

## FLASH PHOTOLYSIS OF CATTLE RHODOPSIN IN SOLUTION: DETERMINATION OF KINETIC ACTIVATION PARAMETERS

B. D. GUPTA\* and I. C. GOYAL

*Physics Department, Indian Institute of Technology, Delhi, New Delhi 110 029 (India)*

(Received February 9, 1979; in revised form April 30, 1979)

### Summary

Flash photolysis of cattle rhodopsin in solution has been analysed theoretically and the results are found to be in good agreement with the experimental results of Williams (1970). The values of various kinetic activation parameters (*i.e.* activation energy, free energy of activation, enthalpy of activation, entropy of activation and the pre-exponential factor of Arrhenius equation) for the reactions metarhodopsin I  $\rightarrow$  metarhodopsin I', metarhodopsin I'  $\rightarrow$  metarhodopsin I, metarhodopsin I'  $\rightarrow$  metarhodopsin II and metarhodopsin II  $\rightarrow$  metarhodopsin I' have been determined. Also the quantum efficiency of the conversion of metarhodopsin I into rhodopsin has been determined.

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### 1. Introduction

Williams [1, 2] has studied experimentally the effect of intense flashes of various durations on digitonin solutions of cattle rhodopsin at different temperatures. He found that the upper limits\*\* to bleaching depend upon (1) the duration of the flash, (2) the temperature of the solution and (3) the presence or absence of UV light in the flash. The upper limits to the bleaching by flashes of different durations (0.9 ms, 2 ms and 60 ms) reported by him were 50% at low temperatures and 88% at high temperatures. The 50% bleach is obtained when the thermal reaction is not so important and therefore the photo-equilibrium takes place between the visual pigment and its intermediate during the course of the flash. This was first observed by Hagins [3] in the case of rabbit rhodopsin. The 88% bleach implies that the system of visual pigment and intermediates comes into

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\*B.D. Gupta is associated with the School of Bioscience Studies.

\*\*By upper limit to bleaching we mean that the percentage bleaching after the flash is unaffected by increasing the intensity of the flash. Such a flash intensity we shall hereafter term the saturated intensity.



$$\frac{dR}{dt} = -\gamma_{R,M_1} J_R(t) + \gamma_{M_1,R} J_{M_1}(t) \quad (2)$$

$$\frac{dM_1}{dt} = \gamma_{R,M_1} J_R(t) - \gamma_{M_1,R} J_{M_1}(t) - k_1 M_1(t) + k_2 M_1'(t) \quad (3)$$

$$\frac{dM_1'}{dt} = k_1 M_1(t) - (k_2 + k_3) M_1'(t) + k_4 M_2(t) \quad (4)$$

$$\frac{dM_2}{dt} = k_3 M_1'(t) - k_4 M_2(t) \quad (5)$$

where  $\gamma_{A,B}$  represents the quantum efficiency of the conversion of A-type molecules into B-type molecules and  $J_X(t)$  represents the absorption rate of the species X at time  $t$  (in photons absorbed per cubic centimetre per second) and is given by

$$J_X(t) = \int_{\lambda_1}^{\lambda_2} J_X(\lambda, t) d\lambda \quad (6)$$

where  $\lambda_1 - \lambda_2$  is the wavelength range of the bleaching flash falling on the cell containing the solution and  $J_X(\lambda, t) d\lambda$  represents the number of photons with wavelengths between  $\lambda$  and  $\lambda + d\lambda$  absorbed by X-type molecules per cubic centimetre per second.

