ELECTRIC FIELD EFFECTS IN BACTERIORHODOPSIN

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ABSTRACT Exposure of aqueous suspensions of fragments of the purple membrane of *Halobacterium halobium* to electric field pulses leads to transient linear dichroism phenomena. The effects are interpreted in terms of field-induced alignments of the bacteriorhodopsin chromophore. Two observed relaxation times (τ) are attributed to rotation of the whole membrane fragments ($\tau_s \sim 100$ ms), and to a much faster reorientation of the chromophore within the membrane ($\tau_f \sim 260\ \mu$s).

Electrooptical phenomena in biological systems are frequently associated with molecular reorientation induced by external fields, giving rise to dichroism or birefringence (1, 2). Direct effects on optical absorption bands are also known, usually due to the electrochromic effect (3–5), serving as an internal probe for the voltage across photosynthetic membranes (6, 7). Chemical field effects have been reported for reactions involving changes in dipole moments or dissociation of weak electrolytes (1, 2). In the present work we report phenomena associated with the exposure of aqueous suspension of fragments of the purple membrane of *Halobacterium halobium* (8) to the strong transient electric field applied in a (Joule heating) temperature-jump instrument (6-70 Messanlagen, G.m.b.H., Göttingen). The experimental effects are attributed to field-induced orientation and thermal rotational relaxation of membrane fragments, as well as to orientation of the bacteriorhodopsin (BR) chromophore within the membrane. The data bear both on properties of the purple membrane and on the spectroscopy of the BR chromophore.

After the $\sim 10^{-5}$-s electric discharge in dark-adapted aqueous purple membrane (*H. halobium* M_1) solutions, transient decay patterns in the region of the main 565-nm absorption band are observed (Fig. 1*a*). To rationalize the observed phenomena, two different effects should be considered: (*a*) absorbance changes induced by the rapid heating of the solution (T-jump), due to evolution of chemically reacting species towards the equilibrium concentrations defined by the new temperature; and (*b*) electric field effects as outlined above, especially those associated with partial orientation of species which have large permanent or induced

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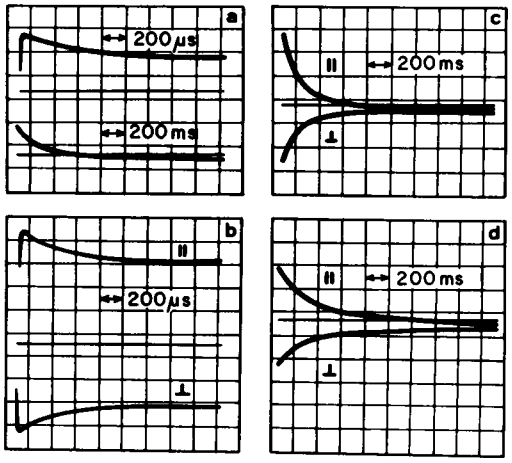

FIGURE 1 Characteristic oscillograms showing relaxation phenomena in aqueous (dark-adapted) purple membrane solutions exposed to electric field pulses ([BR] $\cong 1.5 \times 10^{-5}$ M, $t = 25°C$) *a*. Time-dependent absorbance changes recorded with an unpolarized monitoring beam. *b–d*. Changes recorded with the above minotiring beam, polarized in parallel ($\parallel$) or perpendicular ($\perp$) to the applied electric field. *b, c* in water. *d*, in a 32% glycerol-water solution. In all experiments the straight horizontal lines correspond to the base line before or several seconds after the pulse. The light-to-dark deflection was $V_0 = 4.3$ V. The salt concentration was 0.1 M. All measurements were performed with a 570-nm monitoring beam. (Vertical sensitivity: 200 mV/div.)

dipole moments. The latter effects have been extensively investigated for linear polyelectrolite and nucleic acid molecules in solution (9, 10). To discriminate between T-jump and orientational electric-field effects (see ref. 10 and its references for a comprehensive discussion), experiments were carried out in which the analyzing light beam was plane-polarized parallel or perpendicular to the applied electric field (~ 10 kV/cm). Characteristic results of such experiments are shown in Fig. 1 *b–c*. It is evident that the transient changes in absorbance are almost exclusively due to a time-dependent linear dichroism (expressed as a function $R(t)$ proportional to $D_\parallel(t) - D_\perp(t)$, where $D_\parallel(t)$ and $D_\perp(t)$ are the absorbance changes for light polarized parallel or perpendicular to the field, respectively). The small residual absorbance change observed at long time, when $R(t) \rightarrow 0$, is due to T-jump or to electric field (other than orientational) effects and will be discussed elsewhere.

