Angle of the retinal of bacteriorhodopsin in blue membrane

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ABSTRACT The electric dichroism of purple and cation-depleted (blue) membrane was measured in a.c. electric fields at saturation. A decrease of 5.5° in the direction of the chromophore transition moment with respect to the membrane normal was found upon removal of cations from purple membrane.

INTRODUCTION

The integral protein bacteriorhodopsin (bR) from purple membrane (PM) functions as a light-driven proton pump (Oesterhelt and Stoeckenius, 1971, 1973). The retinal chromophore bound to the protein moiety via a protonated Schiff base linkage (Lewis et al., 1974; Rothschild et al., 1982) is involved in the proton pumping.

A variety of treatments, including deionization (Kimura et al., 1984a), acidification (Oesterhelt and Stoeckenius, 1971; Moore et al., 1978; Edgerton et al., 1978), and lipophilic anions (Kamo et al., 1987) have been reported to result in a reversible red shift of the bR absorption maximum by ~30 nm. bR in blue membrane does not pump protons (Mowery et al., 1979).

Two current interpretations are found in the literature: (a) the purple-blue transition is caused by protonation of the Schiff base counterion due to the changing surface charges (Chang et al., 1986) or (b) by conformational changes of the protein (Szundi and Stoeckenius, 1987; Corcoran et al., 1987). In a recent paper, Szundi and Stoeckenius (1988) point out that at high proton concentration (low pH or low cation concentration at higher pH, which seem to be equivalent) on or near the membrane, surface groups which stabilize the native conformation are protonated, causing an imbalance in interactions of charged groups inside the protein. This leads to conformational changes resulting in a small change in the Schiff base counterion distances and consequently a red shift in the absorption.

In this paper we attempt to evaluate the change of the angle of the retinal to the membrane normal. We have found that the purple-blue transition induces a change in this angle from ~70 to ~65°.

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MATERIALS AND METHODS

PM fragments were isolated from *Halobacterium halobium* strain ET 1001 according to Oesterhelt and Stoeckenius (1974). Cation-depleted (blue) membranes were prepared by passing PM suspension on a cation exchange column of well-washed resin Dowex 50 (W-Fluka AG, Buchs, Switzerland) (Kimura et al., 1984a). Absorption spectra were recorded on a UV-VIS spectrophotometer (model UV-160, Shimadzu Scientific Instruments, Inc., Columbia, MD). The concentration of purple and blue membrane was determined using the extinction coefficients (bR$_{568}$ = 63,000 [Oesterhelt and Hess, 1973] and bR$_{605}$ = 60,000 [Kimura et al., 1984a], respectively).

The method used to determine the angle of the retinal was to measure the linear dichroism of the membrane fragments after ordering by a.c. field. According to the theory (Fredericq and Houssier, 1973),

$$\frac{\Delta A_{\parallel,\perp}}{A} = \frac{1}{A} \lg \left(1 + \frac{\Delta I_{\parallel,\perp}}{I} \right), \qquad (1)$$

where $\Delta A_{\parallel,\perp}$ and $\Delta I_{\parallel,\perp}$ are the changes of absorbance and light intensity, respectively, due to the applied electric field in case of parallel ($\parallel$) and perpendicular ($\perp$) polarized light in relation to the direction of the field. $A = \lg I_0/I$ is the absorbance in the absence of the electric field; I_0 and I are light intensity without and with the sample, respectively.

The $\Delta A/A$ values (called reduced dichroism) at any field strength can be written as

$$\frac{\Delta A_{\parallel}}{A} = (3 \cos^2 \Theta - 1) \, \Phi(\beta,\gamma) \qquad (2a)$$

$$\frac{\Delta A_{\perp}}{A} = \left(\frac{3}{2} \sin^2 \Theta - 1 \right) \Phi(\beta,\gamma), \qquad (2b)$$

where Θ is the angle of the chromophore, $\beta = \mu E/kT$, $\gamma = \alpha E^2/2kT$, μ and α being the permanent electric dipole moment and polarizibility of the particles, respectively, E the electric field strength, k the Boltzmann constant, and T the temperature. In case of a.c. field $\beta = 0$, and the limiting value of $\Phi(0,\gamma)$ for high E is $-\frac{1}{2}$ (Shah, 1963).

