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Dynamics of Proton Translocation in the Primary Process of Vision

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Abstract: Proton translocation may play an important part in the formation of bathorhodopsin, an intermediate species formed at an early stage of the visual process. A quantum mechanical model of the visual chromophore that permits calculation of the rate constant for translocation is presented. The proton is assumed to move in a double-minimum potential well, the zero-order states being determined in the double-adiabatic approximation. The rate constant is given within the linear response approximation as a closed expression in terms of four rate parameters. A fit of experimental data to this expression accounts for strong non-Arrhenius thermal and deuterium isotope effects in the measured rate constant for formation of bathorhodopsin.

I. Introduction

The early photochemical events in the process of vision remain a topic of vigorous research and discussion (for reviews, see ref 1–6). The process is initiated with the absorption of a photon by the 11-*cis*-retinal chromophore of rhodopsin; the electronically excited rhodopsin then rapidly decays to produce an intermediate species bathorhodopsin (prelumirhodopsin). The precise mechanism of formation of bathorhodopsin is, however, a matter of continuing controversy. It was originally suggested by Yoshizawa and Wald,[7] and subsequently has been argued by others,[8-10] that bathorhodopsin results from the isomerization of 11-*cis*-retinal to the all-trans form. The finding by Busch et al.[11] that bathorhodopsin is formed at room temperature in less than 6 ps raised doubts about whether isomerization of the relatively bulky retinal chromophore could take place so rapidly and inspired suggestions[12-15] that proton translocation may play an important role. Later picosecond measurements[8] and molecular dynamics studies[16,17] indicated that photoinduced isomerization of retinal can, indeed, occur on a picosecond time scale. However, other picosecond time-resolved measurements[18] of the thermal and deuterium-isotope dependence of the rate constant for formation of bathorhodopsin strongly suggest the involvement of proton translocation. More recent work by Becker and Freedman[19] also supports this hypothesis. Thus, while there seems to be little doubt that both cis–trans isomerization and proton translocation are involved in the primary process, the detailed mechanism remains obscure.

A key experiment is that of Peters et al.[18] who observed a marked non-Arrhenius thermal dependence of the rate constant k_H for formation of bathorhodopsin. Parallel determinations of k_D for deuteriated samples showed a strong isotopic effect on the rate constant, namely $k_H/k_D \sim 7$, at low temperatures, in addition to a non-Arrhenius thermal dependence of k_D. On the basis of these findings, Peters et al.[18] suggest that the rate-determining step in the conversion of rhodopsin to bathorhodopsin is proton translocation *to* the Schiff base nitrogen of retinal. Moreover, that proton translocation may follow isomerization[20] does not invalidate this suggestion.

In the remainder of this article, we focus on the possible role of proton translocation in the early events of the visual process. Our principal point is to present a general expression for the translocation rate constant that may account for the non-Arrhenius thermal behavior and deuterium isotope effect of the observed rate constant. Our model invokes the double-adiabatic approximation to separate the electronic, protonic, and heavy-nuclear motions. We then consider the electronically adiabatic case, in which the protonic eigenstates are determined by solving an effective Schrödinger equation with the heavy-nuclear coordinates as parameters. The modes of the heavy-atom matrix are found for each protonic eigenstate within the harmonic approximation. The translocation of the proton, which corresponds to transitions between relatively localized protonic states, is induced by vibrations of the heavy-atom matrix. The translocation rate constant, which is computed within the linear-response approximation, is characterized by four parameters.

In section II we outline the general theory. We present in section III nonlinear least-squares fittings of the kinetic data of Peters et al.[18] to our theoretical expression, obtaining two different sets of rate parameters. The isotopic and thermal dependences of these parameters are discussed.

