ON THE PHOTOCYCLE AND LIGHT ADAPTATION OF DARK-ADAPTED BACTERIORHODOPSIN

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ABSTRACT Pulsed Nd laser (25 ns, 530 nm) photolysis experiments were carried out at room temperature in aqueous suspensions of dark- and light-adapted fragments of the purple membrane of *Halobacterium halobium.* It is shown that the (50%) 13-*cis* isomeric component ($BR_{13\text{-}cis}$) of dark-adapted bacteriorhodopsin (BR_{DA}) undergoes a photocycle involving a characteristic transient absorbing in the neighborhood of 610 nm. At relatively high excitation intensities $BR_{13\text{-}cis}$ is converted to the same 410 nm (M) transient that characterizes the photocycle of the all-*trans* isomer (BR_{trans}) of light-adapted bacteriorhodopsin (BR_{LA}). This process, which competes with the generation of the "610" species, is attributed to the photo-induced conversion, during the pulse, of $BR_{13\text{-}cis}$ (or of its primary photoproduct "X") to a species in the BR_{trans} photocycle. The relationship between these observations and the mechanism of $BR_{DA} \xrightarrow{h\nu} BR_{LA}$ adaptation at low excitation intensities (for which a quantum yield limit, $\phi \leq (3.5 \pm 0.7) \times 10^{-2}$, is established) is discussed.

Bacteriorhodopsin, the pigment of the purple membrane of *Halobacterium halobium,* exists in two interconvertible forms (1): a light-adapted modification (BR_{LA}) with maximum absorbance at 570 nm containing an all-*trans* retinyl polyene residue (BR_{trans}), and a dark-adapted form (BR_{DA}) (λ_{max} = 560 nm) composed of equal amounts of all-*trans* (BR_{trans}) and 13-*cis* ($BR_{13\text{-}cis}$) isomers (2).[1] The photochemical cycle of BR_{LA} has been extensively investigated (3–9), especially in relation to the light-driven proton pump in the membrane (1, 3, 4). However, essentially no information was available on the details of the BR_{DA} photochemistry, including the mechanism of light adaptation (see ref. 10 for a preliminary report on a recent work in this respect). The latter problem is of importance also in relation to the analogies between the photochemical mechanism in bacteriorhodopsin and visual pigments (11). The present communication presents results related to the yield of light adaptation and to some aspects of the BR_{DA} photocycle.

Aqueous suspensions of purple membrane fragments (*H. halobium,* M_1, [BR] = 7.5 μM) were exposed at room temperature to the pulsed output (25 ns, 530 nm) of a Q-switched, frequency-doubled, Nd laser. The details of the experimental setup, including a general analysis of light intensity effects and the precautions taken for minimizing the effects of the monitoring beam, have been previously described (7–9, 12, 13).

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[1] Pettei, M. J., A. P. Yudd, K. Nakanishi, and W. Stoekenius. 1976. Private communication.

After the laser pulse, transient changes in absorbance were recorded in both light-adapted (ΔD_{LA}) and dark-adapted (ΔD_{DA}) solutions. When analyzing the results we have made use of the fact that $\mathrm{BR}_{\mathrm{DA}}$ solutions contain equal amounts of the $\mathrm{BR}_{13\text{-}cis}$ and BR_{trans} isomers, i.e., $[\mathrm{BR}_{13\text{-}cis}] = [\mathrm{BR}_{trans}] = \frac{1}{2}[\mathrm{BR}_{\mathrm{total}}]$ (2, 10).[1] Since the absorption spectra of $\mathrm{BR}_{\mathrm{LA}}$ and $\mathrm{BR}_{\mathrm{DA}}$ are characterized by an isobestic point very close to our 530-nm excitation wavelength, we have estimated the absorbance changes exclusively due to the photochemistry of the 13-*cis* chromophore ($\Delta D_{13\text{-}cis}$), using the expression: $\Delta D_{13\text{-}cis} = \Delta D_{\mathrm{DA}} - \frac{1}{2}\Delta D_{\mathrm{LA}}$. This procedure assumes that the photochemical patterns in both systems are independent (i.e., additive) and that the all-*trans* component of dark-adapted solutions is identical to that present in $\mathrm{BR}_{\mathrm{LA}}$.

