ARRHENIUS PARAMETERS OF THE BACTERIORHODOPSIN PHOTOCYCLE IN DRIED ORIENTED SAMPLES

G. VÁRÓ AND L. KESZTHELYI
Institute of Biophysics, Biological Research Center, Szeged, H-6701, Hungary

ABSTRACT In dried oriented samples of purple membranes isolated from *Halobacterium halobium* the Arrhenius parameters of the photocyle showed an abrupt change at a water content of ~80 H_2O molecules per bacteriorhodopsin molecule. This makes probable the existence of a water-dependent conformational change of the protein. This result underlines the importance of water in the proton-conduction mechanism inside the protein. The effect of the external electric potential on the rate constants of the photoelectric signals was also measured. The data demonstrate that the membrane potential affects the steps of the proton transport during the photocycle.

INTRODUCTION

The bacteriorhodopsin molecules (bR) in dried samples obtained from purple membranes (PM) of *Halobacterium halobium* preserve some features of their activity for light excitation. The light–dark adaptation (Korenstein and Hess, 1977*a*) and the photocycle of the bR (Korenstein and Hess, 1977*b*) were studied in dried PM at different sample humidities. The photoelectric activity of partially oriented samples in continuous light has been reported (Nagy, 1978). A study of the flash-excited photoelectric-response signals associated with the light-absorption changes has already been described (Váró and Keszthelyi, 1983). Here we report the results of the investigation of the Arrhenius parameters of the photoelectric and light-absorption signals and their dependence on the externally applied electric potential.

MATERIALS AND METHODS

PMs used in the preparation of the dried oriented samples were obtained by the standard procedure from *Halobacterium halobium* (*H. halobium*) strain ET 1001 (Oesterhelt and Stoeckenius, 1974). The preparation of the dried oriented samples has been described in detail elsewhere (Váró, 1982). The system for measuring the photoelectric and light-absorption signals reported earlier (Váró and Keszthelyi, 1983) was extended. The closed sample holder, in which the humidity was controlled using various saturated salt solutions (Weast, 1979), was attached to a Peltier element that could vary the temperature of the sample in the range of −15° – +40°C. The change of the water content of the sample, due to temperature changes, was negligible during the measurements.

The external potential (up to 200 V) was supplied by batteries connected in series with the sample and the measuring resistance. The sample was light-adapted by illumination with a mercury lamp (HBO 200; Carl Zeiss, Inc. Jena, German Democratic Republic) through heat and green glass filters (transmission maximum at λ = 550 nm). The measured electrical and optical signals were computer fitted by two exponentials as described (Váró and Keszthelyi, 1983). The decompositions gave good fits to the original curves. The lifetime values at different temperatures were fitted to an Arrhenius expression

$$\tau = \frac{1}{f} e^{\Delta H/RT}, \quad (1)$$

where f is the preexponential factor, ΔH the activation enthalpy, T the absolute temperature, and R the universal gas constant.

RESULTS AND DISCUSSION

Temperature Dependence

The electric-response signals and absorption signals at λ = 410 nm were measured in a broad temperature range for five different humidities. The electric signals were assigned to different transitions in bR photocycle. The assignments for the L–M transition and M decay were made as referred to previously (Váró and Keszthelyi, 1983) using the coincidence of the time constants for electric and absorption signals.

The activation enthalpies and preexponential factors for the two transitions were calculated for the five humidities. They are presented in Fig. 1 *a*, *b* (L-M transition, electric and absorption signals); Fig. 2 *a*, *b* (M decay, electric and absorption signals).

