EVIDENCE FOR CONFORMATIONAL TRANSITIONS IN BACTERIORHODOPSIN

W. HOFFMANN, M. GRACA-MIGUEL, P. BARNARD* and D. CHAPMAN

Royal Free Hospital School of Medicine, Department of Biochemistry and Chemistry, 8 Hunter Street, London, WC1N 1BP and **Chelsea College, Department of Chemistry, Manresa Road, London, SW3 6LX, England*

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1. Introduction

After light absorption, bacteriorhodopsin undergoes a series of spontaneous reactions before returning to its initial state [1–7]. The number of molecules reacting via the phototransient $'O'_{640}$ has been shown to be temperature dependent [5]. The reaction mechanism proposed earlier [5] to account for this behaviour requires a single exponential decay for the phototransient M_{410}. Two exponential decay functions for this species have been claimed recently [8,9]. We show in this paper that the ratio between the amplitudes of these two decay functions, as well as the amount of $'O'_{640}$ are strongly temperature dependent. This is consistent with the hypothesis that bacteriorhodopsin exists in a temperature-dependent equilibrium and the two forms are determining two different reaction pathways. This hypothesis accounts for the double exponential decay behaviour of M_{410} as well as for the temperature dependence for transient $'O'_{640}$.

2. Materials and methods

Growth of *Halobacterium halobium* R1 (kindly supplied by Dr R. Henderson) and purple membrane preparation was according to the procedure in [10]. The collected purple membranes from the density gradient were washed in 0.1 M sodium acetate (pH 5) and then used in this condition.

We have studied the temperature dependence of the yield of the transient $'O'_{640}$ and the decay behaviour of M_{410} using flash photolysis of light-adapted purple membrane fragments. A detailed description of the apparatus will be given elsewhere. Briefly, a laser (JK laser system, 20 ns pulse width, 530 nm, 50 mJ energy) was used for excitation. After passing white measuring light through the sample it was focused on to a monochromator (Jobin Yvon, 8 nm band width) and then detected by a photomultiplier (Hamamatsu R 928). The time-dependent voltage changes were then recorded by two transient recorders (Datalab DL 905). The most convenient wavelength to monitor the species $'O'_{640}$ was at 660 nm. The decay of M_{410} was determined from $A_{400\ nm}$ values.

3. Results and discussion

A quantitative description of the decay of the depletion signal in the kinetic traces measured at 660 nm is difficult, therefore, the difference between the positive maximum and the baseline (A_{660}^{max} see fig.1(a)) was assumed to be proportional to the concentration of $'O'_{640}$. The total signal amplitude, measured at 400 nm, A_{400}^{tot}, proportional to the concentration of M_{410} molecules, can be used as a measure of reacting bacteriorhodopsin molecules. Any change in the ratio $A_{660}^{max}/A_{400}^{tot}$ is then related to a concentration change of $'O'_{640}$, caused by a parameter change (i.e., temperature). The values $A_{660}^{max}/A_{400}^{tot}$ as a function of temperature at pH 5 are summarised in fig.1(b).

In every case the decay of phototransient M_{410} could be fitted to the sum of two exponential decay functions, consisting of a fast and a slow half-life time, respectively. We have assumed that these kinetics result from the independent decay of two forms of the M_{410} species, consequently the ampli-

