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Electrogenic transport properties of bacteriorhodopsin containing chemically modified retinal analogues

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The electrical activity of bacteriorhodopsin (BR) containing the 13-substituted retinal analogues 13-demethyl and 13-methoxy as well as the naturally occurring retinal carrying a methyl group at C13 is compared. White membrane patches reconstituted with the different retinals are attached to a black lipid membrane, and the dependency of the photocurrent on light intensity is measured. This allows a comparison of the overall photocycle time and the number of protons transported per cycle for the various preparations. From previous work (Gärtner, W., Towner, P., Hopf, H. and Oesterhelt, D. (1983) Biochem. 22, 2637–2644, see also Gärtner, W. and Oesterhelt, D., unpublished data) the equilibrium isomeric distribution (all-*trans* and 13-*cis*) of the different retinals in the binding site is known. Taking into account that only all-*trans* retinal BR contributes to the pumping activity (Fahr, A. and Bamberg, E. (1982) FEBS Lett. 140, 251–253), it is shown, that the cycle time for the modified BRs is moderately changed, whereas the number of protons transported per cycle and transporting all-*trans* BR molecule is not affected by the substituent. It is concluded, that substituting the methyl group at position 13 of the retinal molecule by a hydrogen atom or a methoxy group only slightly affects the pumping activity of the *trans*-photocycle, but rather controls the biological function of BR via the equilibrium isomeric distribution of the retinal molecule in the binding site.

Glossary

BR,	bacteriorhodopsin;
C_m,	capacity of underlying black lipid membrane;
C_p,	capacity of reconstituted white membrane from retinal-deficient mutants;
f^{tr},	fraction of *trans* isomer;
FCCP,	carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone;
G_m,	conductance of underlying black lipid membrane;
G_p,	conductance of reconstituted white membrane from retinal-deficient mutants;
$h\nu$,	energy of incident light quanta;
Hepes,	4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid;
HPLC,	high performance liquid chromatography;

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$i_{p0}(t)$,	current density generated by the pump at zero potential;
I_{p0},	current density generated by the pump in the stationary state at zero potential;
I_0,	initial current density measured at $t = 0$;
I^s,	saturation value of initial current density;
$j(t)$,	light intensity;
J,	light intensity at $t = 0$;
$J_{1/2}$,	light intensity at half saturation of I_0;
J_{max},	light intensity in the absence of attenuating neutral density filters;
n,	number of protons transported per cycle per bacteriorhodopsin molecule;
p_0,	probability of finding the system in BR_0;
p_x,	probability of finding the system in BR_x;
q,	charge translocated per cycle;
T_c,	decay time constant of BR_x;
η,	quantum efficiency;
λ_{max},	wavelength of maximal absorbance;
ρ,	density of BR molecules on the black lipid membrane;
σ,	absorption cross section;
τ,	time for the pump to reach its stationary state;
13-dm,	13-demethyl;
13-OCH_3,	13-methoxy;
13-dm BR,	bacterioopsin reconstituted with 13-dm retinal;
13-OCH_3 BR,	bacterioopsin reconstituted with 13-OCH_3 retinal.

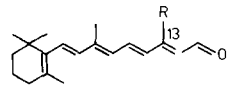
Introduction

In the membrane of *Halobacterium halobium* distinct patches, the so-called purple membranes are found, which consist of only one protein species together with the lipid. This protein, named bacteriorhodopsin (BR), contains as its prosthetic group one molecule of retinal, which is covalently bound as a protonated Schiff base to the ϵ-amino group of lysine 216 in the polypeptide chain (for a review, see Refs. 1 and 24). Depending on the illumination conditions, different isomers of retinal occur in the binding site. In the light-adapted state, $\lambda_{max} = 568$ nm, almost exclusively the all-*trans* isomer is present, whereas in the dark-adapted state, $\lambda_{max} = 558$ nm, a 1 : 1 mixture of 13-*cis* and all-*trans* isomers is present [2]. Comparison with the absorption maximum of a model compound (retinal *n*-butylamine protonated Schiff base, $\lambda_{max} = 440$ nm), reveals that the absorption maximum of BR is further red shifted by about 130 nm. Other noncovalent interactions between retinal and aromatic or charged amino-acid side chains of the binding site are assumed to be responsible for this effect [3].

