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Quantum-Mechanical Kinetic Study of the Primary Reaction of the Photochemical Cycle of *Halobacterium halobium*

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Abstract. A two-frequency oscillator model for the primary photochemical reaction bacteriorhodopsin → *batho*-bacteriorhodopsin (K_{610}) is proposed. According to this model two conformational changes in the reaction are considered to take place: the first one is a distortion of the retinal in the bacteriorhodopsin active site and the second one is a conformational transition of the bacterioopsin, affecting the native structure hydrogen bonds. On the basis of this model the temperature dependences of the rate constants for normal and deuterated reactants are calculated in good agreement with the available experimental data. The relations of the reaction considered to the primary photochemical reaction of vision are discussed.

Key words: Bacteriorhodopsin – Rhodopsin – Quantum mechanics – Reaction kinetics

Bacteriorhodopsin (bR), a purple membrane protein of 26,000 molecular weight, has some analogies to the visual photoreceptor rhodopsin (R) but, in contrast to it, upon light illumination undergoes several consequtive photochemical changes and returns to its initial state. bR (light adapted) is composed of all-*trans* retinal complexed to a protein (bacterioopsin) by a protonated Schiff-base linkage.

The primary photochemical reaction of light adapted bR

$$\text{bR} \rightarrow \textit{batho}\text{-bR } (K_{610}) \tag{1}$$

is similar to the primary photochemical event in vision

$$\text{R} \rightarrow \textit{batho}\text{-R } (\textit{prelumi}\text{-R}) \tag{2}$$

which involves some kind of *cis-trans* isomerization of retinal.

Applebury et al. (1978) have investigated the kinetics of reaction (1) in a wide temperature range (1,8–300 K) with both normal and deuterated reagents.

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The temperature dependences of the rate constants $k_H(T)$ and $k_D(T)$ are similar to those of reaction (2), as measured by the same authors (Peters et al. 1977), and show considerable deviation from the classical Arrhenius relation. For both reactions the same mechanism of proton (deuteron) tunneling along the hydrogen bond, connecting the Schiff-base nitrogen atom with a second electronegative atom of some amino acid side chain, was proposed (Peters et al. 1977).

The above mechanism, however, cannot explain the rate constant temperature dependences for reactions (1) and (2). The minimum value of the N−H vibration frequency is $\nu = 1{,}500\ \mathrm{cm}^{-1}$ ($h\nu = 0{,}186$ eV) so that in the temperature range 1,8−77 K the probability of population of the first excited vibration state $W_{0,1} = \exp(-h\nu/kT)$ is less than 10^{-12}; therefore, the N−H oscillator will remain in the ground vibrational state. We conclude that a vibration of considerably lower frequency has to be included in the reaction mechanism in order to explain the temperature dependences of the rate constants.

The mechanism of *cis-trans* isomerization proposed by Hubbard and Kropf (1958) and discussed recently by Rosenfeld et al. (1977) also can not explain the $k(T)$ dependence if we assume that it occurs in the excited singlet state since, according to the theoretical calculations of Salem and Bruckmann (1975) and Warshel (1976), there is no energy barrier for such an isomerization in this state; consequently, there will be no temperature dependence of the reaction rate.

In order to find the probable mechanism of reaction (1) or (2) we must take into account more completely the available theoretical and experimental information about the structural changes of bR and *batho*-bR during the reaction.

Tokunaga et al. (1976) assume that the conversion of bR to *batho*-bR may be some kind of isomerization. Oseroff and Callender (1974) speak about "intermediate conformation" between *cis* and *trans,* while Pettei et al. (1977) state that retinal could adopt "an intermediate shape" which is neither 13-*cis* nor all-*trans*.

Marcus and Lewis (1978) indicate that small structural adjustments take place in the retinilidene chromophore in going from bR to K, but there is no evidence that they have included a photochemically-driven *trans* to *cis* isomerization as suggested by Hurley et al. (1977) and Honig et al. (1979). The essential result of photoisomerization would be the charge separation due to breaking of a salt bridge (Honig et al. 1979).