Since the experiment was performed on the solution of rhodopsin [1],  $J_X(\lambda, t)$  can be written as (see for example ref. 4)

$$J_X(\lambda, t) = \frac{\alpha_X(\lambda) X(t) I(\lambda, t)}{H} (1 - e^{-H}) \quad (7)$$

where

$$H = \{\alpha_R(\lambda) R(t) + \alpha_{M_1}(\lambda) M_1(t) + \alpha_{M_1'}(\lambda) M_1'(t)\} l \quad (8)$$

$\alpha_X(\lambda)$  represents the extinction coefficient of species X at wavelength  $\lambda$  (in square centimetres per chromophore),  $I(\lambda, t)$  represents the spectral-temporal distribution of the intensity of the flash (in photons per square centimetre per second) and  $l$  represents the length of the cell containing the solution of rhodopsin (in centimetres). It may be noted that we have not taken  $M_2$  into consideration in eqn. (8); this is due to the fact that it does not absorb in the region  $\lambda_1 - \lambda_2$  used in the experiment [1].

In the case of solutions of visual pigments the absorbance  $H$  is generally small. Therefore the self-screening effect will be negligible. Assuming  $H$  to be small in Williams' experiment [1] we can for simplification neglect the higher order terms in the expansion of the exponential in eqn. (7) and we obtain

$$J_X(\lambda, t) = \alpha_X(\lambda) X(t) I(\lambda, t) \quad (9)$$

For the spectral distribution of the intensity of the flash independent of time  $t$  we can write

$$I(\lambda, t) = f(t)I(\lambda) \quad (10)$$

where  $f(t)$  represents the normalized temporal distribution of the flash. The initial conditions for solving eqns. (2) - (5) are

$$R = R_0 \quad M_1 = M'_1 = M_2 = 0 \quad \text{at } t = 0$$

To solve eqns. (2) - (5) the following parameters need to be known:

(1) the extinction coefficients of rhodopsin ( $\alpha_R(\lambda)$ ) and metarhodopsin I ( $\alpha_{M_1}(\lambda)$ );

(2) the spectral distribution  $I(\lambda)$  of the intensity of the flash;

(3) the temporal distribution  $f(t)$  of the intensity of the flash;

(4) the quantum efficiencies  $\gamma_{R, M_1}$  and  $\gamma_{M_1, R}$  and rate constants  $k_1$ ,  $k_2$ ,  $k_3$  and  $k_4$ .

In the present analysis it is assumed that the extinction spectra of rhodopsin and metarhodopsin I are identical to the extinction spectra obtained corresponding to their  $\lambda_{\max}$  from the nomogram in refs. 5 and 6. The spectra obtained can be very well approximated by the following simple relations:

$$\alpha_R(\lambda) = \alpha_R(\lambda_{\max}) \exp\left\{-\left(\frac{\lambda - 502}{77}\right)^2\right\} \quad \lambda < 498 \text{ nm}$$

$$= \alpha_R(\lambda_{\max}) \exp\left\{-\left(\frac{\lambda - 498}{54}\right)^2\right\} \quad \lambda \geq 498 \text{ nm}$$

$$\alpha_{M_1}(\lambda) = \alpha_{M_1}(\lambda_{\max}) \exp\left\{-\left(\frac{\lambda - 482}{71.5}\right)^2\right\} \quad \lambda < 478 \text{ nm}$$

$$= \alpha_{M_1}(\lambda_{\max}) \exp\left\{-\left(\frac{\lambda - 478}{49}\right)^2\right\} \quad \lambda \geq 478 \text{ nm}$$

where [7, 8]

$$\alpha_R(\lambda_{\max} = 498 \text{ nm}) = 40\,600 \text{ l cm}^{-1} \text{ mol}^{-1}$$

and

$$\alpha_{M_1}(\lambda_{\max} = 478 \text{ nm}) = 45\,000 \text{ l cm}^{-1} \text{ mol}^{-1}$$

The wavelength dependence of the intensity of the bleaching flash used in Williams' experiment [1] and reported in ref. 9 can be fitted very well by the following simple relations:

$$I(\lambda) = 8.3 \times 10^{13} \eta (\lambda - 390) \quad \lambda \leq 444 \text{ nm}$$

$$= 3.15 \times 10^{15} \eta \exp\left(\frac{509 - \lambda}{108.9}\right)^2 \quad 444 \text{ nm} \leq \lambda \leq 509 \text{ nm}$$

$$= 3.15 \times 10^{15} \eta \exp\left(\frac{\lambda - 509}{93.5}\right)^2 \quad \lambda \geq 509 \text{ nm}$$

where  $\eta$  is independent of wavelength and decides the intensity of the flash.

