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Electrogenic photocycle of the 13-*cis* retinal-containing bacteriorhodopsin with an M intermediate involved

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It has been found that bacteriorhodopsin containing 13-*cis*-retinal as the chromophore (13-*cis*-bR) is competent in a one-photon photocycle which involves an M-type intermediate. Such an effect can be shown in 13-*cis*-bR obtained by means of 13-*cis*-retinal treatment of white bacterioopsin membrane sheets, as well as in dark-adapted purple sheets, solubilized bR and bR proteoliposomes. The 13-*cis*-bR photocycle with the M intermediate is found to be proton-motive, the electrogenic phases resembling those of the all-*trans*-bR photocycle. All of these effects are observed only when the pH is above some critical value, namely pH 8.5–9.5 for the bR sheets, 7.6 for proteoliposomes and 7.0 for the solubilized bR.

Bacteriorhodopsin; Photocylce; 13-*cis*-Retinal; Light-dark adaptation; M intermediate; (*Halobacterium halobium*)

1. INTRODUCTION

It is known that light-adapted bR contains only all-*trans*-retinal as the chromophore. After dark adaptation, some bR molecules were found to contain all-*trans*-retinal, and others 13-*cis*-retinal. Adaptation takes place in purple membrane sheets, proteoliposomes and solubilized bR [1–5]. The all-*trans*-bR photocycle is proton-motive, electrogenic and includes a short-wavelength intermediate M. Conversely, the 13-*cis*-bR photocycle is assumed to be electrically silent, and to involve only a long-wavelength intermediate [6–10]. This intermediate has a difference spectrum maximum at 610 nm (room temperature) [6]. At $t > -140$°C, the intermediate decay results in the formation of a mixture of 13-*cis*- and all-*trans*-bR. At room temperature, the decay takes about 40 ms [6]. The quantum yield for formation of the long-wavelength intermediate amounts to 0.3 [10], whereas that of 13-*cis*-bR ⟶ all-*trans*-bR is about 0.03 [11].

Kalisky et al. [11] showed that a nanosecond laser flash of high intensity gives rise to an M intermediate from 13-*cis*-bR. The authors assumed this M intermediate to participate in the all-*trans* photocycle. A two-photon pathway between the two photocycles was postulated.

Here, we have found that 13-*cis*-bR forms M in a single-photon fashion, when the pH of the medium is above some critical value. This 13-*cis*-bR photocycle proved to be electrogenic and proton-motive.

2. MATERIALS AND METHODS

Purple and white membrane sheets were isolated from *Halobacterium halobium* ET1001 and JW5, respectively [12]. To obtain dark-adapted purple sheets, they were kept for 24 h in distilled water at 23°C. In some cases, dark-adapted purple sheets were solubilized by means of treatment with 2% (w/v) Triton X-100 for 24 h in the dark at pH 7.0 and 23°C. To regenerate purple from white sheets, the latter were incubated with a 2-fold excess of 13-*cis*-retinal in the dark for 10 min at 0°C. The methods for reconstitution of bR proteoliposomes, measurements of the fast kinetics of M formation, pH changes

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and electric response in the 'bR proteoliposome-collodion film' system are described in [8,13,14].

The following light sources were employed: a 90 W halogen lamp, a photoflash ($t_{1/2}$ = 0.4 ms, 30 mJ) and a Quantel YG-481 ND-YAG Q-switched laser with frequency doublers (λ = 532 nm, $t_{1/2}$ = 15 ns). The light intensity of laser flashes was varied by means of neutral filters, thus covering the range 5–15 mJ.

Triton X-100 was from Serva and asolectin from Sigma (type IIS).

3. RESULTS AND DISCUSSION

Figs 1 and 2 show the formation and decomposition of the M intermediate. In fig.1 white membrane sheets containing bacterioopsin were treated with 13-*cis*-retinal in the dark so that 13-*cis*-bR was obtained. One observes that a 15 ns laser flash causes no formation of M in 13-*cis*-bR at pH 6.1. bR becomes competent at producing M after light adaptation. This can be accounted for by the light adaptation-induced 13-*cis*-bR ⟶ all-*trans*-bR transition. At pH 9.8, not only preilluminated but also non-preilluminated 13-*cis*-retinal-regenerated bR is observed to form M.

As can be seen in fig.2A, the magnitude of M formation in light-adapted purple sheets is approx. 2-fold greater than that in dark-adapted ones (pH 7.5). This difference is most probably due to partial all-*trans* ⟶ 13-*cis* isomerization of the bR chromophore on dark adaptation. At the same time, bR solubilized with 2% Triton X-100 is equally effective at producing M in its light- and dark-adapted forms (fig.2B). The same figure also shows that decomposition of M is more rapid in dark-adapted vs light-adapted bR, since a proportion of the short-lived form of M in the total of pool M increases after dark adaptation. It was found that the difference spectra of short- and long-lived M intermediates are similar (λ_{max} ~ 410 nm). Other spectral measurements confirmed that light adaptation of Triton-solubilized bR is accompanied by a 4–8 nm red-shift of the ground-state spectral maximum (not shown). As shown previously by Stoeckenius and co-workers [5], dark adaptation of Triton-solubilized bR results in all-*trans* ⟶ 13-*cis* isomerization occurring similarly to that in purple sheets or proteoliposomes.