The decay of the linear dichroism exhibits two distinct kinetic stages. A fast initial drop with a lifetime of $\tau_f \sim 260$ μs (Fig. 1 *b*) is followed by a much slower process in the 10^2-ms range (Fig. 1 *c*). The latter stage can be described by at least two exponential decays with rate constants differing by a factor of ~ 2. In water at 25°C, the central fraction ($\sim 75\%$) of the slow process is characterized by a lifetime of $\tau_s \sim 100$ ms. The last stages of the decay exhibit even longer lifetimes.

In keeping with the assignment of the above transient phenomena to electric field orientational effects is the dependence of the corresponding absorbance changes on the magnitude of the applied voltage. A linear dependence of the amplitude of the

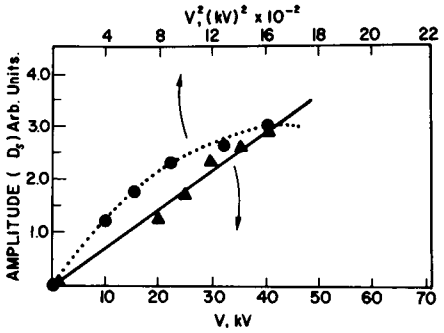

FIGURE 2 Voltage effect on the absorbance change D_s (measured with a nonpolarized monitoring beam) induced at 25°C in the T-jump instrument. [NaCl] = 0.1 M, C = 0.02 μF, λ = 590 nm. [Br] $\cong 1.4 \times 10^{-5}$ M.

slow relaxation (D_s) on V is observed (Fig. 2), contrary to the proportionality to $1/2CV^2$ (where C is the discharge circuit capacitance) characteristic of T-jump (heating) effects. A further test was carried out by varying the capacitance in the discharge circuit, as well as the salt concentration in the BR sample. The data of Table I indicate that, as long as the RC value is maintained constant (the resistance, R, is inversely proportional to the salt concentration, M), the amplitude associated with D_s is independent of C. A decrease in C without a compensating increase in R causes a drop in D_s, implying that the magnitude of the electric field effect depends on the pulse duration which increases with RC. i.e., D_s depends on the time during which the solution is exposed to the external field. It is therefore evident that the changes induced by the field are not instantaneous, as for e.g. the electrochromic effect, and that the associated field-induced orientation time is of the order of the pulse duration ($\sim 10^{-5}$ s).

Assignments of the two principal relaxation times to specific processes could be made on the basis of the respective sensitivities to the macroscopic medium viscosity

TABLE I

Effects of circuit capacitance (C) and cell conductance (NaCl concentration) on the amplitude of the (slow) change (D_s, measured with a nonpolarized monitoring light beam) induced in aqueous ($\sim 1.4 \times 10^{-5}$ M) bacteriorhodopsin in the T-jump instrument.

C	[NaCl]	C/[NaCl]	Relative initial amplitude
μF	M	$\mu F/M (\times 10)$	
0.01	0.1	1.0	1.3 ± 0.15
0.02	0.1	2.0	2.0 ± 0.1
0.02	0.25	0.8	0.9 ± 0.15
0.05	0.25	2.0	2.0 ± 0.1

All measurements were carried out at 25°C with a constant voltage of 30 kV.

$\eta \cdot \tau_s$ was found to be markedly affected by η. For example, when the latter was varied at 25°C by a factor of 2.7, e.g., by passing from water to a 32% glycerol-water mixture, a comparable increase in τ_s was observed (Fig. 1 *d*). However, absolutely no external medium effects on τ_s could be detected. The rotational relaxation time of purple membrane fragments, represented as oval disks with a diameter r, may be semi-quantitatively represented (11) by Perrin's formula $\tau_s = \eta 2\pi r^3/3kT$. Thus, the observed dependence of τ on η identifies τ_s as a rotational relaxation time of the whole field-oriented purple membrane fragments suspended in the aqueous solution. For such fragments (which are ~50 Å thick) $r \cong 0.5$ μm (12, 13), so that the expected τ_s value is of the order of 10^2 ms, in agreement with our present results.