Using the foregoing expressions for  $\alpha_X(\lambda)$  and  $I(\lambda)$  and  $\lambda_1 = 390$  nm and  $\lambda_2 = 600$  nm (the values used in the experiment), we obtain the following values for the absorption rates

$$\begin{aligned} J_R(t) &= 3.743 \times 10^{17} \alpha_R(\lambda_{\max}) \eta f(t) R(t) \\ J_{M_1}(t) &= 3.655 \times 10^{17} \alpha_R(\lambda_{\max}) \eta f(t) M_1(t) \end{aligned} \quad (12)$$

where

$$\alpha_R(\lambda_{\max}) = 1.58 \times 10^{-16} \text{ cm}^2 \text{ chromophore}^{-1}$$

It is clear from eqn. (12) that eqns. (2) - (5) cannot be solved analytically in terms of  $f(t)$ . We have solved them using the following method.

The number of incident quanta per sample area per flash is given by

$$Q(\tau) = \int_0^\tau \int_{\lambda_1}^{\lambda_2} I(\lambda, t) d\lambda dt = \int_{\lambda_1}^{\lambda_2} I(\lambda) d\lambda \int_0^\tau f(t) dt \quad (13)$$

where  $\tau$  represents the duration of the flash. Using [1]

$$Q(\tau = 2 \text{ ms}) = 1.7 \times 10^{17}$$

$$Q(\tau = 60 \text{ ms}) = 5.1 \times 10^{18}$$

and eqn. (13) we obtain

$$\eta \int_0^{\tau=2 \text{ ms}} f(t) dt = 0.22278$$

and

$$\eta \int_0^{\tau=60 \text{ ms}} f(t) dt = 6.68351 \quad (14)$$

If we substitute

$$f(t) = f_0 = \text{a constant} \quad 0 \leq t \leq \tau$$

and

$$f_0 = \frac{1}{\tau} \int_0^\tau f(t) dt \quad (15)$$

in eqn. (12), the solutions of eqns. (2) - (5) can be obtained. We have verified that the results so obtained at saturated intensities are same to those obtained after substituting eqn. (12) in eqns. (2) and (3) and solving them numerically using the Runge-Kutta method. This is also expected from the fact that the experimentally measured concentrations of the various reactants after flashes of saturated intensity are independent of the intensity. Using eqns. (14) and (15), eqn. (12) can be written as

$$\begin{aligned} J_R(t) &= 6587.53R(t) \\ J_{M_1}(t) &= 6432.66M_1(t) \end{aligned} \tag{16}$$

The solutions of eqns. (2) - (5) so obtained can be written as

$$R = R_0 \left\{ \sum_{i=1}^3 U_i \left( n_i^2 + n_i z_1 + z_2 + \frac{z_3}{n_i} \right) - U_4 z_3 \right\} \tag{17}$$

$$M_1 = aR_0 \left\{ \sum_{i=1}^3 U_i \left( n_i + z + \frac{k_2 k_4}{n_i} \right) - U_4 k_2 k_4 \right\} \tag{18}$$

$$M'_1 = aR_0 k_1 \left\{ \sum_{i=1}^3 U_i \left( 1 + \frac{k_4}{n_i} \right) - U_4 k_4 \right\} \tag{19}$$

and

$$M_2 = aR_0 k_1 k_3 \left\{ \sum_{i=1}^3 \frac{U_i}{n_i} - U_4 \right\} \tag{20}$$