Although there is no complete agreement in opinion about the detailed mechanism of reactions (1) and (2), it seems well established that the retinal in *batho*-R and *batho*-bR is in a distorted conformation. Speaking about "partial cis-trans isomerization" (Gochev and Christov 1979) should actually mean a process related to such a distorted conformation.

Normal coordinate calculations for a distorted structure show drastically altered vibrational modes below $900\ \mathrm{cm}^{-1}$ (Sulkes et al. 1978). These calculations suggest that the most probable geometrical changes are ring displacements and a rotational displacement of the whole molecule (concerted rotation about two bonds) which enhance modes with non-zero Franck-Condon

factors in the 800--950 cm^{-1} region (Warshel 1976, 1977). Cookingham et al. (1978) conclude from their normal coordinate calculations that the bending vibrations of the polyene have frequencies in the range 750–950 cm^{-1}.

All these indings suggest that the primary photochemical event in vision and in *Halobacterium* involve a distortion of the chromophore which is related to vibration frequencies in the 800–950 cm^{-1} range.

On the other hand, Fransen et al. (1976) have found that D_2O has no access to the region of direct protein-chromophore interaction in rhodopsin. According to Englander and Englander (1977) bR is deuterated to a lower extent that rhodopsin; moreover as shown by Schreckenbach et al. (1977) the retinal in bR is in a hydrophobic environment. We may, therefore, conclude that D_2O treatment will not affect the vibration frequencies in the retinal chain of bR, too. This conclusion agrees with the experiments of Marcus and Lewis (1978) which show that incorporating of protonated retinal into fully deuterated bacterioopsin does not cause any changes in the fingerprint spectral range (1,000–1,400 cm^{-1}) which is sensitive to the various retinal isomerizations (Marcus and Lewis 1978).

These facts suggest that the vibration frequencies of the chromophore in the active site are not influenced by deuteration in D_2O. Therefore, the models which take into account only some kind of *cis-trans* isomerization of the chromophore can not explain the kinetic isotope effect. This conclusion also holds for models, which consider the retinalprotein interaction only in a small region around the active site.

All these findings suggest that in both reactions (1) and (2) at least two molecular vibrations take place: the first one, with a higher frequency, is not influenced by an isotope substitution in D_2O and the second one, with a lower frequency, determines the temperature dependence and the isotope effect.

Vibrations with a lower frequency ν_x which are influenced by isotope substitution, can be assigned as the lattice protein-globule vibration, related to H-bonds of the native structure, for which $\nu_x \leqq 50\ cm^{-1}$ (Brown et al. 1972). There will be only a small change of this frequency by the isotope substitution because of the great effective vibrating mass ($1 < \nu_x^H/\nu_x^D < \sqrt{2}$). Since bR can be deuterated in a smaller extent than the R (Englander and Englander, 1977) we can expect a weaker isotope effect in reaction (1) than in reaction (2).

In a previous work (Gochev and Christov 1979) a quantum-mechanical treatment of the primary photochemical reaction of vision (2) was presented. Using the two-frequency oscillator model for reactions in condensed media (Christov 1977), based on the general quantum-mechanical reaction rate theory (Christov 1972, 1974, 1980), the temperature dependences $k_H(T)$ and $k_D(T)$ for reaction (2) were calculated. The good quantitative agreement of the theoretical curves with the experimental results of Peters et al. (1977) supports the physical model used. This model is consistent with the above assumptions that two conformational changes of the rhodopsin structure take place: the first one is a distortion of the retinal in the active site and the second one is a conformational transition of the opsin affecting the hydrogen bonds of its native structure.

We accept here the same two-frequency oscillator model for reaction (1) by assuming again that the reaction heat at 0° K is zero ($Q = O$)[1], in agreement withthe common excited state hypothesis (Rosenfeld et al. 1977). This hypothesis implies that the relaxation from the excited state to the ground state of *batho*-bR is a very fast process. However, it should be noted that, in principle, the model proposed is equally well applicable to both cases in which the reaction occurs in either the ground or the excited states.