The unique absorption properties of BR allow a very efficient conversion of light energy into the electrochemical energy of a proton gradient. The incident light drives a cycle of photochemical and thermal isomerization reactions of the retinal moiety, which are accompanied by de- and reprotonations of the protonated Schiff base. During this photocycle, which is completed within milliseconds, one or two protons are transported to the outer side of the membrane [4]. Although photochemical activities were detected for both possible isomeric conformations of the retinal molecule (13-*cis* and all-*trans*) only the all-*trans* form is electrogenically active [5–7].

The spectroscopic properties of BR are well described, but much less information is available about the interactions between retinal and the amino acid residues of the binding site. A powerful approach to this problem is the use of chemically modified retinals, which can occupy the binding site of the retinal-free apoprotein bacterioopsin, thus reconstituting a BR analogue [7–10]. The activity of BR can be tested on intact cells, liposomes or black lipid membranes. Since adsorption of membrane patches containing electrogenically active proteins to black lipid membranes has proved to be a versatile and sensitive

13-substituted retinal analogues



$R=CH_3$ retinal

R=H 13-demethyl retinal

$R=OCH_3$ 13-methoxy retinal

Fig. 1. Structures of retinal analogues modified at position 13.

method to investigate their electrical behaviour [11–14], we applied this method to BR analogues carrying the modified retinals 13-demethyl retinal (13-dm), and 13-methoxy retinal (13-OCH_3) instead of the naturally occurring retinal (Fig. 1).

Material and Methods

Sample preparation. The synthesis and characterization of 13-dm retinal is described elsewhere [15]. 13-OCH_3 retinal was synthesized by Natsias and Hopf [16]. All retinal compounds were purified by high-performance liquid chromatography (HPLC) prior to use. Solvents and chemicals were of analytical grade. White membrane derived from retinal deficient *Halobacteria* mutants was prepared according to Ref. 7. BR analogues were reconstituted by the addition of retinal analogues to the apoprotein in a molar ratio of 0.9 : 1.0, to allow a complete reconstitution of the added retinal. No absorption peak around 360 nm, indicating the presence of unreacted retinal, persisted. Also further addition of retinal did not significantly increase the absorption of the chromophore. The influence on the electrical measurements of photoexcitation of traces of free retinal in the sample was tested by removing the cut-off filter (see below) from the light beam. The additional near ultraviolet light ($\lambda < 350$ nm) had no effect on the signal. Alternatively, BR analogues were produced by growing up retinal-deficient mutant cells under addition of retinal analogues and isolation of the BR analogue containing purple membrane. Also in these samples no free retinal could be detected spectroscopically. No differences in the electrogenic properties between BR isolated from bacteria and BR prepared by reconstitution from white membrane from retinal-deficient mutants were found. Routinally reconstituted BR samples were used, in order to prevent artifacts due to different sizes of the membrane patches or contaminating protein content. For determination of the isomeric distribution, retinals were extracted from the binding sites and analyzed as described by Ref. 7.

Electrical measurements. Optically black lipid membranes with an area of $8 \cdot 10^{-3}$ cm^2 were formed in a teflon cell (7 ml) filled with a solution of 0.1 M NaCl and 1 mM Hepes (pH 6.0). The black lipid membrane was formed from a solution consisting of 1.5% (w/v) diphytanoylphosphatidylcholine and 0.025% (w/v) octadecylamine dissolved in *n*-decane [17]. It was connected to an external measuring circuit via Ag|AgCl electrodes, which were separated from the cell by salt bridges [13]. The current amplifier (Keithley model 427) was set to a gain of 10^9 V/A and a rise time (10–90%) of 10 ms.