where

$$\begin{aligned} U_1 &= \frac{\exp(n_1 t)}{(n_1 - n_2)(n_1 - n_3)} & U_2 &= \frac{\exp(n_2 t)}{(n_2 - n_1)(n_2 - n_3)} \\ U_3 &= \frac{\exp(n_3 t)}{(n_3 - n_1)(n_3 - n_2)} & U_4 &= \frac{1}{n_1 n_2 n_3} \\ a &= 6587.53 \gamma_{R, M_1} & b &= 6432.66 \gamma_{M_1, R} \\ z_1 &= b + z + k_1 & z_2 &= y + bz \\ z_3 &= b k_2 k_4 & z &= k_2 + k_3 + k_4 \\ y &= k_1(k_3 + k_4) + k_2 k_4 \end{aligned}$$

$n_1, n_2$  and  $n_3$  are the roots of the equation

$$p^3 + \alpha p^2 + \beta p + \gamma = 0 \tag{21}$$

where

$$\begin{aligned} \alpha &= a + z_1 & \beta &= a(k_1 + z) + bz + y \\ \gamma &= ay + z_3 \end{aligned}$$

The roots of eqn. (21) are [10] :

$$\begin{aligned} n_1 &= f \cos \frac{\theta}{3} - \frac{\alpha}{3} \\ n_2 &= f \cos \left( \frac{\theta}{3} + 120^\circ \right) - \frac{\alpha}{3} \end{aligned}$$

$$n_3 = f \cos\left(\frac{\theta}{3} + 240^\circ\right) - \frac{\alpha}{3}$$

where

$$\theta = \arccos\left(\frac{3g}{ef}\right)$$

$$e = \beta - \frac{\alpha^2}{3}$$

$$f = 2\left(-\frac{e}{3}\right)^{1/2}$$

and

$$g = (2\alpha^3 - 9\alpha\beta + 27\gamma)/27$$

In the present analysis the quantum efficiency  $\gamma_{R, M_1}$  of the forward reaction has been chosen to be 0.67 [11, 12]. Only five unknown parameters,  $\gamma_{M_1, R}$ ,  $k_1$ ,  $k_2$ ,  $k_3$  and  $k_4$ , have to be varied to obtain a good agreement of the theoretical results with the experiment data [1].

### 3. Results

The values of  $R$ ,  $M_1$ ,  $M'_1$  and  $M_2$  after flashes of two different durations (2 ms and 60 ms) and at various temperatures corresponding to saturated intensity are known experimentally [1]. Therefore, in solving equations (17) - (20) we adjusted by trial and error the values of unknown parameters (rate constants and quantum efficiency) so as to obtain values of  $R$ ,  $M_1$ ,  $M'_1$  and  $M_2$  equal to the experimentally known values. Another restriction on the choice of these parameters is that they should obey the Arrhenius equation, i.e.  $\log k = \log F - E_a/2.303RT$ , where  $k$  represents the rate constant (in reciprocal seconds) of the reaction at temperature  $T$  (in kelvins),  $E_a$  the activation energy (in joules per mole),  $R$  the gas constant (in joules per mole per kelvin) and  $F$  the pre-exponential factor (in reciprocal seconds). The values of the rate constants so determined have been plotted for the reactions  $M_1 \rightarrow M'_1$  and  $M_2 \rightarrow M'_1$  in Fig. 1 and for  $M'_1 \rightarrow M_1$  and  $M'_1 \rightarrow M_2$  in Fig. 2. It can be seen from these figures that the values of rate constants obtained at different temperatures for four different reactions obey the Arrhenius equation. However,  $k_3$  and  $k_4$  obtained at 10 °C and 15 °C show a slight disagreement. The straight lines drawn are obtained from the least square analysis of the Arrhenius plots. The value of the quantum efficiency  $\gamma_{M_1, R}$  obtained is 0.76.