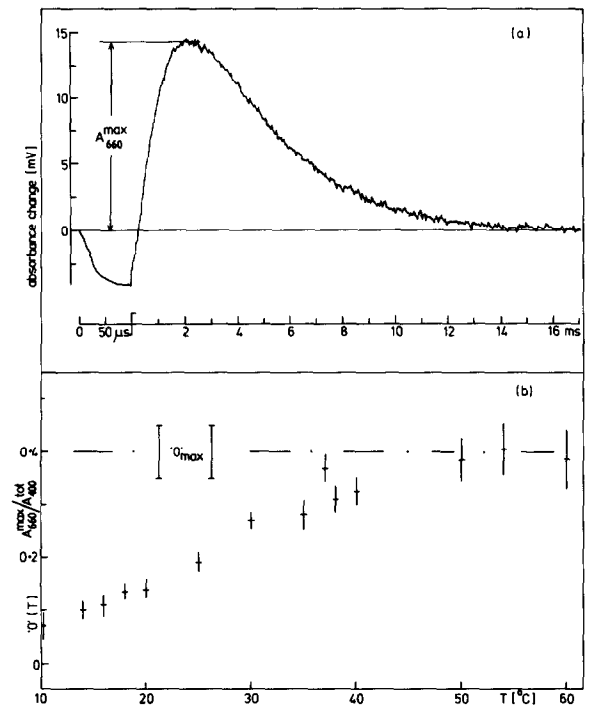

Fig.1.(a) Kinetic signal at 660 nm measuring wavelength, T = 38°C, flash at t = 0, sodium-acetate buffer 0.1 M, pH 5, bacteriorhodopsin 2 × 10^{-6} M. (b) Temperature dependence of the ratio $A_{660}^{max}/A_{400}^{tot}$ which is proportional to the concentration of transient $'O'_{640}$.

tudes of the two exponential terms are proportional to the initial concentrations of these two forms. Figure 2 shows that a change in temperature results in a change in the ratio of these two forms, but not in their sum. Both the amount of the product $'O'_{640}$ and the fast:slow component amplitude ratio of the M_{410} decay are reversible with respect to temperature. The sigmoid curvature obtained for the temperature dependence of transient $'O'_{640}$ together with the fact that the total concentration of its precursor, M_{410}, is independent of temperature, are characteristics of an equilibrium process. We propose that two bacteriorhodopsin conformers are initially in equilibrium, resulting in two parallel reaction pathways, as indicated by the double exponential decay of M_{410}. The concentration of $'O'_{640}$, occurring in only one of the two pathways, is assumed to be controlled by this equilibrium.

If we denote $'O'_{max}$ as the maximum ratio obtained in fig.1(b), i.e., $'O'_{max} = (A_{660}^{max}/A_{400}^{tot})_{max}$, and $'O'(T') = (A_{660}^{max}/A_{400}^{tot})(T)$ as the ratio obtained at the respective temperature T, then the equilibrium constant for this proposed equilibrium is given by $K(T) = 'O'(T)/('O'_{max} - 'O'(T))$. The temperature dependence for the equilibrium constant is presented in the van't Hoff plot in fig.3. From the slope of this graph a reaction enthalpy of 16.7 ± 2.3 kcal/mol is calculated. This value is of the same magnitude as those reported for transitions between conformational states of rhodopsin [11,12].