According to our model the potential energies of the initial and final states are described by two elliptical paraboloids $V_1(x, y)$ and $V_2(x, y)$ where x and y are vibration coordinates of bacterioopsin and retinal, respectively. If the two frequencies ν_x and ν_y are not very different, the reaction occurs with the highest probability along a dynamically separable coordinate u normal to the intersection plane of the two paraboloids. The effective vibration frequency along u is given by the equation

$$\nu_u = (\tfrac{1}{2}\pi^2\mu_u)^{1/2}(f_x^2 E_r^x + f_y^2 E_r^y)/(f_x E_r^x + f_y E_r^y)^{1/2} \tag{3}$$

$(\nu_x < \nu_u < \nu_y)$ and the corresponding "energy of reorganization" is

$$E_r^u = (f_x/f_y)^2 E_r^x + E_r^y , \tag{4}$$

where f_x and f_y are force constants while E_r^x and E_r^y are the reorganization energies along x and y, respectively. Since $\nu_x < \nu_y$ and $f_x \leqq f_y$ from the relation $\nu = (1/2\pi)\,(f/\mu)^{1/2}$ it follows that

$$(\mu_x/\mu_y) \leqq (\nu_y/\nu_x)^2 , \tag{5}$$

where μ_x and μ_y are the effective masses of the x- and y-vibrations. This inequality corresponds to our assumption that ν_x is the frequency of the protein lattice vibration (large effective mass) while ν_y is the one of the retinal vibration [small effective mass (Warshel 1976)].

The temperature dependences of the rate constants $k_H(T)$ and $k_D(T)$ of reaction (1) were calculated using the expression (Christov 1975)

$$k(T) = \nu_u \exp(h\nu_u/kT) \sum_{n_1} W_{n_1, n_2}(E_{n_1}) \exp(-E_{n_1}/kT) , \tag{6}$$

where $E_{n1} = (n_1 + 1/2)h\nu_u$, n_1 and n_2 are the vibrational quantum numbers of reactants and products, respectively, and $W_{n1,\,n2}(E_{n1})$ is the probability of the transition $(n_1 \rightarrow n_2)$ along the u-coordinate $(E_{n1} = E_{n2})$. For an adiabatic reaction the formula (Christov 1975)

$$W_{n_1, n_2} = [(\pi F^2)/(2^{n_1 + n_2} n_1 ! n_2 !)] \exp[-(n_1 - n_2)^2 h\nu_u/E_r^u] \exp(-E_r^u/h\nu_u) \tag{7}$$

[1] For reaction (2) the assumption $Q = 0$ is confirmed by the experimental fact that in the temperature range 77–300 K the rates of the direct and reverse reactions are independent of temperature which is explained by the absence of an energy barrier for the retinal isomerization (Rosenfeld et al. 1977). However, this fact can also be understood if we assume the exsistence of a *low* symmetric barrier for some kind of isomerization in the excited state which could be detected below 77 K. We assume a symmetric barrier ($Q = 0$) for the similar reaction (1) but the height of this barrier does not need to be small since the rate of reaction (1) is temperature dependent from 50–300 K

applies, where F is a function containing the Hermite polinomials of order n_1, n_1-1, n_2, and n_2-1.

The isotope substitution does not affect the electronic energy, therefore, the energy of reorganization along any coordinate remains unaltered. The kinetic isotope effect is connected only with the change of the vibration frequency along the x-coordinate, due to the different masses of H and D ($\nu_x^D < \nu_x^H$). Since $Q = 0$ ($n_1 = n_2$) we need three adjustable parameters, E_r^u, ν_u^H, and ν_u^D for calculating $k_H(T)$ and $k_D(T)$ by (6). Taking into account that $\nu_x < \nu_u < \nu_y$ ($\nu_x \leqq 50\ \mathrm{cm}^{-1}$, $\nu_y \geqq 800\ \mathrm{cm}^{-1}$), we use the value $\nu_{eu}^D = 221{,}65\ \mathrm{cm}^{-1}$ in order to compute the reorganization energy E_r^u from the experimental rate constant at 4 K, which yields $E_r^u = 0{,}2908$ eV. Using a single experimental value for k_H we find $\nu_u^H = 239{,}32\ \mathrm{cm}^{-1}$ in agreement with the condition $1 < \nu_u^H/\nu_u^D < \sqrt{2}$.