The parameter τ_f, insensitive to the viscosity of the aqueous environment, is attributed to the relaxation of the BR chromophore oriented by the field within the membrane itself. Whether the oriented species consists of the whole BR molecule or only of a limited section carrying the retinal residue is still an open question. If the first mechanism applies, then in view of the high (75%) protein content of the membrane (12, 13), such an internal field-induced orientation may be regarded as a membrane-shearing process. According to this interpretation, the BR molecules are associated with a high permanent (or induced) individual dipole moment (note the value of ~700D measured for the rhodopsin molecule [14]). This moment may also be responsible for the net cross-membrane dipole moment which causes the whole fragment orientation. Until the exact nature of the fast intramembrane orientational relaxation is unambiguously established, we hesitate to apply Perrin's equation to the fast decay, τ_f. It is likely, however, that this parameter may serve as a probe for the internal membrane viscosity, like that obtained for frog rhodopsin from the transient dichroism induced by polarized light pulses (15). The above interpretations of the field-induced relaxations are in complete agreement with the very recent observations of Lozier and Niederberger (16), who reported a two-component decay (~0.3 ms and ~70 ms) of a polarization anisotropy, after the polarized (laser) excitation of purple membrane fragments. Further work will be required for a comprehensive application of both techniques to the study of dynamic aspects of the purple membrane, as well as to the spectroscopy of the BR chromophore.

The authors are grateful to Dr. K. Rosenheck, Professor S. R. Kaplan and A. Danon of the Weizmann Institute of Science, and to Professors B. Perlmutter-Hayman and J. Hayman, and Dr. B. Honig of the Hebrew University for helpful discussions. They are indebted to Mrs. A. Danon for her valuable help in the preparation of the BR samples.

Received for publication 1 June 1976 and in revised form 29 December 1976.

REFERENCES

1. ILGENFRITZ, G. 1971. Electric field jump relaxation method. *In* Probes of Structure and Function of Macromolecules and Membranes. Academic Press, Inc. New York. Vol. 1 p. 505.
2. DE MAEYER, L. 1969. Electric field methods. *Methods Enzymol.* **16**:80.
3. LIPTAY, W. 1969. Electrochromism and solvatochromism. *Angew. Chem. Int. Ed. Engl.* **8**:177.

4. REICH, R., and S. SCHMIDT. 1972. Über den Einfluss elektrischer Felder auf das absorption Spektrum von Farbstoffmolekulen in Lipidschichten. I. Theorie. *Ber. Bunsenges. Phys. Chem.* **76**:589.
5. REICH, R., and S. SCHMIDT. 1972. Über den Einfluss elektrischer Felder auf das absorption Spektrum von Farbstoffmolekulen in Lipidschichten. III. Elektrochromic eines carotinoids. *Ber. Bunsenges. Phys. Chem.* **76**:1202.
6. JUNGE, W., and H. T. WITT. 1968. On the ion transport system of photosynthesis. Investigation on a molecular level. *Z. Naturforsch.* **23b**:244.
7. WITT, H. T. 1971. Coupling of quanta, electrons, fields, ions and phosphorylation in the functional membrane of photosynthesis. *Q. Rev. Biophys.* **4**:365.
8. OESTERHELT, D., and W. STOECKENIUS. 1971. Rhodopsin-like protein from the purple membrane of *Halobacterium halobium. Nat. New Biol.* **232**:149.
9. ALLEN, F. S., and K. E. VAN HOLDE. 1971. Dichroism of TMV in pulsed electric fields. *Biopolymers.* **10**:865.
10. DOURLENT, M., J. F. HOGREL, and C. HÉLÈNE. 1974. Anisotropy effects in temperature-jump relaxation studies on solutions containing linear polymers. *J. Am. Chem. Soc.* **96**:3398.
11. SHERMAN, W. V., M. A. SLIFKIN, and S. R. CAPLAN. 1976. Kinetic studies of phototransients in bacteriorhodopsin. *Biochim. Biophys. Acta.* **423**:238.
12. BLAUROCK, A. E., and W. STOECKENIUS. 1971. Structure of the purple membrane. *Nat. New Biol.* **233**: 152.
13. HENDERSON, R. 1975. The structure of the purple membrane from *Halobacterium halobium:* analysis of the x-ray diffraction pattern. *J. Mol. Biol.* **93**:123.
14. PETERSON, D. C., and R. A. CONE. 1975. The electric dipole moment of rhodopsin solubilized in Triton X-100. *Biophys. J.* **15**:1181.
15. CONE, R. A. 1972. Rotational diffusion of rhodopsin in the visual receptor membrane. *Nat. New Biol.* **236**:39.
16. LOZIER, R. H., and W. NIEDERBERGER. The photochemical cycle of bacteriorhodopsin. To be published.