The following characteristics of the activation process are demonstrated by the data. (*a*) The values of activation enthalpy (ΔH) and preexponential factor (f) change abruptly at a water content of 80–100 H_2O molecules per bR molecule. (*b*) The ΔH values do not differ significantly for the two time constants of L-M transition and of the M

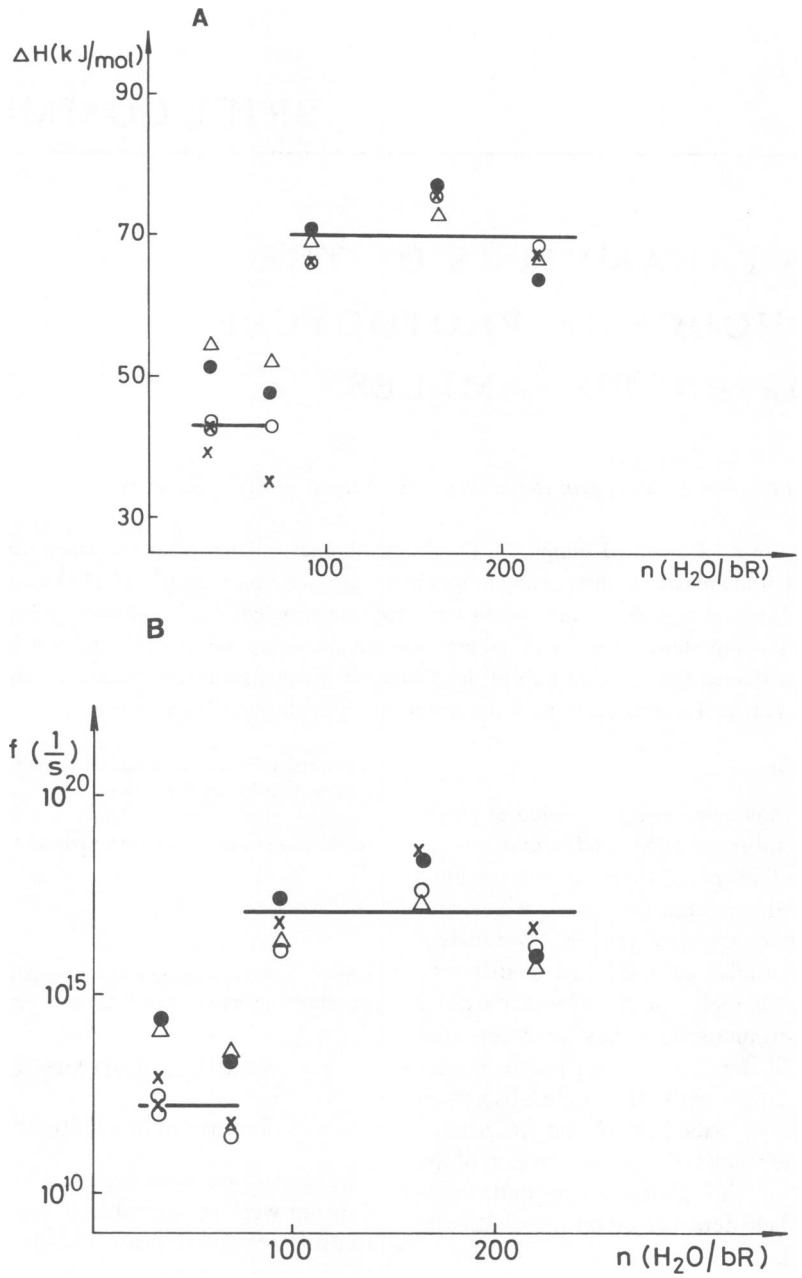

FIGURE 1 Dependence (*a*) of the activation enthalpy and (*b*) of the frequency factor of the L-M transition on the water content. *x*, electric signal fast component; *o*, electric signal slow component; ●, absorption signal fast component; Δ, absorption signal slow component; λ = 400 nm. The lines are to guide the eye.

decay. (*c*) The ratio of the two time constants of L-M transition and/or of the M decay is about 10, which is manifested in the preexponential factor if the activation enthalpies are the same. This difference, however, is negligible in the scale of representation (Figs. 1 *b*, 2 *b*). (*d*) The ΔH values of L-M transition at high water content agree well with the data measured in suspension (Beece et al., 1981). (*e*) There are two values for ΔH and f in Fig. 2 *a*, *b*, respectively, at high H_2O/bR. They reflect the difference of the Arrhenius curve below and above the freezing point of water (Fig. 3). The higher values were calculated for temperatures below 0°C in both figures (Fig. 2 *a*, *b*). The high value of ΔH corresponds well with the ΔH measured for the M-O transition in suspension, whereas the lower value is similar to ΔH for the O-bR transition (Beece et al., 1981).