Light from a 150 W tungsten lamp equipped with a heat protection filter and a cut off filter ($\lambda > 435$ nm) was focussed on to the membrane. With this setup the light intensity at the membrane was 2.0 W/cm^2 corresponding to a spectral irradiance of $2.6 \cdot 10^{-3}$ $W \cdot cm^{-2} \cdot nm^{-1}$ at $\lambda =$ 575 nm. Neutral density filters could be applied to measure the dependence of the photocurrent on the light intensity. For the determination of the action spectrum narrow band interference filters ($\Delta\lambda = 10$ nm) could be inserted into the light beam.

1.5 nmol of the reconstituted protein in 50 μl of distilled water was added to the rear compartment of the cell, whereas the light entered through the front compartment (Fig. 2). For all experiments the same amount of chromoprotein was used. After stirring for 15 min the photoresponse of the black lipid membrane-BR system developed and remained constant after 60 min. For details of the method, see Ref. 13.

Results and Discussion

The attachment of BR to a black lipid membrane results in a sandwich-like structure [13]. The BR patches adsorb to the bilayer, but do not integrate into the membrane. The resulting arrangement is shown in Fig. 2a, and can be

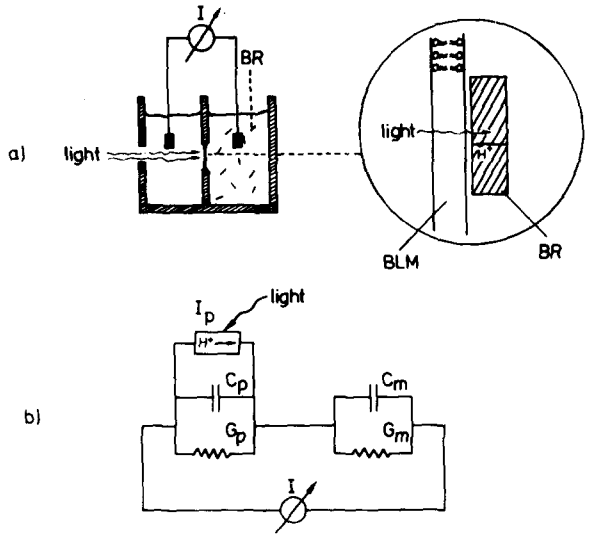


Fig. 2. (a) Experimental set-up for the measurement of photocurrents and schematic representation of the arrangement of BR patches adsorbed to the black lipid membrane (BLM). (b) Equivalent circuit for the BR-black lipid membrane system.

described by a circuit diagram as given in Fig. 2b. The analysis of the diagram shows that activation of the proton pump results in a high initial current decaying exponentially to a stationary value, when the capacitors C_p and C_m are charged [12,13]. The initial current density I_0 is related to I_{p0}, the current density generated by the pump at zero potential [13] via:

$$I_0 = I_{p0}\frac{C_m}{C_p + C_m} \tag{1}$$

The value of the stationary current depends on the conductivity G_m of the black lipid membrane and the shunt conductivity, G_p. G_m can be changed from 10^{-9} to 10^{-6} $S \cdot cm^{-2}$ by the addition of the uncoupler FCCP and the electrically silent Na^+/H^+ exchanging carrier monensin to both sides of the black lipid membrane. The stationary currents observed under these conditions prove the continuous pump activity of the protein. Even after the addition of the ionophores, a certain resistance of the black lipid membrane remains. At high light intensities this may lead to a potential across the attached BR patches and could distort quantitative measurements of the light intensity dependence of the proton pump activity. Therefore, instead of monitoring the stationary current density we determined the initial current density I_0, which is directly proportional to the pump current density I_{p0}, to characterize the electrogenic properties of the pump.

Typical traces of the light induced current densities of BR, 13-dm BR and 13-OCH_3 BR in the presence of the uncouplers are shown in Fig. 3. The stationary photocurrents demonstrate continuous proton-pumping activity for all three preparations. The modified retinals, however, generate an approx. 20-times lower stationary current density than the naturally occurring retinal.