From the slopes of the lines drawn in Figs. 1 and 2 which are equal to  $-E_a/2.303R$  we have determined the values of activation energies for four different reactions. The values determined are given in Table 1. In addition to this, we have determined the free energy of activation  $\Delta F^\ddagger$ , the enthalpy of activation  $\Delta H^\ddagger$ , the entropy of activation  $\Delta S^\ddagger$  and the pre-exponential factor  $F$  of the Arrhenius equation from the following formulas [13]:

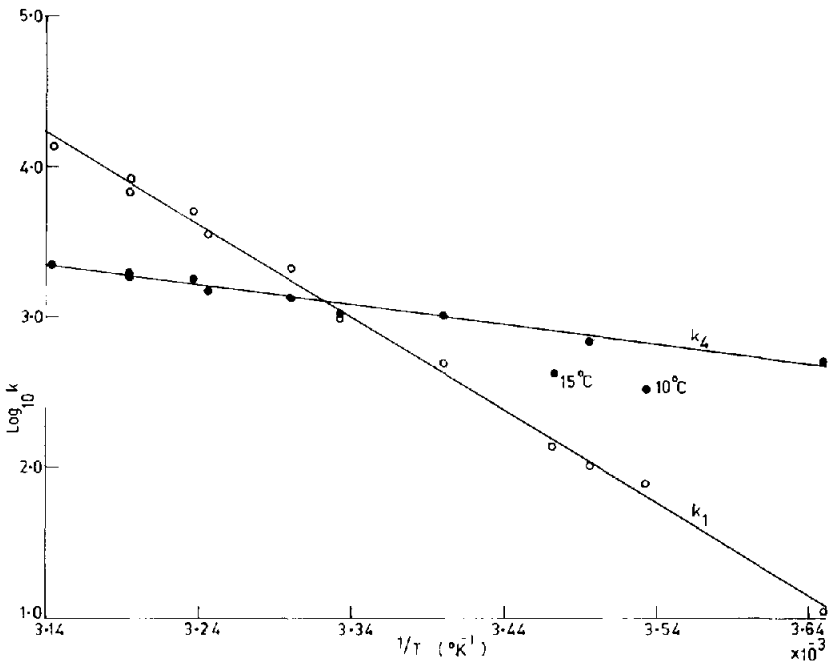


Fig. 1. Arrhenius plots of  $k_1$  (○) and  $k_4$  (●).

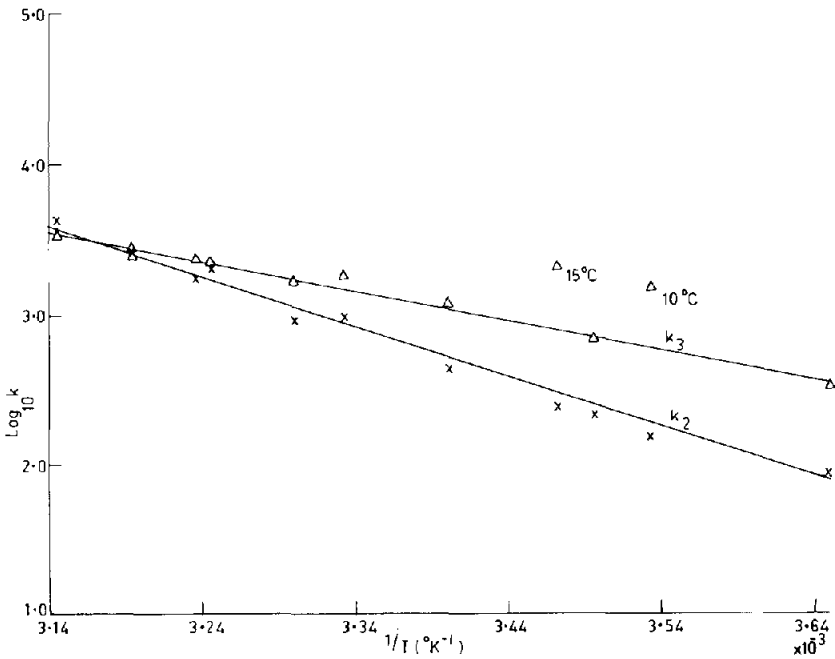


Fig. 2. Arrhenius plots of  $k_2$  (x) and  $k_3$  (△).