These observations call attention to the importance of water for the functioning of the bR molecule. It has already been pointed out that the bR does not pump protons if the water content is below 80–100 H_2O molecules per bR molecule, and the proton pump gradually builds up above this value (Váró and Keszthelyi, 1983).

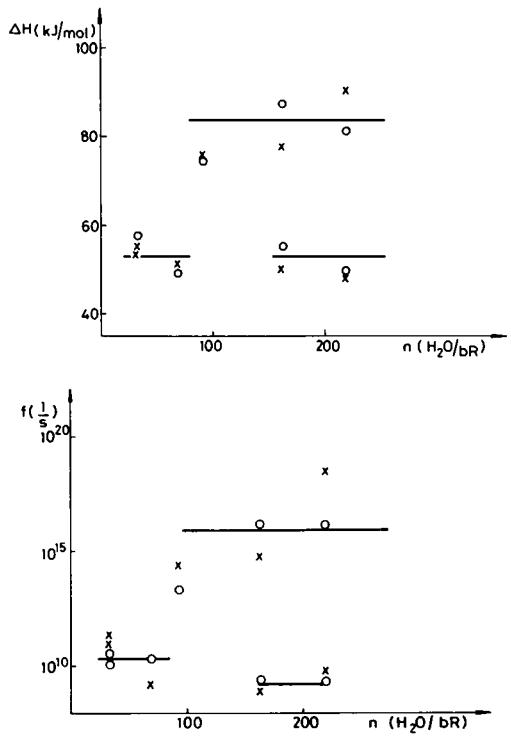

FIGURE 2 Dependence (*a*) of the activation enthalpy and (*b*) of the frequency factor of M decay on the water content. Values calculated from the time constants of the electric signals. *x*, fast components; *o*, slow components. The lines are to guide the eye.

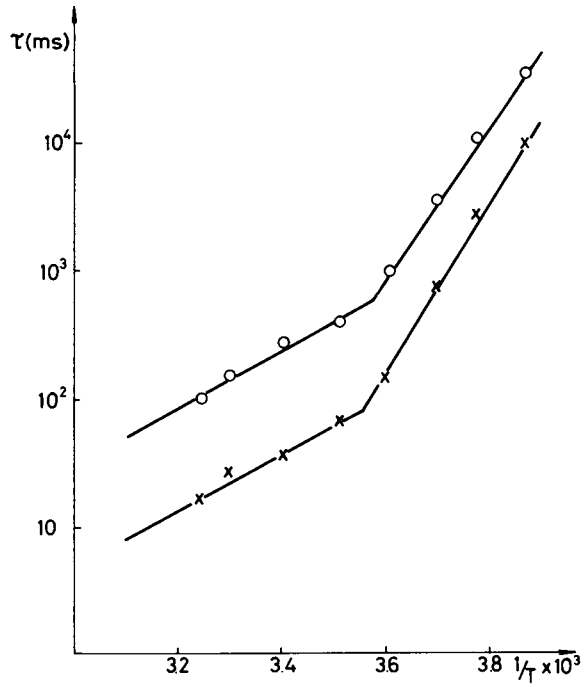

FIGURE 3 Temperature dependence of the time constants of electric signals for M decay. Relative humidity P/P_o = 0.85 (corresponds to n[H_2O/bR] = 210), *x*, fast components; *o*, slow components.

We now see the substantial increase of the Arrhenius parameters around this H_2O/bR ratio and the restoration of the activation enthalpies characterizing the bRs in suspension.

The freezing of water has a profound effect on the Arrhenius parameters at high H_2O/bR ratios (Figs. 2 *a*, *b*, 3). The data give evidence that fluid bulk water is necessary for the O state, which is needed for the proton pump. The absorbance changes characteristic for the O decay also appear at these H_2O/bR ratios at room temperature (Váró and Keszthelyi, 1983).

For long-range transport of protons through bR molecules, there are two current models. The so-called "charge injection model" elaborated by Nagle et al. (see a review by Nagle and Tristram-Nagle, 1983) assumes the existence of hydrogen-bonded chains built up from special amino-acid side chains of the bR. The protons are conducted through this "proton wire" as in the ice after they are "injected" by the absorbed photons.