As shown in the appendix, cycle time and pumping stoichiometry may be obtained by measuring the dependence of the pumping current on the light intensity. When applying light of different intensities J to the BR-black lipid membrane system, kinetics of Michaelis-Menten type are expected:

$$I_0 = I^s\frac{J}{J + J_{1/2}} \tag{2}$$

I^s is the saturation current density and $J_{1/2}$ is the light intensity at half saturation. By combining the two Eqns. A-6 (see Appendix) the phenomenological parameters I^s and $J_{1/2}$ can be correlated to the microscopic properties of the pumping system:

$$T_c = \frac{h\nu}{\eta\sigma}\frac{1}{J_{1/2}}$$
$$n = \frac{h\nu}{\eta\sigma}\frac{1}{q\rho}\frac{I^s}{J_{1/2}} \tag{3}$$

T_c is the time needed to complete a pumping cycle, while transporting n protons across the membrane. The other variables are: the energy of the incident light quanta $h\nu$, the quantum efficiency η, the absorption cross section σ, the proton charge q and the density of BR molecules on the black lipid membrane.

Clearly the calculation presented above only applies for $t \gg \tau$, where τ is the time the pump needs at zero potential to reach its stationary state (see Appendix, Eqn. A-4). As suggested above, the initial current density I_0 is used to measure the electrogenic activity of the protein. I_0 was determined by extrapolating the exponential decay shown in Fig. 3 to $t = 0$, which is the time when

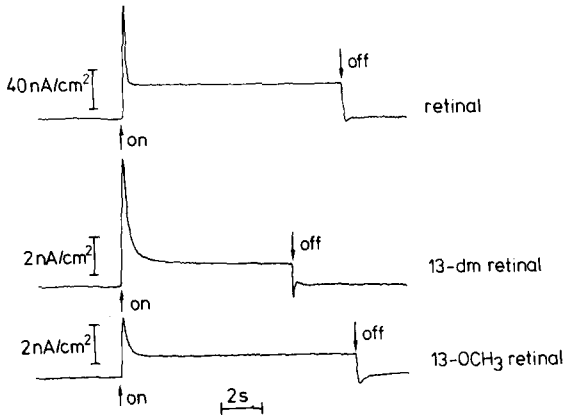


Fig. 3. Light-induced current densities of BR, 13-dm BR and 13-OCH_3 BR. The solution contained 0.1 M NaCl, 1 mM Hepes (pH 6.0), 0.1 μM FCCP and 10 μM monensin. The arrows indicate switching on and off of the light. Light intensity was 290 mW/cm^2 within the BR absorption band (520–630 nm).

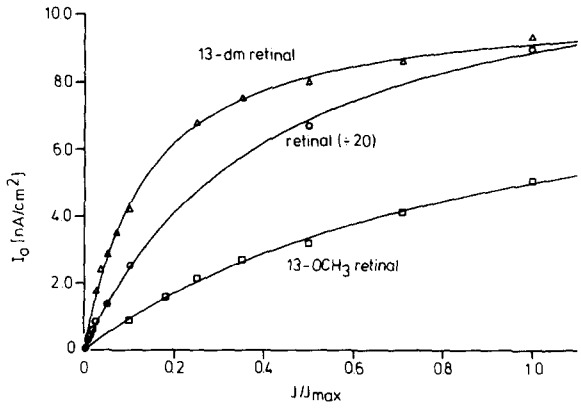


Fig. 4. Initial current density I_0 vs. light intensity J/J_{max} for BR (○), 13-dm BR (△) and 13-OCH_3 BR (□). J_{max}, the maximal light intensity used, was 290 mW/cm^2 within the BR absorption band (520–630 nm). The solid line is a fit to the data with a Michaelis-Menten type saturation curve (see Eqn. 2). Note the different scale for unmodified BR.

the light was switched on. In order to determine the properties of the pump in the stationary state ($t \gg \tau$), only data points with $t > 200$ ms were used for the extrapolation. This must be compared with τ, which at maximal light intensity used here has a value of $\tau < 20$ ms. Of course, for long cycle times ($T_c > 200$ ms) and low light intensities the condition $t \gg \tau$ might not be fulfilled. On the other hand, for $t \ll \tau$ a linear instead of a saturating intensity dependence would be expected *, which is in contradiction to our experimental results.