TABLE 1

Kinetic parameters of the decay of  $M_1$ ,  $M'_1$  and  $M_2$ 

Reaction	$E_a$ (kJ mol <sup>-1</sup> )	$F$ (s <sup>-1</sup> )	$\Delta F^\ddagger$ (kJ mol <sup>-1</sup> )	$\Delta H^\ddagger$ (kJ mol <sup>-1</sup> )	$\Delta S^\ddagger$ (J mol <sup>-1</sup> K <sup>-1</sup> )
$M_1 \rightarrow M'_1$	118.09	$3.96 \times 10^{23}$	57.44	115.66	198.71
$M'_1 \rightarrow M_1$	63.28	$9.25 \times 10^{13}$	56.66	60.85	14.29
$M'_1 \rightarrow M_2$	37.60	$5.28 \times 10^9$	54.78	35.16	-66.96
$M_2 \rightarrow M'_1$	25.14	$2.93 \times 10^7$	54.98	22.70	-110.16

$$\Delta F^\ddagger = -RT \ln \frac{kh}{k_B T}$$

$$\Delta H^\ddagger = E_a - RT$$

$$\Delta S^\ddagger = \frac{\Delta H^\ddagger - \Delta F^\ddagger}{T}$$

and

$$F = k \exp(E_a/RT)$$

where  $h$  represents Planck's constant and  $k_B$  Boltzman's constant. The values of the parameters determined are given in Table 1. The activation energy  $E_a$  and the pre-exponential factor  $F$  give the rate of the reaction while the entropy of activation (determined using the values of free energy of activation and enthalpy of activation) determines the conformational change of the molecule. From Table 1 it can be seen that the entropy of activation of the reaction  $M_1 \rightarrow M'_1$  is largest. Thus we can conclude that the metarhodopsin I to metarhodopsin I' transition is accompanied by very great conformational change.

Baker and Williams [14] have reported that  $M_1 \rightarrow M'_1$  and  $M'_1 \rightarrow M_2$  processes involve an entropy change of 42.3 cal mol<sup>-1</sup> K<sup>-1</sup> and 7.325 cal mol<sup>-1</sup> K<sup>-1</sup> respectively. Also, they have reported the enthalpy changes for these two reactions to be 11.3 kcal mol<sup>-1</sup> and 1.8 kcal mol<sup>-1</sup> respectively. From the results reported in Table 1 we can see that the entropy changes for  $M_1 \rightarrow M'_1$  and  $M'_1 \rightarrow M_2$  processes are 44.1 cal mol<sup>-1</sup> K<sup>-1</sup> and 10.3 cal mol<sup>-1</sup> K<sup>-1</sup> respectively and the enthalpy changes for these two reactions are 13.1 kcal mol<sup>-1</sup> and 3.0 kcal mol<sup>-1</sup> respectively. It can be seen that these values obtained from our results are in good agreement with those reported by Baker and Williams [14].

In conclusion, we have determined the rate constants of the four reactions metarhodopsin I  $\rightarrow$  metarhodopsin I', metarhodopsin I'  $\rightarrow$  metarhodopsin I, metarhodopsin I'  $\rightarrow$  metarhodopsin II and metarhodopsin II  $\rightarrow$  metarhodopsin I' at various temperatures. From these rate constants, the values of kinetic activation parameters have been determined. We have also

determined the value of the quantum efficiency of the conversion of meta-rhodopsin I into rhodopsin.

### Acknowledgments

The authors are grateful to Professor M. S. Sodha and Professor A. K. Ghatak for useful suggestions and stimulating discussions. This work was partially supported by the Department of Science and Technology (India).

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