A transient difference-spectrum of $\Delta D_{13\text{-}cis}$, recorded after 1 ms under relatively high intensity excitation conditions, is presented in Fig. 1. It exhibits the 410-nm band, characteristic of the "M_{410}" transient of the $\mathrm{BR}_{\mathrm{LA}}$ photochemistry (1, 3–9) (its decay time, $\tau_{1/2}$ = 6.5 ms, was found identical to that of the 410-nm transient in the light-adapted cycle), a minimum at ~ 540 nm representing the depletion of $\mathrm{BR}_{13\text{-}cis}$, and a peak at 610 nm due to a new transient, characterized by a half-life of ~ 40 ms, which is not detectable in the photocycle of $\mathrm{BR}_{\mathrm{LA}}$. The occurrence of a transient absorbing at 610 nm in the photolysis of $\mathrm{BR}_{13\text{-}cis}$ has recently been reported by Dencher et al. (10). We have previously established criteria for discriminating between primary and secondary photochemical effects in the pulsed laser excitation of visual (13) and purple mem-

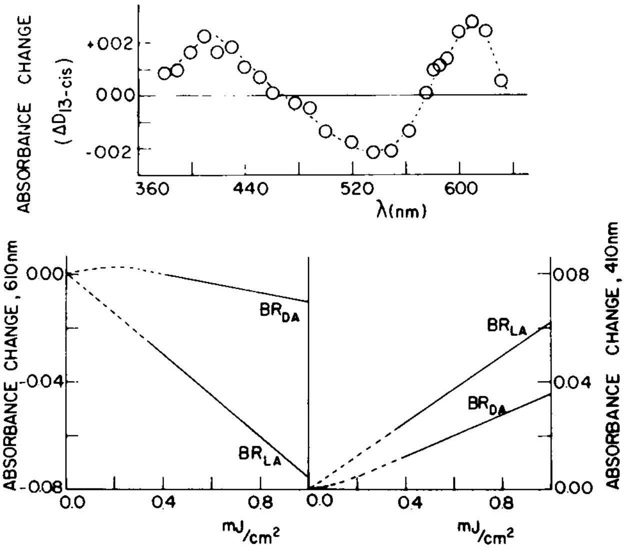

FIGURE 1 *Top:* Calculated difference spectrum ($\Delta D_{13\text{-}cis}$), 1 ms after pulsed (530-nm) excitation of dark-adapted bacteriorhodopsin. The experiments were carried out under relatively high pulse intensity conditions (~ 150 mJ/cm²). *Bottom:* Observed absorbance changes in dark- and light-adapted bacteriorhodopsin, at 610 nm and 410 nm, as functions of the laser pulse intensity. Each curve is the result of a linear regression analysis using 75 points. The dotted sections are qualitative estimates.

TABLE I

EFFECT OF THE LASER PULSE INTENSITY ON THE ABSORBANCE RATIO $\Delta D_{13\text{-}cis}$ (610 nm)/$\Delta D_{13\text{-}cis}$ (410 nm). THE PARAMETER IS EVALUATED FROM THE DATA OF FIG. 1 AS DESCRIBED IN THE TEXT.

Intensity	$\Delta D_{13\text{-}cis}$ (610 nm)/$\Delta D_{13\text{-}cis}$ (410 nm)
mJ/cm^2	
0.4	60 ± 8
0.5	35 ± 6
0.6	13 ± 3
0.7	8.7 ± 1.5
0.8	8.4 ± 1.5
0.9	7.3 ± 1.5
1.0	6 ± 1.5
150	1.0 ± 0.5

brane (7, 8) pigments. At relatively high excitation intensities, saturation conditions may prevail, under which a photoequilibrium is attainable between the pigment, its primary photoproduct, and secondary species generated from the excited photoproduct, e.g., rhodopsin, bathorhodopsin, and isorhodopsin, respectively (13). Being aware of the feasibility of such effects in the present BR_{DA} system, we carried out experiments in which ΔD_{DA} and ΔD_{LA} were measured (1 ms after pulsing) as function of the pulse intensity. The results are shown in Fig. 1, while the relative intensity dependence of $\Delta D_{13\text{-}cis}$ at 610 and 410 nm is presented in Table 1.