The different activities of the three BRs become more obvious in Fig. 4, which shows the dependence of I_0 on the light intensity given as the ratio J/J_{max}. There is a striking difference (a factor of 20) between the saturation current densities I^s of the samples reconstituted with the naturally occurring retinal compared to those reconstituted with 13-dm or 13-OCH_3 retinal. The half saturation intensities $J_{1/2}$ indicate that the cycle times T_c for the three preparations increases in the order 13-OCH_3 retinal, retinal, 13-dm retinal. For a quantitative comparison the data were fitted with a saturation curve (solid line in Fig. 4) as given by Eqn. 2. The cycle time T_c and the number of protons translocated per cycle can be calculated according to Eqn. 3. Unfortunately, the amount of BR adsorbed to the membrane is not known. Also the values of C_m and C_p in Eqn. 1 could not be determined. Therefore all results were normalized

* This can be easily verified by writing down Eqn. A-4 (see Appendix) for $t \ll \tau$:

$$i_{p0}(t, J) = nqp\eta\sigma t \frac{J}{h\nu T_c}$$

TABLE I

PARAMETERS OF THE FIT SHOWN IN FIG. 4 $J_{1/2}/J_{max}$ (J_{max} = 290 mW/cm^2) AND I^s

The parameters allow the calculation of the relative cycle time $T_c^{rel} = J_{1/2}(BR)/J_{1/2}(x)$ and the relative number of protons transported $n^{rel} = J_{1/2}(BR)/I^s(BR) \cdot I^s(x)/J_{1/2}(x)$ with x = 13-dm BR or x = 13-OCH_3 BR (see Eqn. 3). These relations are strictly valid only under the assumption, that η and σ are the same for BR and the modified preparations. For comparison the relative concentration of *trans* isomer f^{tr} and the absorption wavelength of *trans* BR λ^{tr}_{max} have been included.

	$\frac{J_{1/2}}{J_{max}}$	I^s (nA/cm^2)	T_c^{rel}	n^{rel}	f^{tr}	λ^{tr}_{max} (nm)
retinal	0.42	250	1	1	0.91 [a]	570 [a]
13-dm retinal	0.14	10.4	3	0.13	0.16 [a]	565 [a]
13-OCH_3 retinal	0.97	9.9	0.4	0.02	< 0.02 [b]	550 [b]

[a] From Ref. 7.

[b] Gärtner, W. and Oesterhelt, D., unpublished data.

to the values obtained for unmodified BR. The relative quantities T_c^{rel} and n^{rel} are summarized in Table I. Clearly, this procedure is only applicable under the assumption that η and σ as well as ρ, C_m and C_p are the same for the three different preparations.

The white membrane from retinal-deficient mutants used for the reconstitution showed a residual electrogenic activity with a half saturation intensity similar to BR, but a current density at maximal light intensity which was less than 10% of the current density of 13-OCH_3 BR under the same conditions. The contribution of the white membrane from retinal-deficient mutants to the electrical signal was therefore neglected. The action spectrum of the white membrane is identical to the action spectrum of BR within the error limits, and, as for the other samples, a stationary current could be measured in the presence of the ionophores. The electrogenic activity of the white membrane therefore must be probably attributed to residual BR in the preparation.

To estimate the errors associated with the relative quantities given in Table I, the experiments were repeated several times under identical conditions. It turned out that the absolute currents measured could be reproduced with an accuracy of about $\pm 20\%$. The influence of FCCP and monensin on the photoelectric properties of the three samples, BR, 13-dm BR and 13-OCH_3 BR was investigated by performing the experiments in the presence and in the absence of the ionophores. In both cases the parameters n^{rel} and T_c^{rel} obtained were the same within the error limits.