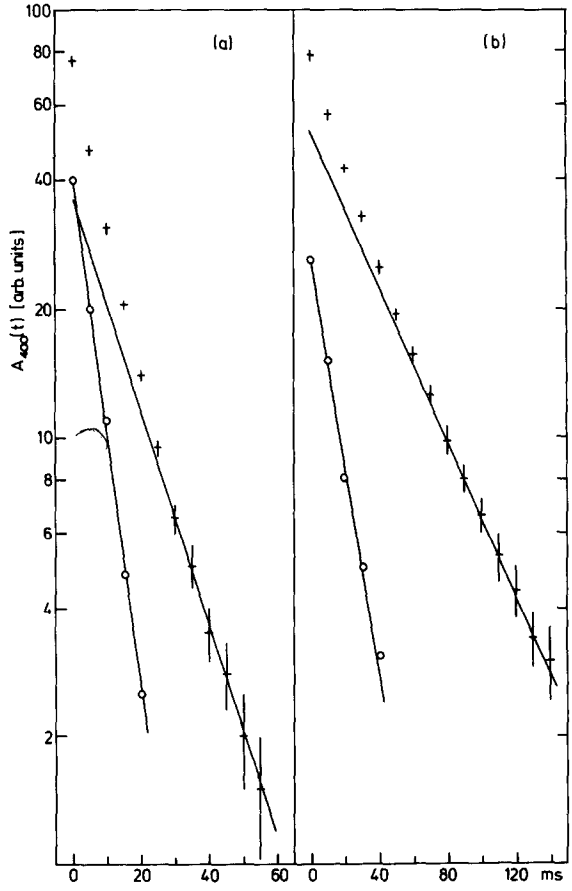

Fig.2. Decay of M_{410} at (a) T = 25°C and (b) T = 11°C. (+) indicate values of $A_{400}(t)$. Extrapolation of the linear portion of this function yields at t = 0, the slow component amplitude. (○) indicate differences between $A_{400}(t)$ and the corresponding values on the extrapolated line. At t = 0, the fast component amplitude is obtained.

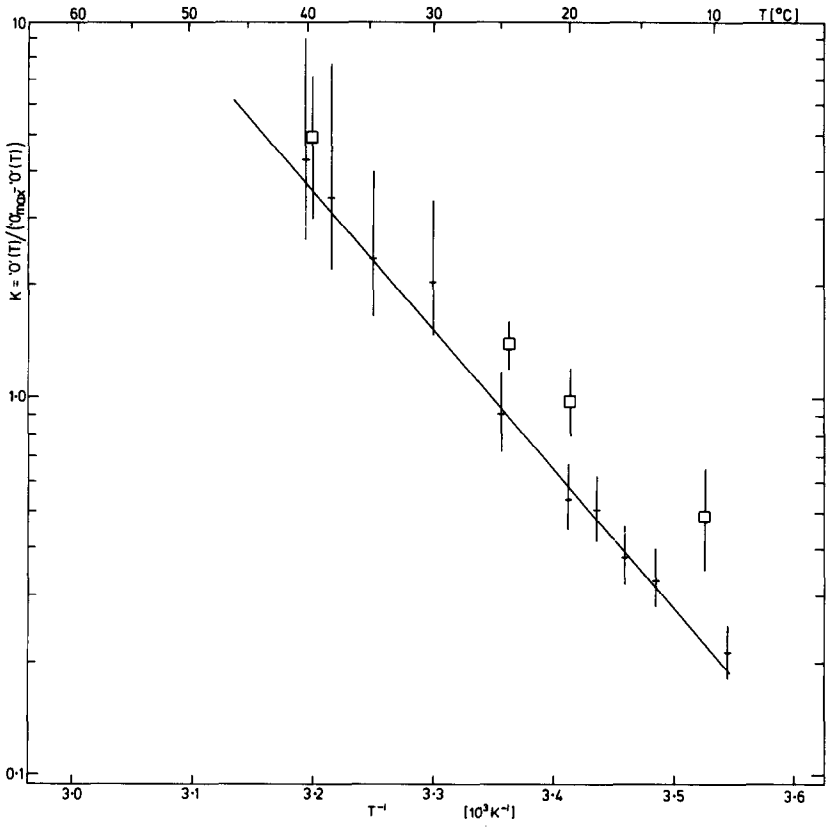

Fig.3. Van't Hoff plot for the proposed equilibrium between two bacteriorhodopsin conformers. Equilibrium constant $K(T)$ given by $'O'\ (T)/('O'_{max} - 'O'\ (T))$ (+) and fast:slow component amplitude ratio (□).

According to our hypothesis of two conformationally altered bacteriorhodopsin molecules, the above defined equilibrium constant is also given by the fast:slow component amplitude ratio of the M_{410} decay. Consequently, the same temperature dependence would be expected. A comparison in fig.3 shows that the equilibrium constants at a particular temperature, determined in the two different ways, agree rather well. It should be noted, however, that a slightly lower reaction enthalpy is calculated from the temperature dependence of the fast:slow component amplitude ratio (13.7 ± 2.5 kcal/mol). The systematic deviations are accounted for by neglecting the absorbance contributions due to the decay of the depletion signal when calculating the $\Delta_{660}^{max}/\Delta_{400}^{tot}$ value.

Our hypothesis is also consistent with results from a recent spin label study [13] where the authors tentatively suggested a conformational change of bacteriorhodopsin.

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