Further experiments revealed a pronounced pH dependence of the novel 13-*cis*-bR photocycle involving the M intermediate. It was found that there exists a critical pH below which this cycle is arrested. For solubilized bR, bR proteoliposomes, and purple sheets at high and low ionic strength, the pH values were shown to be 7.0, 7.6, 8.5 and 9.5, respectively. At higher pH values, the magnitudes of M formation in dark- and light-

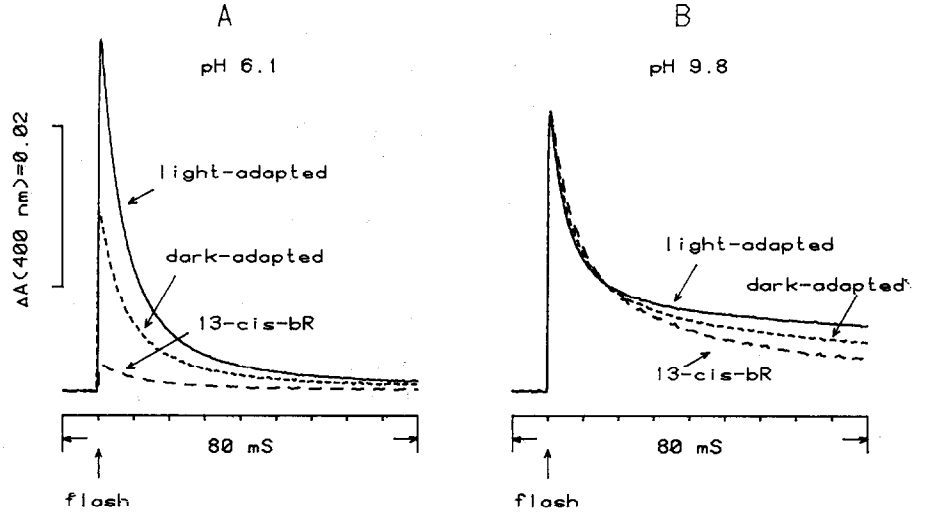

Fig.1. Formation and decay of the M intermediate in purple membrane sheets regenerated from white sheets and 13-*cis*-retinal. 13-*cis*-bR response to the first flash after dark regeneration is shown. (Light-adapted) Response to flash after 5 min preillumination of regenerated sheets. (Dark-adapted) Response to flash after 3 h incubation of light-adapted sample at pH 6.1. Incubation mixture contained 0.1 M potassium citrate-phosphate-borate buffer, and 13-*cis*-retinal-regenerated purple sheets (1.2×10^{-5} M bR); at 23°C.

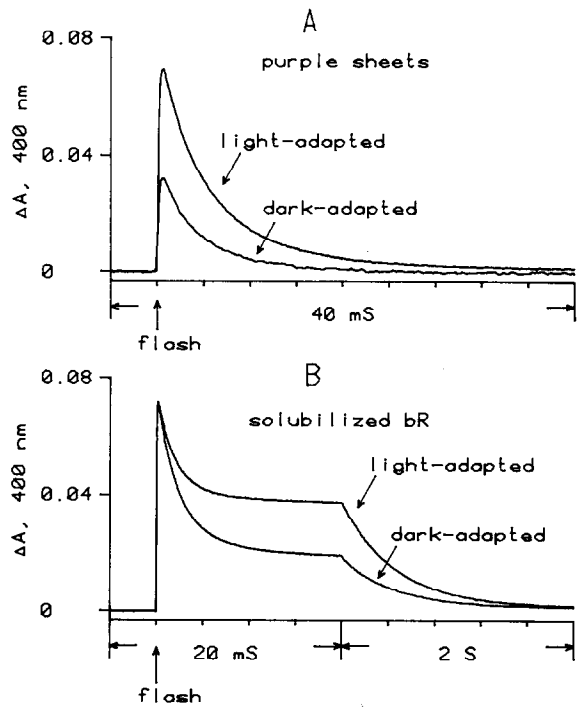

Fig.2. Formation of the M intermediate in purple sheets and Triton X-100 solution of bR. Incubation mixture: 60 mM potassium citrate-phosphate-borate buffer, pH 7.5, and 1.25×10^{-5} M bR.

adapted samples were equal in proteoliposomes and in Triton X-100 bR solution (see fig.2B). For the purple sheets, this magnitude in the light-adapted sample was 1.4-fold greater compared to the dark-adapted one at pH values above the critical value and 2-fold higher below it.

In all cases, flash-induced M formation from 13-*cis*-bR could be demonstrated at low light intensity (0.2–0.8 mJ). Under these conditions the magnitude of M formation was a linear function of the light. The facts indicate that the observed phenomenon cannot be accounted for by a two-photon process.

In the latest series of experiments, it has been shown that the 13-*cis*-bR photocycle is proton-motive and electrogenic.