The assumptions involved in the analysis given above merit more detailed discussion. C_m and C_p are determined by the geometrical arrangement of the white membrane from retinal-deficient mutants on the black lipid membrane and must be very similar for different experiments. Also, the absorption cross sections of the electrically active isomer (*trans*) of the different BRs are close together (Ref. 7; see also Gärtner, W. et al., unpublished data). As it is obvious from Eqn. 3 a reduced η for the modified BRs would lead to an increased n and n^{rel}. This is inconsistent with the values of f^{tr} obtained by extraction experiments (see Table I), since $n^{rel} > f^{tr}$ is only possible if the modified compound transports more protons per cycle per pumping molecule than unmodified BR. Recent results for the quantum efficiency of BR give a lower bond of $\eta \geqslant 0.6$ [18]. Therefore an acceptable range $0.6 \leqslant \eta < 1$ for the quantum efficiency of the modified BRs is obtained. This range results in a variation for T_c and n of $\pm 25\%$, which is comparable with the error as estimated above.

Proton pumping activity of BR analogues has been studied in the past mainly by light-induced pH changes in reconstituted vesicle suspensions [7,19,20]. Data obtained for 13-dm BR using this method are presented in Ref. 7 and are in reasonable agreement with our results. The authors of Ref. 20, however, failed to detect any proton-pumping activity of 13-dm BR. The same is true for 13-OCH_3 BR, which did not show light-induced pH changes in vesicle suspension (Gärtner, W. and Oesterhelt, D., unpublished data). On the other hand, our measurements demonstrate proton-pumping activity for these modified BRs. We attribute this discrepancy to the insufficient sensitivity of the liposomal system, because our data show that, at low light intensities, the proton-pumping activity of 13-OCH_3 BR can be as low as 2% of the activity of unmodified BR (Table I).

A surprising result of our measurements is that the photocycle is only retarded by a factor of 3 for 13-dm BR and even speeded up by approx. a factor of 2 for 13-OCH_3 BR. The drastically reduced stationary current density of the two analogues compared to BR as presented in Fig. 3 is explained by the low number of protons transported (n^{rel}) for the modified BR. Here we emphasize that n represents the number of protons transported per cycle per BR molecule, regardless of the conformation of the chromophore present in the binding site. It is not to be confused with the stoichiometry of the pumping process, the number of protons translocated per cycle per pumping BR molecule (*trans*).

Considering the present state of knowledge about the transport mechanism in BR, only the *trans* isomer translocates a net charge across the membrane [6]. For this reason, in comparing n for the different BR analogues we must also consider f^{tr}, the fraction of *trans* isomer present in the binding site. ($f^{tr} = n^{tr}/(n^{tr} + n^{cis})$; n^{tr} and n^{cis} are the number of *trans* and *cis* isomers, respec-

tively). f^{tr} has therefore been included in Table I. Comparison of n^{rel} and f^{tr} demonstrates another surprising fact: the number of protons transported in one cycle of the pumping species (*trans*) is approximately the same for the unmodified and the modified BR. This means that substituting the methyl group at position 13 by a hydrogen atom or a methoxy group only slightly affects the pumping *trans*-photocycle, but rather controls the biological function of BR via the equilibrium isomeric distribution of the retinal molecule in the binding site. Data about the photocycle of 13-dm BR obtained by following the flash photolytic absorbance changes have been recently obtained [21]. A time constant of 20 ms for the rate-limiting step of the *trans*-photocycle has been found in these measurements. This agrees well with a value of $T_c = 30$ ms, which is obtained from Table I by assuming a cycle time of 10 ms for unmodified BR, a value that is generally accepted.