II. Theory

The approximate quantum mechanical treatment of proton translocation via Kubo's linear-response theory[21] requires that we first determine a set of unperturbed (zero-order) energy eigenstates of the system. The rate of translocation can then be expressed in terms of matrix elements of the perturbation between these unperturbed states. In our model we shall find the zero-order states by the double-adiabatic approximation,[22] in which the

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(1) Honig, B. *Annu. Rev. Chem.* **1978**, *29*, 31–57.
(2) Birge, R. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 315–354.
(3) Applebury, M. L. *Photochem. Photobiol.* **1980**, *32*, 425–431.
(4) Alfano, R. R. *Biological Events Probed by Ultrafast Spectroscopy*; Academic: New York, 1982; pp 239–316.
(5) Rentzepis, P. M. In *Spectroscopy and the Dynamics of Molecular Biological Systems* Academic: London, 1985; pp 379–401.
(6) Sandorfy, C.; Vocelle, D. *Can. J. Chem.* **1986**, *64*, 2251–2266.
(7) Yoshizawa, T.; Wald, G. *Nature (London)* **1963**, *197*, 1279–1286.
(8) Green, B. H.; Monger, T. G.; Alfano, R. R.; Aton, B.; Callender, R. H. *Nature (London)* **1977**, *269*, 179–180.
(9) Rosenfeld, T.; Honig, B.; Ottolenghi, M.; Hurley, J.; Ebrey, T. G. *Pure Appl. Chem.* **1977**, *49*, 341–351.
(10) Honig, B.; Ebrey, T.; Callender, R. H.; Dinur, U.; Ottolenghi, M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2503–2507.
(11) Busch, G. E.; Applebury, M. L.; Lamola, A. A.; Rentzepis, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2803–2806.
(12) Mathies, R.; Stryer, L. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *73*, 2169–2173.
(13) Thompson, A. J. *Nature (London)* **1975**, *254*, 178–179.
(14) van der Meer, K.; Mulder, J. J. C.; Lugtenberg, J. *Photochem. Photobiol.* **1976**, *24*, 363–367.
(15) Fransen, M. R.; Luyten, W. C. M. M.; van Thuijl, J.; Lugtenberg, J.; Jansen, P. A. A.; van Breugel, P. J. G. M.; Daemen, F. J. M. *Nature (London)* **1976**, *260*, 726–727.
(16) Warshel, A. *Nature (London)* **1976**, *260*, 679–683.
(17) Birge, R. R.; Hubbard, L. M. *J. Am. Chem. Soc.* **1980**, *102*, 2195–2205.
(18) Peters, K.; Applebury, M. L.; Rentzepis, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3119–3123.
(19) Becker, R. S.; Freedman, K. A. *J. Am. Chem. Soc.* **1985**, *107*, 1477–1485.
(20) Warshel, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 2558–2562.
(21) Kubo, R. *J. Phys. Soc. Jpn.* **1957**, *12*, 570–586.
(22) Levich, V. G.; Dogonadze, R. R.; German, E. D.; Kuznetsov, A. M.; Kharkats, Y. I. *Electrochim. Acta* **1970**, *15*, 353–367.

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motion of the electrons is separated from that of all nuclei and, in turn, the motion of the proton is separated from that of the heavier nuclei. Within a *single electronic manifold* (e.g., the ground electronic state), transitions among the protonic eigenstates are induced by movements of the heavier nuclei.

To describe the proton-transfer process rigorously, it is necessary to determine the time-dependent reduced density matrix of the protonic subsystem, from which any desired property of the proton, specifically its expected location, can be computed. However, in the system with which we are concerned, namely the visual pigment, we shall *assume* the relevant proton moves in an asymmetric double well, the lowest two states of which are localized near either minimum. In other words, in the ground state ($i = 0$), the proton is localized near one minimum, whereas in the first excited state ($i = 1$) it is localized near the other minimum. It can be shown that for a suitable choice of potential energy this situation may indeed prevail.[23] It is therefore reasonable to describe the translocation as a transition from localized state ϕ_1 to localized state ϕ_0 and to neglect all other protonic states. This is accomplished by use of projection-operator techniques[24,25] to derive a generalized master equation for the populations (i.e. *diagonal* elements of the reduced density matrix) of the states ϕ_0 and ϕ_1. Solving this equation in the long-time, weak-coupling limit,[25,26] one finds that the population of state ϕ_1 decays exponentially with a time constant τ given by