Using pyranine to monitor rapid pH changes, we found that the 13-*cis*-bR photocycle of solubilized bR at pH > 7 is accompanied by reversible pH release resembling that of the all-*trans*-bR photocycle (not shown). The only difference is that the contribution of the fast component of the pH change decay increases in 13-*cis*-bR compared with all-*trans*-bR. This is in agreement with a similar effect on M decay (see fig.2B).

Fig.3 depicts the photoelectric response of dark- and light-adapted bR proteoliposomes at pH 6.0

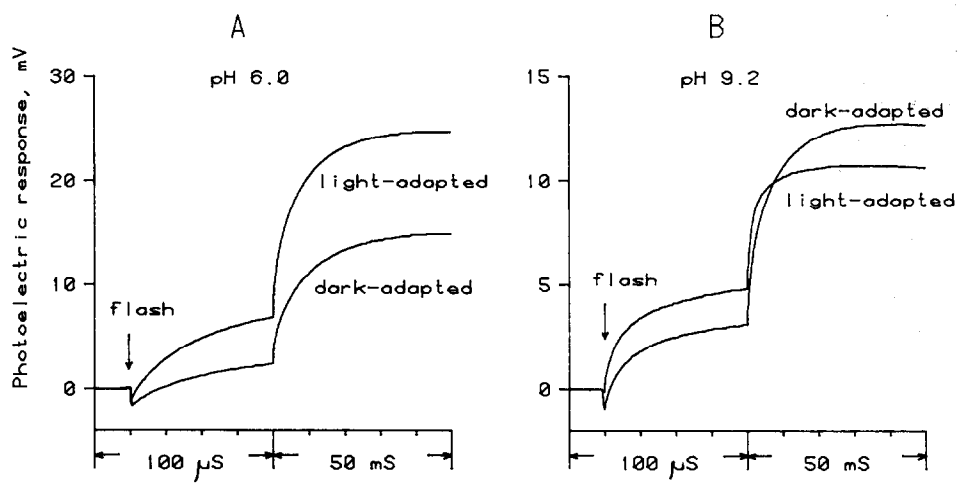

Fig.3. Photoelectric responses of bR in proteoliposomes adsorbed onto an asolectin-impregnated collodion film. Adsorption of proteoliposomes was carried out in 100 mM NaCl, 15 mM $CaCl_2$, 5 mM Mes, pH 6.0, at 23°C for 12 h in the dark. The incubation mixture was then replaced by 0.1 M potassium citrate-phosphate-borate buffer of pH 6.0 (A) or 9.2 (B).

and 9.2, i.e. below and above the critical pH. To monitor electrogenesis, proteoliposomes were adsorbed onto an asolectin-impregnated collodion film. The electric potential difference across the film was measured using two electrodes on both sides of the film. At pH 6.0, light adaptation resulted in approx. 2-fold increase in magnitude of the flash-induced electric response, which is in agreement with the assumption that the proton-motive 13-*cis*-bR photocycle is not operative [8]. At the same time, under alkaline conditions the magnitudes of the electric responses of light- and dark-adapted samples were similar as if not only all-*trans*- but also 13-*cis*-bR photocycles were electrogenic.

Fig.4 shows a tentative scheme explaining the above data. It is suggested that in 13-*cis*-bR, the outward H^+-conducting pathway is not operative when the pH is below some critical level. It should be mentioned in this context that in all-*trans*-bR, the outward H^+ pathway can also be inactivated by the acidic pH shift but it occurs at much lower pH than in 13-*cis*-bR, namely pH 3.5 [13]. The simplest assumption is that there is a protolytic group (y) in the outward H^+ pathway which is deprotonated in the bR ground state and accepts H^+ when M is formed. The outward H^+ pathway is not operative when y is already protonated in the bR ground state, i.e. when pH is below the pK of this group. This pK is $\leqslant 3.5$ in all-*trans*-bR and $\geqslant 7$ in 13-*cis*-bR. In the latter case, the pK strongly depends upon the state of bR preparation. In bR trimers (purple sheets) it amounts to 9.5 for low and 8.5 for high ionic strength. In bR monomers, i.e., in proteoliposomes and solubilized bR, it is shifted to 7.6 and 7.0, respectively.

In fig.4 it is also shown that the M intermediate formed from 13-*cis*-bR in a single-photon process regenerates 13-*cis*-bR rather than all-*trans*-bR. We found that this is the case at pH values above the critical values mentioned.

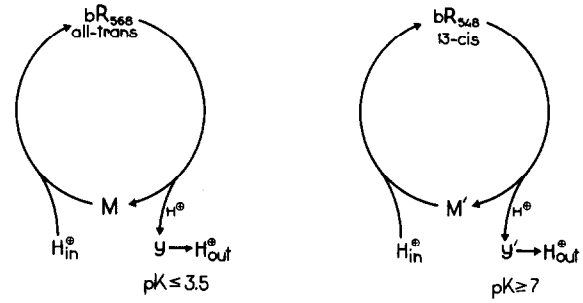

Fig.4. A tentative scheme of photocycles of all-*trans*-bR and 13-*cis*-bR. (y) A protolytic group in the outward H^+-transfer pathway.

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