Interestingly, the results for the modified retinals support a prediction derived from theoretical calculations [22]. The authors of Ref. 22 report thermal 13-*cis*/all *trans* isomerisation barriers for retinal (48.1 kJ/mol), 13-dm retinal 68.7 kJ/mol and 13-OCH_3 retinal (18.0 kJ/mol) in the protein-binding site. According to these values and taking into account that the rate-limiting step of the *trans*-cycle of BR involves a thermal 13-*cis*/all-*trans* isomerisation, the cycle times for the three compounds are predicted to increase in the order: 13-OCH_3 retinal, retinal, 13-dm retinal. This is in fact observed (see Table I).

The very unusual biological properties of 13-OCH_3 BR are illustrated most clearly by Fig. 5, which represents the action spectrum, i.e., the dependence of the electrogenic activity on the wavelength of the incident light. Whereas for light adapted BR and for 13-dm BR the maximal activity coincides with the absorption maximum within the error limits (data not shown), we find for 13-OCH_3 BR that the maximal absorption is around 515 nm but the peak of maximal activity is focused around 550 nm (Fig. 5). The apparent discrepancy between the absorption and the action maxima can be explained by the observed isomeric distribution of 13-OCH_3 retinal in the binding site: the abundant 13-*cis* and 9,13-di*cis* isomers give rise to the absorption maximum at

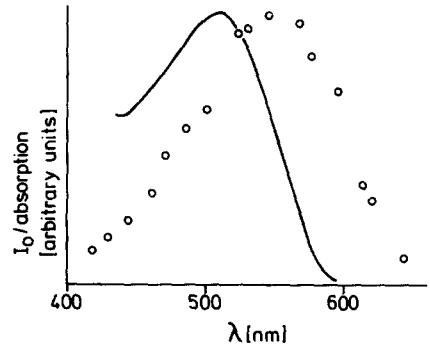


Fig. 5. Action spectrum of 13-OCH_3 BR (○). For comparison the absorption spectrum of the sample has been included (solid line).

515 nm, whereas the peak of the action spectrum around 550 nm reflects the very small amount of the all-*trans* isomer (less than 2%), which could not be detected by extraction experiments. The presence of the all-*trans* isomer is assumed from the results cited in the introduction, and an absorption maximum of 550 nm for all-*trans* 13-OCH_3 BR is proposed from Fig. 5.

The results of 13-OCH_3 BR must be compared with data which have been obtained for 13-ethyl BR by the same method. Since the results of this preparation greatly resemble the data obtained for BR, we do not present any detailed measurements. Interestingly, the ethyl and the methoxy group require about an equal amount of space within the binding site, as was concluded from the comparison of their Van der Waals radii. The stronger electronegativity and the lone pair electrons of the oxygen atom of the OCH_3 substituent, however, force the molecule into a 13-*cis* configuration with an in planar arrangement of the methoxy group and the polyene chain [22]. Therefore, these electronic interactions, which are not present in 13-ethyl retinal, serve as an explanation for the very small amount of the all-*trans* form.

Conclusions

Measurements of the electrogenic properties of retinal analogues, incorporated into the binding site of bacterioopsin have been performed by attaching the reconstituted membrane patches to a black lipid membrane. This method was used to demonstrate the effect of electronic and steric

modifications at position 13 of the retinal molecule, on the photocycle of the corresponding BR analogue. In particular we find that substituting the methyl group at position 13 with a hydrogen atom or a methoxy group, changes the cycle time of the modified BR by a factor of 3 and 0.4, respectively. In addition, our measurements demonstrate that the number of protons transported per cycle per pumping molecule (*trans*) is not affected by the substituents. The moderate modification of cycle time contrasts with the drastic effects of the substituent on the isomeric distribution of the retinal in the binding site, which accounts for the 20-fold reduction in biological activity of the analogues.

Appendix. Time and intensity dependence of a relaxation model for a light-driven ion pump

BR may be considered as an enzyme driven by the 'substrate' light [23]. Consequently, the Michaelis-Menten formalism of enzyme kinetics can be applied to BR [23,12]. In this appendix we discuss the response of a light-driven ion pump to a step in light intensity, and derive expressions which relate the phenomenological parameters of the light intensity dependence of the photocurrent to the overall cycle time and the number of charges translocated per cycle.