$$\tau^{-1} = W_{1\to 0} = (\beta\langle N_1\rangle)^{-1} \int_0^\infty \mathrm{d}t \int_0^\beta \mathrm{d}\lambda\ \langle \dot{N}_1(0)\ \dot{N}_1(t + i\hbar\lambda)\rangle \tag{1}$$

where N_1 is the occupation probability operator associated with localized state ϕ_1 of the proton, $\beta = 1/k_BT$, k_B is Boltzmann's constant, and the broken brackets denote a canonical ensemble average. The rate constant for the translocation is then taken to be $W_{1\to 0}$.

To evaluate expression 1 explicitly, we follow the prescription of Fong[27] for the treatment of electronic radiationless relaxation in solids. Our basic assumption is that the heavy nuclei oscillate harmonically about fixed equilibrium positions, which shift only slightly as the proton undergoes translocation. After lengthy, yet straightforward, manipulations, we arrive at the explicit result

$$W_{1\to 0} = (2\pi)^{1/2}\hbar^{-2}|C_{10}|^2\omega_m^{-1} \sum_{\nu=-2}^{2} \Lambda_\nu(p_m - \nu)^{-1/2} \exp[-g_m^2(2\bar{n}_m + 1)] \exp\{-(p_m - \nu)[\ln[(p_m - \nu)/g_m^2(\bar{n}_m + 1)] - 1]\} \tag{2}$$

where

$$C_{10} = -(\hbar^3\omega_m/2)^{1/2}\Delta\epsilon_{10}^{-1}\left[\int \mathrm{d}r\ \phi_1^*(r;Q)\ (\partial V/\partial Q_m)\ \phi_0(r;Q)\right]_{Q=0} \tag{3}$$

$$\Delta\epsilon_{10} \equiv \epsilon_1(0) - \epsilon_0(0) \tag{4}$$

and the remaining quantities p_m, Λ_ν, g_m, and $\bar{n}_m$ are given in the Appendix.

The significant rate parameters are $\Delta\epsilon_{10}$, the difference between the minima of the potential energy surfaces corresponding to the ground ($i = 0$) and first excited ($i = 1$) *states of the proton*; ω_m, the normal-mode frequency; g_m, which is proportional to the shift in the equilibrium position of the normal mode; and C_{10}, which is proportional to the strength of the coupling between the protonic states.

III. Connection between Theory and Experiment

We focus here on the experiment of Peters et al.[18] who used time-resolved picosecond spectroscopy to measure the rise time of the intermediate bathorhodopsin. The formation of intermediate is essentially exponential, and the rate constant is taken to be the reciprocal of the $1/e$ time (i.e. rise time). An Arrhenius plot of the experimental rate constant is shown (discrete points with error bars) in Figure 1. The two most salient features of the plot are the sharply non-Arrhenius thermal dependence and the strong deuterium isotope effect.

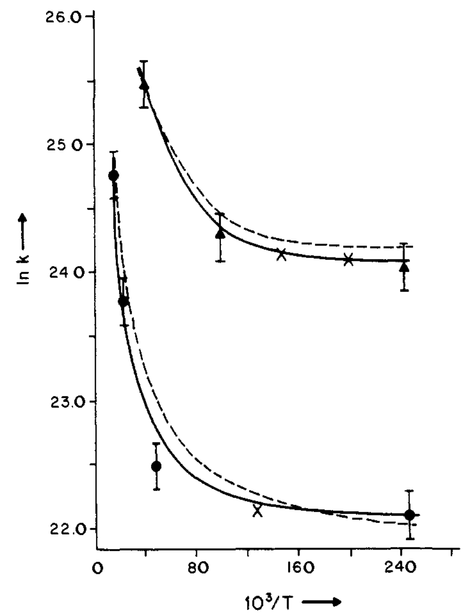

Figure 1. Arrhenius plots for rate of formation of prelumirhodopsin. The two curves shown correspond to two different sets of parameters given in Table I: experiment,[18] ●, ▲; interpolated, ×; fitting 1, —; fitting 2, - - -.

