COMMENTS

Analysis of an Activation-Energy Spectrum and the Existence of Myoglobin Conformers

P. H. Fang
Department of Physics, Boston College, Chestnut Hill, Massachusetts 02167

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The continuous activation-energy spectrum of the photodissociation of carbonmonoxy myoglobin reported by Austin *et al.* is investigated. I find that the existence of the continuous spectrum, and thereby the implication of myoglobin conformers, could be a consequence of their assumptions in the analysis, and that the result, therefore, is ambiguous.

In a recent work on the photodissociation of carbonmonoxy myoglobin, Austin *et al.*[1] measured the transient curves of temperature dependence of the rebinding of carbon monoxide to myoglobin. From these curves, they stated that the reaction is governed by a continuous energy spectrum, and not by a single activation energy. I seek to show in the present paper that (1) on the basis of their evidence, one cannot rule out the possibility of two discrete activation energies, and that (2) a continuous energy spectrum could have resulted from an implicit assumption in their analysis.

Austin *et al.* found that the fraction of myoglobin molecules that have not rebound at time t after the photoflash, $H(t)$, satisfies the relation[1]

$$H(t) = H(t,T)/H(0,T) + (1 + t/t_0)^{-n}, \tag{1}$$

where T is the absolute temperature of reaction, and t_0 and n are adjustable parameters which are dependent on T.

(1) Equation (1) can be derived from a familiar reaction-kinetics equation[2]:

$$dH(t,T)/dt = -c[H(t,T)]^{\alpha}, \tag{2}$$

where $c = nH(0,T)^{-1/n}/t_0$ and α is the *order of reaction.* The solution of Eq. (2) with the initial condition that $H(t,T) = H(0,T)$ at $t=0$ leads to Eq. (1) with an identification

$$n = 1/(\alpha - 1)$$

except when $\alpha = 1$. The case where $\alpha = 1$ will be discussed in connection with Eq. (5).

Now, Eq. (1) has two temperature-dependent parameters, t_0 and n. From Fig. 1 of the experimental data of Ref. 1, one can introduce two discrete activation energies. The experimental data give

$$t_0 = 4.16 \times 10^{-8}\, e^{0.069/kT} \text{ sec}, \tag{3}$$

$$n = 1.15\, e^{-0.010/kT}; \tag{4}$$

that is, the activation energy for t_0 is 0.069 eV and that for n is 0.010 eV. With these two discrete energy values, one can fit $H(t)$ curves at all t and T without assuming a *continuous energy spectrum.*

(2) The question now is the origin of the continuous spectrum of Austin *et al.* I traced the answer to their usage of a formalism developed originally by Primak[3]:

$$H(t) = \int_0^{\infty} g(E)\, e^{-\lambda t}\, dE. \tag{5}$$

Here $\lambda = (\text{frequency factor}) \times \exp(-E/kT)$, with E the activation energy and $g(E)$ the distribution function of E. From Eq. (5), $g(E)$ can be found as an inverse Laplace transform of $H(t)$, as done by Austin *et al.* According to Eq. (5), if and only if $H(t)$ is an exponential function, that is, $H(t)$ obeys first-order kinetics, can $g(E)$ be a δ function, i.e., a single activation energy. In general, with a very mild restriction on the existence of an inverse Laplace transform, Eq. (5) says that $H(t)$ can be interpreted as the result of the first-order kinetics with a distribution of E. On the other hand, one can formulate a formalism similar to that of Primak with second-order kinetics ($\alpha = 2$), and replace Eq. (5) by the following:

$$H(t) = \int_0^{\infty} [g_2(E)/(1 + \lambda t)]\, dE. \tag{6}$$

In this case, a new $g_2(E)$ can be determined from the Stieltjes transform of the same $H(t)$.[4] However, g_2 would be different from g.

Equations (5) and (6) are special cases of a general representation problem:

$$H(t, E_0) = \int_0^\infty g(E)\bar{H}(t, E)\,dE. \tag{7}$$

In Eq. (7), if $\bar{H}(t, E)$ is linearly proportional to $H(t, E)$ in t space, then $g(E)$ is a δ function, i.e., a null distribution; if not, $g(E)$ becomes continuous. Therefore, the existence of a continuous distribution is dependent on a prior knowledge of α which determines $\bar{H}(E, t)$. In the work of Austin *et al.*, the value of α is not discussed except in their adaptation of Eq. (5) which is equivalent to assuming that $\alpha = 1$. On the other hand, we found[5] that macromolecular reactions usually involve complex kinetics; and instances are known where the reaction does not obey first-order kinetics. Without having direct physical evidence at hand, I have looked into the following problem: Based on $H(t)$, is there a mathematical principle to determine $g(E)$ independently from $\bar{H}(t, E)$? I found the answer to be negative.[6] Therefore, it seems that the proposed myoglobin conformer of Austin *et al.*, intuitively plausible or biologically important as it may be, is not unambiguously established.

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Dependence of the Electronic Transition Moment on Internuclear Separation in Na_2: A Quantum Mechanical Treatment

P. F. Williams and D. L. Rousseau
Bell Laboratories, Murray Hill, New Jersey 07974

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We present a quantum mechanical treatment of laser-induced atomic fluorescence from molecular sodium. It is shown that such fluorescence data may be analyzed with an r-centroid approximation and an accurate determination of the electronic transition moment may be made.

The proper interpretation of the optical spectra of simple molecules requires knowledge of the variation of the electronic transition moment with internuclear separation. This variation must be considered when analyzing absorption, emission,[1] and resonance Raman data.[2] Experimental measurements of the separation dependence of the transition moment have been scarce and have relied for the most part on r-centroid analyses of discrete vibrational spectral progressions.[3] Recently Gersten[4] has described a new technique for measuring the transition moment by monitoring the atomic fluorescence resulting after transitions from a ground electronic state to a dissociative excited state. Callender *et al.*[5] extended this method of induced atomic fluorescence to apply to cases in which the upper electronic state has an attractive potential. Their technique, if accurate, would appear to be preferable to previous methods in that it does not require numerical calculations of matrix elements and needs only approximate knowledge of the two potential functions involved.

In the experiments reported by Callender *et al.*[5] a laser beam was passed through a cell containing sodium dimers. The laser induces energy-conserving transitions between the bound vibrational-rotational states of the ground electronic state $X(^1\Sigma_g{}^+)$ and levels of the vibrational-rotational manifold of the excited electronic state $B(^1\Pi_u)$. If the laser induces a transition to a state above the B dissociation limit, the excited dimer dissociates producing excited free sodium atoms because of the very short lifetime for dissociation as compared to molecular fluorescent re-emission. The fluorescence intensity of the