The other model does not need any special structure because it is based on the general properties of proteins (Keszthelyi et al., 1982; Keszthelyi, 1984). It is well known from H–D exchange experiments that any part of the protein is accessible to water because of the fluctuations of the protein structure (for a review, see Woodward et al., 1982). This is a rather fast process (in the milliseconds range). We assume that the protons in the M state are taken over by the H_2O molecules in the protein and the H_3^+O ions are driven out to the external side and taken up from the internal side by the existing internal electric field due to the primary charge separation.

This second model is supported by the present data. The substantial changes of the Arrhenius parameters and, rather directly, the necessity of fluid water for the appearance of O state, i.e., a normal proton pumping, underline the correctness of the model.

However, the model of Nagle et al. may not be discarded on the basis of the present data. Though one would expect an H-bonded chain to be similar to ice, i.e., to conduct better in a presumably more rigid state without water molecules, it may also be expected that some water molecules are necessary to complete the chain or fluctuation in the components of the H-bonded chain, assured by the loose water environment of the protein, may be needed for conduction.

Effect of the External Electric Potential

The membrane potential increases the lifetime of M decay in cells and envelope vesicles of *H. halobium* (Dancshazy et al., 1983). The study of such effects is straightforward in dried oriented samples. Positive (i.e., what the pumped protons would produce) and negative potentials were applied to the sample and the lifetimes of the electric signals associated with the L-M transition were measured. In Fig. 4 it is clearly shown that this transition is affected by the external electric field. The results of a voltage-

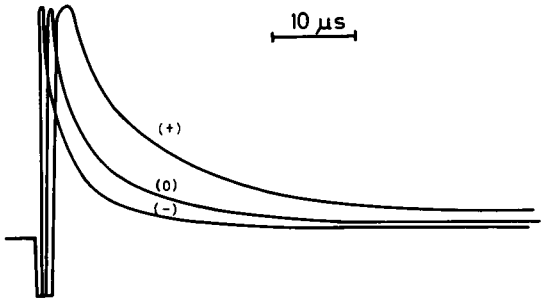

FIGURE 4 Electric potential dependence of the electric signals for the L-M transition (+) and (−) signals positive (with respect to what the pumped protons would produce) and negative potentials, (0) without.

dependent study are represented for the two lifetimes associated with the L-M transition (data points in Fig. 5).

The simplest model for the effect of potential on the decay is based on the assumption that a fraction of potential should be added to or subtracted from the activation enthalpy (Eq. 1)

$$\Delta H(V) = \Delta H + FV. \tag{2}$$

Here F is the Faraday constant and

$$V = \frac{1}{2}\frac{d}{D} \cdot V_o. \tag{3}$$

V_o is the potential on a single PM. It is calculated by dividing the externally applied voltage by the average number of PM layers determined from the measured absorbance. D is the membrane thickness (5 nm) and d is the thickness of the individual barrier, the distance the charges move during the transition. Eqs. 2 and 3 are given by Läuger et al. (1981) for symmetric barriers. Combining Eqs. 1, 2, and 3 gives

$$\tau(V_o) = \frac{1}{f}\exp\left[\frac{\Delta H + \left(F\frac{d}{2D}V_o\right)}{RT}\right]$$

$$= \tau(0)\exp\left[\frac{\left(F\frac{d}{2D}V_o\right)}{RT}\right]. \tag{4}$$

The lines in Fig. 5 are the results of fitting the data points to Eq. 4 with $d_1 = 0.6$ nm and $d_2 = 0.4$ nm for the two components of the L to M transition. The distance determined for the L−M transition by Keszthelyi and Ormos (1980) is ~ 0.5 nm. Therefore, we may consider our data as independent confirmation of the previous result. We note that the two components are probably not different transitions but the result of a somewhat distributed potential barrier (Váró and Keszthelyi, 1983).

The authors are grateful to I. Szundi for helpful discussions, and to Zs. Dancshazy and E. Hegedüs for supplying the PM suspension.