Table I. Results of Nonlinear Least-Squares Fitting of Expression 2 to Experimental Rate Data[18]

fitting	isotope	ω_m, cm^{-1}	$\lvert C_{10}\rvert^2$, erg^2	g_m	$\Delta\epsilon_{10}$, cm^{-1}	ρ
1	H	38.2	0.11 (−24)[a]	2.68	1200	4.1
	D	34.3	6.60 (−27)	2.58	930	3.8
2	H	26.9	1.94 (−26)	0.77	277	13.9
	D	23.2	3.34 (−28)	0.64	197	15.8

[a] Numbers in parentheses indicate powers of 10 by which entry should be multiplied.

Now, if we assume that the rate-determining step in the formation of bathorhodopsin is translocation of a proton, then the experimentally determined rate constant can be identified with $W_{1\to 0}$ in the theoretical expression (eq 2). However, evaluation of this expression requires a detailed knowledge of the potential energy surface, which we lack. This fact, combined with the paucity of experimental data and also the rather large error bars associated with them (see Figure 1) make any precise correlation between theory and experiment impracticable. Consequently, we take a semiempirical tack and simply fit the observed rate data to expression 2.

To accomplish the fitting, we employed the Marquardt procedure.[28] Since there are too few experimental data, we generated additional points (indicated by crosses × in Figure 1) by linear interpolation. We obtained two different sets of optimized parameters, which are listed in Table I; the corresponding smooth curves are shown in Figure 1.

Table I reveals that both fittings predict the involvement of a relatively low-frequency (30–40 cm^{-1}) heavy-atom mediating mode. This finding is in agreement with previous suggestions[29] and calculations[30] and is connected with the sharp transition in the temperature dependence of the rate constant in the vicinity $T_0 \sim$ 15–25 K (see Figure 1). The way in which this "transition temperature" T_0 essentially dictates a low value for ω_m will be discussed later.

Another prominent characteristic of the parameters of Table I is their isotopic dependence; ω_m, $|C_{10}|^2$, g_m, and $\Delta\epsilon_{10}$ all decrease upon deuteriation. The isotopic dependence of $\Delta\epsilon_{10}$ seems easily rationalized. For large

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[23] Riveros, O. Ph.D. Thesis, Purdue University, 1984.

[24] Zwanzig, R. *J. Chem. Phys.* **1960**, *33*, 1338–1341.

[25] Diestler, D. J. In *Radiationless Processes in Molecules and Condensed Phases*; Fong, F. K., Ed.; Springer: Berlin, 1976; pp 169–238.

[26] van Hove, L. *Physica* **1955**, *21*, 517–540.

[27] Fong, F. K. *Theory of Molecular Relaxation*; Wiley: New York, 1975; pp 75–90.

[28] Bevington, P. R. *Data Reduction and Error Analysis for the Physical Sciences*; McGraw-Hill: New York, 1969.

[29] Sarai, A.; Kakitani, T.; Kakitani, H. *Photochem. Photobiol.* **1981**, *33*, 875–880.