In the measurement the saturation value of $\Delta A_{\parallel}$ is determined. a.c. electric fields of 100–350 V/cm, frequency 1 kHz, duration 0.8 s were applied to the membrane suspension in a quartz cuvette of 0.1 cm thickness by platinum electrodes in a distance of 1 cm. A tungsten lamp, followed by interference filters ($\lambda = 575$ nm and $\lambda = 600$ nm in the case of purple and blue membrane, respectively), was used as a light source. The photomultiplier signal (light intensity change) was recorded by a

multichannel analyzer (ICA 70, product of Central Research Institute of Physics, Budapest). Five to 10 signals were averaged.

RESULT AND DISCUSSION

A red shift of the absorption maximum of ~35 nm is observed after deionization of PM suspension (not shown). The relative light intensity changes parallel $\Delta I_\parallel/I$ to an a.c. electric field applied to purple and blue membrane suspensions are shown in Fig. 1 in dependence of the field strengths.

Measurements were performed in the range of the electric field strength to reach the saturation of the reduced dichroism. The saturation value is lower for cation-depleted than for purple membrane. This indicates a change in the tilt angle. Table 1 contains data for purple and blue membranes for different preparations. The mean value for purple membranes is 70.6 ± 0.6° in accordance with previous determinations (Barabás et al., 1982; Dencher, 1983; Kimura et al., 1984b; Earnest et al., 1986) and for blue membranes 65.1 ± 0.5°. It can be stated, therefore, that the purple-blue transition is connected with a substantial change of 5.5 ± 0.8° in the orientation of the retinal.

This result is in accord with the hypothesis of Szundi and Stoeckenius (1988) that "the purple-blue transition involves just such a change in the protein conformation which results in alteration of the distance between the counterions and the retinal." The change of the retinal angle can occur only if the geometrical arrangement of the protein alters too. The observed change of the angle may indicate a loosening up of the protein, consequently in a possible change of the distance between the retinal and the counterions as it was suggested.
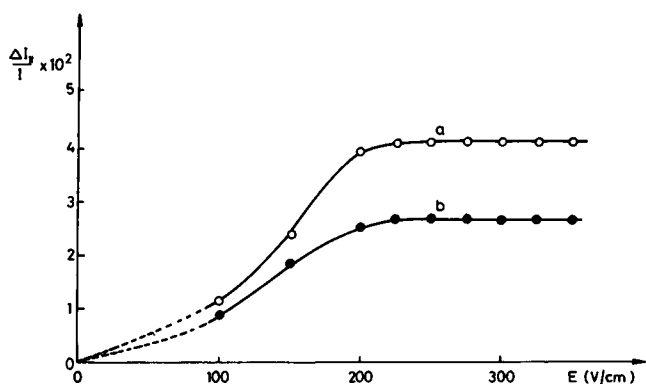

FIGURE 1 Dependence of the $\Delta I_\parallel/I$ value from the applied a.c. voltage. Different saturation values are measured for purple (a) and blue (b) membrane. pH = 5.8, T ≈ 22°C. Lines were drawn to guide the eyes.

TABLE 1   Values of the retinal angle θ determined from the saturation values of $\Delta I_\parallel/I$ for three samples of different A

A	Θ_{PM}	Θ_{BM}
	degrees	*degrees*
0.1	72.3	66.4
	71.7	66.4
	69.5	64.1
0.05	69.2	64.3
0.042	70.4	64.3
Average	70.6 ± 0.6	65.1 ± 0.5
Difference	5.5 ± 0.8	

Eqs. 1 and 2 were used to calculate θ. PM, purple membrane. BM, blue membrane.

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