The theoretical curves calculated with the above parameters are presented on Fig. 1 where the good agreement with the experimental results of Applebury et al. (1978) is seen. More significant deviation from these results are observed only at 300 K, however, the theoretical curve for $k_H(T)$ excelently agrees with the value measured by Ippen et al. (1978) at this temperature. It should be noted, however, that the latter result may be possibly related to the rise time of the precursor of K_{610} but not to that of K_{610} (Ottolenghi 1980).

It is noteworthy to make a comparison between the results of our treatment of the similar reactions (1) and (2) and their relation to some experimental facts. It is known that, in contrast to rhodopsin, bacteriorhodopsin has an extremely rigid structure (Blaurock and Stoeckenius 1971; Henderson 1975) which prevents large conformational changes, allowing fast reversibility and making it

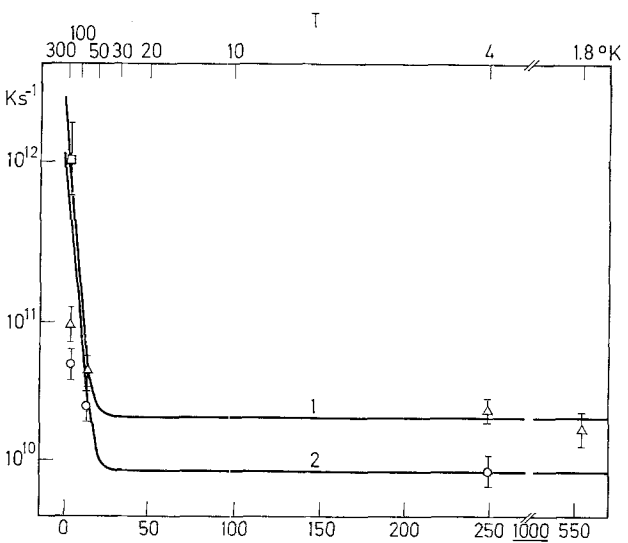

Fig. 1. Temperature dependence of the rate constants k_H (curve 1) and k_D (curve 2) of the isotope reaction bR → *batho*-bR (K_{610}) calculated by $\nu_u^H = 239{,}32\ \mathrm{cm}^{-1}$, $\nu_u^D = 221{,}65\ \mathrm{cm}^{-1}$ and $E_r^u = 0{,}2908$ eV. The points are the experimental results of Applebury et al. (1978) (△, ○) and Ippen et al. (1978) (□)

favourable for energy conversion (Lewis 1977). This menas that the vibrations of bR have higher frequencies than those of R so that their excitation occurs at higher temperatures. These facts are in accordance with our calculations which show that the effective vibration frequency $\nu_u^H(\mathrm{bR}) = 239{,}32\ \mathrm{cm}^{-1}$ is about three times higher than the vibration frequency $\nu_u^H(\mathrm{R}) = 85{,}50\ \mathrm{cm}^{-1}$(Gochev and Christov 1979).

On the other hand, in contrast to reaction (2), the rate of the reaction (1) depends on the temperature in the range 77–300 K as well. Consequently, the potential barrier for reaction (1) should be higher than the one for reaction (2). This conclusion is in accordance with the values of the reorganization energies $E_r^u(\mathrm{bR}) = 0{,}2908$ eV and $E_r^u(\mathrm{R}) = 0{,}0882$ eV used in our calculations, since they are related to the corresponding activation energies E_c^u by the equation (Christov 1975):

$$E_c^u + V_{1,2} = (E_r^u + Q)^2/4\, E_r^u\,, \tag{8}$$

where $V_{1,2}$ is the resonance energy at the barrier peak. Assuming that $V_{1,2}(\mathrm{bR}) \simeq V_{1,2}(\mathrm{R})$ we obtain, $E_c^u(bR) > E_c^u(R)$ $(Q = 0)$. This result is contradictory to the statement of Applebury et al. (1978) that $E_c(\mathrm{bR}) < E_c(\mathrm{R})$.

In the course of publication of our previous work (Gochev and Christov 1979) a paper by Lewis (1978) appeared in which a similar mechanism of reactions (1) and (2) was proposed. Our considerations, based on kinetic studies (Peters et al. 1977), yield an independent justification of this mechanism which provides a quantitative explanation of the experimental results for both reactions (1) and (2).

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