted by a distribution in the energy of activation. This result led to the concept of a distribution of microconformational states of the protein (Frauenfelder et al., 1991), in addition to major conformational populations, each of which can be described by a slightly different activation energy. The critical difference from a homogeneous system is that these micro-conformational states do not equilibrate rapidly relative to the observed overall reaction. Multiple conformational energy minima have also been described by subnanosecond molecular dynamic simulations (Elber and Karplus, 1987), and by kinetic hole burning (Srajer and Champion, 1991) of near-infrared absorption bands.

Carbon monoxide makes a very useful probe of local molecular interactions, since it is highly polarizable and absorbs strongly in the infrared spectral region where few other bands occur. The deviation from the free gas vibrational frequency (2144 cm^{-1}) is a measure of the strength of perturbation by a noncovalently interacting group or by a coordinated metal. Thus in myoglobin, the Fe-CO absorbs near 1945 cm^{-1} (A states, in Frauenfelder's terms), while photodissociated carbon monoxide that is noncovalently associated with a group in the heme pocket absorbs near 2130 and 2118 cm^{-1} (B states). The latter bands are observed only at cryogenic temperatures (e.g., 10 K) or as transients by picosecond spectroscopy. Recent work by the Frauenfelder group (Mourant, et al. (1993), this issue) makes use of a very powerful analytical technique in which the time course of the reaction is monitored at constant rate of temperature change (dT/dt). This technique leads to three-dimensional maps of the reacting species and has been used to determine which molecular species interconvert during the relaxation process. Thus during the relaxation protocol, B_2 (2118 cm^{-1}) converts to B_1 (2130 cm^{-1}), which then recombines to form the heme·CO complex.

The concepts of distribution of activation energies and of microconformational states within a configurational population has been applied to more complex biological systems such as mammalian cytochrome *c* oxidase (Fiamingo et al., 1982, 1986, 1990) and related bacterial enzymes (Shapliegh et al., 1992). The cytochrome oxidases are multi-subunit enzymes that contain an iron-copper binuclear center, at which dioxygen is reduced to water with conservation of chemical energy. Carbon monoxide photodissociated from cytochrome a_3FeCO is trapped by the adjacent Cu_B, forming Cu_BCO, which can thermally dissociate to reform the original a_3FeCO at temperatures above 140 K. In this case, the CO relaxation follows an Arhenius temperature dependence, with a power law time dependence for each of the major configurational components of the enzyme.

Questions for the future: While much of the relaxation process has now been described, several questions remain unanswered. The chemical groups with which photodissociated CO associates in the heme pocket remain undefined, although the possibilities are obvious from molecular models. A more vexing problem may be defining the initial configuration of the heme and conformation of the protein following absorption of light. In case of the first photon absorbed by each heme group, the iron changes spin state, the coordinated CO is ejected, and excess kinetic energy is dissipated by vibrational and translational interactions with surrounding molecular groups of the protein. However, when sufficient light is applied to photodissociate nearly 100% of the carbonyl complexes, there will be a distribution of total energy absorbed per heme such that many of the hemes will have absorbed two or more photons, which translate as kinetic energy or a local temperature increase of several hundred Kelvin (Henry et al., 1986). The result can be a distribution of configurational and conformational states of the heme and surrounding protein toward the deoxy- conformation in the first few picoseconds. Such a distribution would be frozen by rapid cooling to the equilibrium matrix temperature at which CO relaxation kinetics are observed, and would contribute to the observed distribution of activation energies. It may be important to distinguish a distribution of conformational states induced by absorption of secondary photons by the heme from a similar distribution in the ground state produced by cooling from room temperature. The first, but not the second distribution should depend upon the fractional photolysis of the heme-CO complex. We

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look forward to future contributions directed to this problem.

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Filling the Gaps in Ca^{2+} Channel Regulation

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Regulation of the cardiac L-type Ca^{2+} channel has served well as a model for modulation of voltage-gated channels by phosphorylation (Hartzell and Duchatelle-Gourdon, 1992). In heart, norepinephrine released from sympathetic nerves acts on β-adrenergic receptors to increase the Ca^{2+} current (I_{Ca}) via a mechanism that is thought to involve cAMP-dependent phosphorylation of the Ca^{2+} channel. Although phosphorylation clearly controls Ca^{2+} channel function somehow, it has been difficult to prove that the Ca^{2+} channel itself is the ultimate target of phosphorylation and to relate its gating properties to its phosphorylated states. Some steps toward this goal have recently been published, one of which is the paper by Herzig et al. (1993) in this issue.

The problems in understanding how phosphorylation relates to channel gating became evident with the earliest single channel studies. On the basis of macroscopic currents, Reuter & Scholz (1977) proposed in 1977 that the increase in Ca^{2+} current in response to β-adrenergic agonists was due to an "increase in the number of functional conductance channels." When this was first tested at the single channel level in Reuter's (Reuter and Scholz, 1977) and Trautwein's (Brum et al., 1984) labs, however, it appeared that cAMP increased open probability (p_o) by accelerating the rate constants leading to channel opening with no change in the number of channels, N. Shortly thereafter Tsien's lab (Bean et al., 1984) showed that the increase in p_o was not large enough to explain the magnitude of the increase in I_{Ca} and that N increased as well. The apparent contradiction between these studies was resolved by assuming that channels could cycle slowly on the timescale of several seconds between two states, one competent to be opened by voltage and one nonfunctional (Tsien et al., 1986). β-Agonists do not increase the number of channels in the patch, but rather increase the probability that the channel will be in the available state. This conclusion was promoted by the finding (Cavalie et al., 1986; Ochi and Kawashima, 1990) that in a series of depolarizations, sweeps having channel openings ("active sweeps") and sweeps having no openings ("blank sweeps") were clustered in a strikingly nonrandom fashion. Although these studies provided a valuable hypothesis, the goal of elucidating the quantitative relationship between phosphorylation and Ca^{2+} channel function has remained elusive.

One reason for this difficulty is that a quantitative analysis of the clustering of active sweeps has not been accomplished. Traditional continuous-time Markov analysis of open and closed times is useful in analyzing the rapid (millisecond) gating transitions within a short depolarization, but channel inactivation rends traditional Markov analysis inadequate for long depolarizations, because closed time histograms are dominated by transitions from inactivated states. Furthermore, with continuous-time Markov analysis, there is no formal way of dealing with slow transitions that occur in the gaps between the depolarizing steps. Herzig et al. (1993) have filled this gap by applying discrete-time Markov analysis to understanding these slow transitions. This provides the first quantitative framework for dealing with the slow transitions. According to their model, there are multiple gating modes corresponding to permutations of two or more phosphorylation sites. Site 1 must be phosphorylated for the channel to be available to open and yield active sweeps. Phosphorylation of a second and possibly more sites alters the fast gating properties within channels that are available to open. As predicted from this model, isoproterenol increases the forward rate constant for the transition from an unavailable to an available state, and the protein phosphatase inhibitor okadaic acid decreases the backward rate constant for this reaction. In addition, isoproterenol decreases the backward rate constant as expected if cAMP-dependent phosphorylation of protein phosphatase inhibitor-1 were to activate this inhibitor and slow phosphatase activity.

This formal, quantitative analysis of the slow transitions in Ca^{2+} channel gating makes an important biophysical step toward proving Tsien's (Tsien et al., 1986) proposal that "the calcium channel has two phosphorylation sites, one that controls slow transitions between available and unavailable states,

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