[30] Gochev, A. D.; Christov, S. C. *Biophys. Struct. Mech.* **1981**, *7*, 187–201.

internuclear separations $\Delta\epsilon_{10}$ is roughly equal to the energy-level difference between states ϕ_0 and ϕ_1, and we expect this difference to decrease upon deuteriation. If the energy levels remain parallel as the internuclear separation diminishes, then $\Delta\epsilon_{10}$ should also become smaller when H is replaced by D. Likewise, the isotopic dependence of $|C_{10}|^2$ is easily understood. It is helpful to recast eq 3 as

$$|C_{10}|^2 = \hbar^3\omega_m|V_{10}|^2/(2m\Delta\epsilon_{10}^2) \tag{5}$$

where

$$V_{10} \equiv \int \mathrm{d}r\, \phi_1^*(r;Q) \frac{\partial V}{\partial R} \phi_0(r;Q) \tag{6}$$

is evaluated with the heavy nuclei fixed at their equilibrium positions in the protonic ground state ($i = 0$) and m is the effective mass of the normal mode. Then from eq 5 and values of ω_m and $\Delta\epsilon_{10}$ given in Table I, we estimate that $|V_{10}|^2$ decreases by a factor of about 10^3–10^4, which represents a factor of 30–100 in the matrix element V_{10}. From eq 6 we see that V_{10} is approximately proportional to the overlap of the protonic wave functions ϕ_0 and ϕ_1. Since both of these functions are more localized about their respective minima for the heavier deuterium, one would expect the overlap to be smaller for D than for H.

In principle, ω_m should not change upon deuteriation, since the heavy-atom mode does not involve the relevant hydrogen. However, in practice, many, if not all, hydrogens exchange and therefore the "heavy" normal modes involving other (nontranslocating) hydrogens would be expected to become more massive. This might explain the small shifts ($\leq 10\%$) in ω_m.

Finally, the influence of deuteriation on g_m is particularly obscure. It is simply unclear which direction ΔQ_m should shift upon replacement of H by D.

We turn next to the physical basis of the thermal dependence of the rate constant. We observe from eq 2 that the rate constant comprises essentially two factors: (1) the "overlap" factor C_{10} and (2) the remaining factor involving the sum on ν. The first factor governs the isotopic dependence of $W_{1\to0}$ in large part, although not entirely. On the other hand, the second factor completely determines the thermal dependence. Of course, the actual details of the temperature dependence are strongly influenced by the parameters $\Delta\epsilon_{10}$, g_m, and ω_m, and, as we have seen, these are altered by isotopic substitution. In any event, for a given isotope, as the temperature approaches 0 K and $\bar{n}_m \to 0$, the rate constant approaches a constant nonzero value

$$W_{1\to0} = (2\pi)^{1/2}\hbar^{-2}|C_{10}|^2\omega_m^{-1}\sum_{\nu=-2}^{2}\Lambda_\nu(p_m-\nu)^{-1/2}\exp(-g_m^2)\exp\{-(p_m-\nu)[\ln[(p_m-\nu)/g_m^2]-1]\} \tag{7}$$

In this limit, the translocation is assisted strictly by tunneling of the heavy nuclei. As T increases, the population of the normal mode increases and the heavy nuclei become gradually more "activated". If the temperature becomes too high, condition A.8 is violated and expression 2 becomes invalid. It is then necessary to consider the "exact" expression[31] for the rate constant in order to evaluate the high-T limit. The approximate result is

$$W_{1\to0} \simeq (k_BT/\hbar)\{2|C_{10}|^2\pi^{1/2}/[\hbar\omega_m(g_m^2\hbar\omega_m k_BT)^{1/2}]\}\exp(-g_m^2)\exp(-E_a/k_BT) \tag{8}$$

where the "activation energy" is

$$E_a \equiv \Delta\epsilon_{10}[\Delta\epsilon_{10}/(\hbar\omega_m g_m^2) - 1]/2 \tag{9}$$

This corresponds to the usual Arrhenius rate law for thermally activated processes. The "transition temperature" T_0 mentioned above is where the thermal activation sets in and can be roughly estimated from the relation

$$\hbar\omega_m \sim k_BT \tag{10}$$

For T_0 = 15–25 K, eq 10 predicts $\omega_m \sim$ 12–18 cm^{-1}, i.e., a very small frequency. Thus, it is seen that a small T_0 forces ω_m to be small, as is borne out by the results (see Table I).