This work was partially supported by cooperative grants between the Hungarian Academy of Sciences and the National Science Foundation under grant INT 82-17761.

Received for publication 10 April 1984 and in final form 5 July 1984.

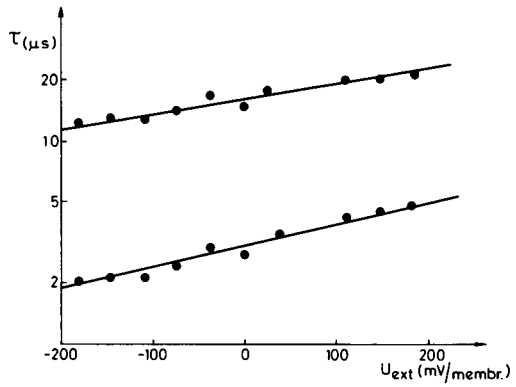

FIGURE 5 Time constants of the electric signals for the L-M transition in externally applied potential. ($T \simeq 300°K$, $n[H_2O/bR] = 70$). The lines are results of computer fits of Eq. 4.

REFERENCES

Beece, D., S. F. Bowne, J. Czege, L. Eisenstein, H. Frauenfelder, D. Good, M. C. Marden, J. Marque, P. Ormos, L. Reinisch, and K. T. Yue. 1981. The effect of viscosity on the photocyle of bacteriorhodopsin. *Photochem. Photobiol.* 33:517–522.

Dancshazy, Zs., S. L. Helgerson, and W. Stoeckenius. 1983. Coupling between the bacteriorhodopsin photocycle kinetics and the protonmotive force. I. Single flash measurements in *Halobacterium halobium* cells. *Photobiochem. Photobiophys.* 5:347–357.

Keszthelyi, L. 1984. Intramolecular charge shifts during the photoreaction cycle of bacteriorhodopsin. *In* Biological Membranes—Information and Energy Transduction in Biological Membranes. E. J. Helmreich, editor. Alan R. Liss Inc. New York. In press.

Keszthelyi, L., and P. Ormos. 1980. Electric signals associated with the photocycle of bacteriorhodopsin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 109:189–193.

Keszthelyi, L., P. Ormos, and G. Váró. 1982. Fast components of the electric response signal of bacteriorhodopsin protein. *Acta Physica Acad. Sci. Hung.* 53:143–157.

Korenstein, R., and B. Hess. 1977*a*. Hydration effects on cis-trans isomerization of bacteriorhodopsin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 82:7–11.

Korenstein, R., and B. Hess. 1977*b*. Hydration effects on the photocycle of bacteriorhodopsin in thin layers of purple membrane. *Nature (Lond.).* 270:184–186.

Läuger, P., R. Benz, G. Stark, E. Bamberg, P. C. Jordan, A. Fahr, and W. Brock. 1981. Relaxation studies of ion transport systems in lipid bilayer membranes. *Q. Rev. Biophys.* 14:513–598.

Nagle, J. F., and S. Tristram-Nagle. 1983. Hydrogen bonded chain mechanisms for proton conduction and proton pumping. *J. Membr. Biol.* 74:1–14.

Nagy, K. 1978. Photoelectric activity of dried oriented layers of purple membrane from *Halobacterium halobium*. *Biochem. Biophys. Res. Commun.* 85:383–390.

Oesterhelt, D., and W. Stoeckenius. 1974. Isolation of the cell membrane of *Halobacterium halobium* and its fractionation into red and purple membranes. *Methods Enzymol.* 31:667–678.

Váró, G. 1982. Dried oriented purple membrane samples. *Acta. Biol. Acad. Sci. Hung.* 32:301–310.

Váró, G., and L. Keszthelyi. 1983. Photoelectric signals from dried oriented purple membranes of *Halobacterium halobium*. *Biophys. J.* 43:47–51.

Weast, R. C. 1979. Handbook of Chemistry and Physics. 57 ed. 46.

Woodward, C., I. Simon, and E. Tüchsen. 1982. Hydrogen exchange and the dynamic structure of proteins. *Mol. Cell Biochem.* 48:135–160.