The results of Fig. 1 and Table I imply that the 610-nm transient is a product characteristic of the photocycle of $BR_{13\text{-}cis}$. This species predominates at low excitation intensities. However, as the intensity is increased, the relative amount of the M(410 nm) transient increases. Such behavior indicates that $BR_{13\text{-}cis}$, or its primary photoproduct (X), are photochemically converted to a species in the BR_{trans} photocycle, presumably to BR_{trans} itself or to its primary photoproduct K_{590} (3–9). This situation is consistent with two photocycles related as shown in Fig. 2. Although our data indicate a direct light-induced transition from either $BR_{13\text{-}cis}$ or X, to BR_{trans} or K_{590}, we cannot

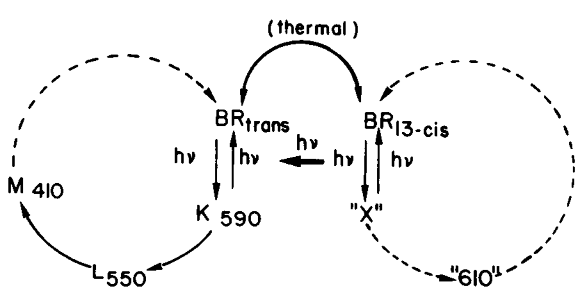

FIGURE 2 Suggested schematic diagram showing the relation between the photocycles of BR_{trans} and $BR_{13\text{-}cis}$. Dotted lines represent steps that may involve additional intermediates.

yet discriminate between the various plausible alternatives (e.g., $BR_{13\text{-}cis} \xrightarrow{h\nu} Br_{trans}$, $BR_{13\text{-}cis} \xrightarrow{h\nu} K_{590}$, $X \xrightarrow{h\nu} BR_{trans}$, $X \xrightarrow{h\nu} K_{590}$). Moreover, the lower limit established by us for the rate of formation of the 610 nm transient ($\tau_{1/2} < 20\ \mu s$), does not preclude the possibility that this species is actually the primary photoproduct of $BR_{13\text{-}cis}$ (i.e., $X \equiv$ the 610-nm intermediate).

By applying a previously described method (8) we have determined a limit for the $BR_{DA} \xrightarrow{h\nu} BR_{LA}$ quantum yield (ϕ), using low intensity pulses and monitoring the net absorbance change at 585 nm after completion of both photocycles. (The details of such experiments will be discussed elsewhere.) Since an approach to photoequilibrium between $BR_{13\text{-}cis}$ and X implies multiple excitations of the same $BR_{13\text{-}cis}$ molecule, the relatively low value obtained ($\phi \leq 0.035 \pm 0.007$) is in keeping with the occurrence of isomerization as a primary step in $BR_{13\text{-}cis}$ (i.e., $BR_{13\text{-}cis} \xrightarrow{h\nu} BR_{trans}$ or K_{590}), in competition with cycling via the 610-nm species. This interpretation is appealing since it involves a *cis-trans* isomerization as a primary photo-process in 13-*cis* bacteriorhodopsin, as it is the case for the 11-*cis* chromophore in the visual photocycle of rhodopsin (see ref. 11 for a discussion of the primary event in vision). However, at the present stage of the investigation, it is still impossible to exclude other (in our opinion less plausible) alternatives, (e.g., that although light adaptation under intense pulsed laser excitation is mainly due to the direct photoisomerization of $BR_{13\text{-}cis}$ or X, that induced by low intensity sources involves the thermal isomerization of X, of the 610 nm transient, or of any other intermediate in the photocycle of $BR_{13\text{-}cis}$).

Another point of interest is associated with the observation (2)[1] that the M(410 nm) transient of BR_{LA} represents a 1:1, 13-*cis*: all-*trans* mixture. This implies that a 13-*cis* transient is generated in the photocycle of BR_{trans}, which is, however, ultimately reverted to the exclusively transoid molecule $BR_{trans} \equiv BR_{LA}$. It would be interesting to establish whether the isomerization process occurs thermally (in K_{590}, L_{550} or M_{410}) or photochemically, being associated with the primary $BR_{trans} \xrightarrow{h\nu} K_{590}$ step. If the K_{590} transient does actually represent a mixture of 13-*cis* and *trans* isomers, it would be reasonable to assume that the process $BR_{13\text{-}cis}$ (or X) $\xrightarrow{h\nu} K_{590}$ is that responsible for the transition from BR_{DA} to BR_{LA}. More work will obviously be needed to elucidate the details of the BR_{DA} photocycle, including its relationship to that of BR_{trans}.

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