IV. Conclusions

Available experimental evidence indicates that proton translocation is probably involved in the early ultrafast photochemical transformations associated with vision. Specially suggestive are picosecond measurements[18] of the rise time of bathorhodopsin. Moreover, recent spectroscopic evidence[32] as well as molecular dynamics calculations[20] seems to indicate that proton transfer is indeed a ground-state process. However, the specific mechanism of translocation remains clouded in controversy.[6]

We have presented here (section II) a general expression for the translocation rate constant without assuming anything about the nature of the particular species involved. We have utilized (section III) this expression to fit the kinetic data,[18] obtaining two reasonable sets of rate parameters, which are interpreted in terms of several mechanisms. This expression describes the main features of the data: (1) the extreme rapidity of the process; (2) the sharply non-Arrhenius thermal dependence of the rate constant; and (3) the strong deuterium isotope effect. These results support, therefore, the involvement of proton transfer in the primary photochemical events of vision.

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Appendix

In deriving eq 2 we assume that the heavy nuclei oscillate harmonically about fixed equilibrium positions. Thus, when the proton is in the ground state ($i = 0$), the heavy nuclei experience the potential energy

$$\epsilon_0(Q) = \sum_\lambda \omega_\lambda^2 Q_\lambda^2/2 \tag{A.1}$$

where Q_λ is a normal mode of fundamental frequency ω_λ. In the first excited state ($i = 1$) the effective potential energy becomes

$$\epsilon_1(Q) = \sum_\lambda [\omega_\lambda^2(Q_\lambda - \Delta Q_{1\lambda})^2/2 + \Delta V_{1\lambda}] \tag{A.2}$$

where $\Delta Q_{1\lambda}$ and $\Delta V_{1\lambda}$ are the shifts in the equilibrium position and potential energy minimum associated with mode λ. The rate expression 2 takes into account only a single mode (m), with which are associated the parameters given in eq A.3–A.6.

$$p_m \equiv \Delta\epsilon_{10}/\hbar\omega_m \tag{A.3}$$

$$\begin{aligned}
\Lambda_{-2} &\equiv g_m^2\bar{n}_m^2 \\
\Lambda_{-1} &\equiv -2g_m^2(2\bar{n}_m + 1)\bar{n}_m + \bar{n}_m \\
\Lambda_0 &\equiv g_m^2[6\bar{n}_m(\bar{n}_m + 1) + 1] \\
\Lambda_1 &\equiv -2g_m^2(2\bar{n}_m + 1)(\bar{n}_m + 1) + \bar{n}_m + 1 \\
\Lambda_2 &\equiv g_m^2(\bar{n}_m + 1)^2
\end{aligned} \tag{A.4}$$

$$g_m \equiv (\omega_m/2\hbar)^{1/2}\Delta Q_m \tag{A.5}$$

$$\bar{n}_m \equiv 1/[\exp(\beta\hbar\omega_m) - 1] \tag{A.6}$$

We note that in arriving at eq 2 we have invoked the weak-coupling limit

$$\rho \equiv (\Delta\epsilon_{10} - \nu\hbar\omega_m)/g_m^2\hbar\omega_m \gg 1 \tag{A.7}$$

Also, the temperature must be sufficiently low that the inequality

$$\rho \sinh(\beta\hbar\omega_m/2) \gg 1 \tag{A.8}$$

is obeyed.

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(31) Fong, F. K.; Lauer, H. V. *J. Chem. Phys.* **1974**, *60*, 274–280.

(32) Birge, R. R.; Murray, L. P.; Pierce, B. M.; Akita, H.; Balogh-Nahir, V.; Findsen, L. A.; Nakanishi, K. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4117–4121.