A sketch of the simple model adopted for this analysis is shown in Fig. A-1. Following the absorption of a light quantum $h\nu$ the molecule is transferred from the ground state BR_0 to the excited state BR_x. Thereupon it relaxes back to BR_0 with lifetime T_c by transporting n protons across the membrane. The absorption process is described by the cross section σ and quantum efficiency η and is assumed to be much faster than T_c. In this simple model, the pumping cycle is described by only one time constant T_c, the overall cycle time. In the case of BR this corresponds to the rate-limiting step M → BR (for the designation of BR photocycle intermediates, see, e.g., Ref. 1) with a time constant of approx. 10 ms.

The model system is described by two coupled linear differential equations:

$$\frac{dp_0}{dt} = -\frac{j(t)}{h\nu}\eta\sigma p_0 + \frac{1}{T_c}p_x$$
$$\frac{dp_x}{dt} = \frac{j(t)}{h\nu}\eta\sigma p_0 - \frac{1}{T_c}p_x \qquad \text{(A-1)}$$

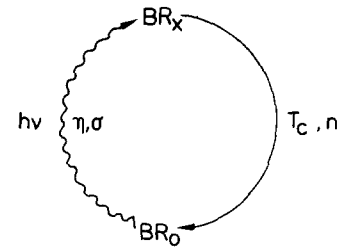


Fig. 6. A-1. Simple relaxation model for a light-driven ion pump. BR_0 = ground state, Br_x = excited state, $h\nu$ = photon energy, η = quantum efficiency, σ = absorption cross section, T_c = relaxation time, n = number of charges translocated.

In Eqn. A-1 p_0 and p_x are the probabilities of finding the system in the state BR_0 and BR_x, respectively, and $j(t)$ is the intensity of the exciting light (quantum energy, $h\nu$).

At time $t = 0$ light of intensity J is switched on:

$$j(t) = \begin{cases} 0 (t < 0) \\ J (t \geq 0) \end{cases} \qquad \text{(A-2)}$$

The current density produced by a planar system of oriented enzyme molecules (area density, ρ) in the absence of a potential across the molecule is given by:

$$i_{p0}(t) = nq\rho\frac{1}{T_c}p_x(t) \qquad \text{(A-3)}$$

Here q is the elementary charge.

Solving the differential Eqn. A-1 under the boundary condition A-2 and calculating the light-induced current density by Eqn. A-3 yields:

$$i_{p0}(t) = \frac{nq\rho}{\frac{h\nu}{J\eta\sigma} + T_c}(1 - e^{-t/\tau})$$
$$\frac{1}{\tau} = \frac{1}{T_c} + \frac{J\eta\sigma}{h\nu} \qquad \text{(A-4)}$$

Eqn. A-4 shows that after switching on the light the current density rises with a time constant, which for small light intensities is equal to the cycle time T_c. It reaches a stationary value given by:

$$I_{p0} = \frac{nq\rho}{T_c}\frac{J}{J + \frac{h\nu}{\eta\sigma T_c}} \qquad \text{(A-5)}$$

Eqn. A-5 represents a Michaelis-Menten saturation behaviour where the saturation current density I^s and half saturation intensity $J_{1/2}$ are:

$$I^s = \frac{nq\rho}{T_c}$$
$$J_{1/2} = \frac{h\nu}{\eta\sigma T_c} \qquad \text{(A-6)}$$

Eqn. A-6 demonstrates that the measurement of the intensity dependence of the photocurrent of an oriented light-driven ion pump allows determination of the overall cycle time T_c and the number of charges translocated per cycle n by fitting a Michaelis-Menten type saturation curve to the data and calculating T_c and n according to Eqn. A-6.

If, as in the case of BR, some of the molecules are electrically silent (*cis*), a slight modification has to be applied to Eqns. A-6: n has to be replaced by $n \cdot f^{tr}$, where f^{tr} represents the fraction of molecules actually contributing to the pumping current (*trans*).

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